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Proceedings of the 17th International Sunflower Conference
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Photography: *Spanish landrace of confectionary sunflower collected by L. Velasco and B. Pérez-Vich in Villarta de San Juan, Ciudad Real, Spain, on October 10, 2007.*

Foreword

The proceedings of the *17th International Sunflower Conference* contain 142 contributions from scientists of 24 countries. They include plenary lectures in several disciplines and regular communications presented in posters during the conference and discussed in the corresponding workshops. The manuscripts are classified by disciplines. They offer a good picture of the current state of the art of sunflower research and cultivation around the world.

The manuscripts in the *Proceedings* have been reviewed by an editorial committee with the main objective of helping the authors to improve their manuscripts through a critical reading. The authors received the edited manuscripts together with the comments of the reviewers and then went on to draft their final version. All the manuscripts received have been published in the *Proceedings*. The contents of the manuscripts are the responsibility of the authors. They should be considered as being privileged communications that require the express consent of the authors to be reprinted in part or as a whole. We wish to thank both the members of the Editorial Committee for their dedication to the task of editing such a large number of manuscripts, as well as all the authors for their collaboration throughout the whole edition process.

The Organizing Committee would also like to thank Diana Badder and José A. Palacios for their excellent editorial assistance in the preparation of these *Proceedings*. We are indebted to the Spanish Association of Sunflower Breeders (Asociación Española de Mejoradores de Girasol), which collaborated actively in the organization of the conference, and, very especially, to Juan Parejo, who was in charge of the financial side.

Finally, we would like to thank all the participants in the conference, who have contributed to its success by a careful preparation and revision of manuscripts and posters, presentation of their research in the workshops, and stimulating discussions throughout the conference on the scientific and technical aspects of sunflower research and cultivation in the world.

The Organizing Committee
17th International Sunflower Conference
Córdoba, Spain. June 8-12, 2008

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PLENARY

DISEASE RESISTANCE AND PATHOLOGY

CROP PRODUCTION – PHYSIOLOGY

Research progress in sunflower diseases and their management

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ABSTRACT

Sunflower diseases are of major concern in the production of this crop worldwide. This is due to the regular and quite often severe attack by different pathogens. As a result considerable yield losses occur or the quality of product lessens. Though the number of pathogens known to attack sunflower is relatively high, only a handful to a dozen are considered important ones depending on region and cultivar. In this review, I am focusing particularly on these significant pathogens. The emphasis will be on new findings and results obtained by researchers related to pathogen biology, ecology, genetics, host resistance, and control. It was interesting to note a considerable shift in the relative dominance of diseases over the last four years as reflecting in the number of publications available. The scientists' efforts have resulted in better understanding of individual diseases and underline their significance in the improvement of sunflower management.

Key words: ecology – diseases – genetics – host resistance – pathogen biology

INTRODUCTION

Sunflower diseases are one of the major constraints in influencing production stability of this crop worldwide. Though there are more than a dozen of pathogens that may attack cultivated sunflower resulting yield losses, just a few are of concern in a particular country or region. In a literature survey of the past four years (2004 to 2007), I found a great number of publications regarding sunflower diseases. Similarly to Felicity Vear's findings reported at the 16th International Sunflower Conference in Fargo (Vear, 2004), there were significant differences in the number of papers dealing with individual pathogens and/or originating from different countries and regions. An overview of reference sources obtained from the Web of Science or kindly provided by individual scientists served as a basis of this present review. A total of 13 sunflower pathogens were the subject of papers available and there were major differences in the proportion of these references (Table 1). Most of the publications dealt with downy mildew (*Plasmopara halstedii*), and broomrape (*Orobanche cumana*), followed by white rot (*Sclerotinia sclerotiorum*), stem canker (*Diaporthe helianthi*), Alternaria blight (*Altermaria helianthi*, *A. helianthinficiens*), rust (*Puccinia helianthi*) and black stem (*Phoma macdonaldii*) in a decreasing sequence of order. Some diseases of local importance represented by several or just a few references are Verticillium wilt (*Verticillium dahliae*), white blister rust (*Albugo tragopogonis*), charcoal rot (*Macrophomina phaseolina*), Fusarium wilt (*Fusarium* spp.), Rhizopus head rot (*Rhizopus arrhizus*), and *sunflower chlorotic mottle virus* (SuCMoV). In addition, information on sunflower diseases is also available in a Progress Report by Masirevic (2005a) based on contributions of the sub-group leaders of the Working Group Sunflower Diseases, presented at the 10th FAO European Research Network on Sunflower Consultation Meeting in Novi Sad in 2005.

Table 1. A list of references concerning sunflower diseases for the period 2004-2007 available for the author

Disease	Pathogen	No. of records
Downy Mildew	*Plasmopara halstedii*	46
Broomrape	*Orobanche cumana*	31
White rot	*Sclerotinia sclerotiorum*	17
Stem canker	*Diaporthe helianthi*	15
Alternaria blight	*Alternaria helianthi*, *A. helianthinficiens*	15
Rust	*Puccinia helianthi*	12
Phoma black stem	*Phoma macdonaldii*	10
Virus	*Sunflower chlorotic mottle virus*	7
Verticillium wilt	*Verticillium dahliae*	5
Charcoal rot	*Macrophomina phaseolina*	4
White blister rust	*Albugo tragopogonis*	4
Fusarium wilt	*Fusarium* spp.	3
Rhizopus head rot	*Rhizopus* spp.	1

DISCUSSION

Downy mildew. Based on a literature survey for the years 2004-2007, most of the publications for this period have dealt with this devastating disease caused by *Plasmopara halstedii* (Farl.) Berl. et de Toni. It continued to occur in almost all parts of the world where sunflower was grown, except for Australia. The biology and ecology of this organism is well-known as are many aspects of its pathogenicity, host – pathogen interaction and genetic and chemical control. Its capacity to diverse, both in virulence and fungicide, however, is very high, giving a continuous challenge to scientists.

The most detailed and up-to-date list of global distribution of *P. halstedii* pathotypes has been compiled by Gulya (2007) in a paper presented at the 2nd International Downy Mildew Symposium, Olomouc, Czech Republic. In this accurate overview he comprised as many as 35 pathotypes (races), an unbelievably high number considering the fact that in most sunflower producing countries from just a few to 12 well-distinguished virulence phenotypes exist. In Europe, France, Germany and Spain reported the highest numbers but the pathogen is rather diverse in the USA, Canada, and in South Africa as well. Furthermore, there are five *P. halstedii* pathotypes (300, 330, 710, 730, 770) that are universally distributed globally, recorded from North and South America, Europe and Africa. Apart from the quantitative aspect of virulence, it is interesting to consider the dynamics of diversity as well, i.e. the changes in a given region over time. In this respect, France leads with the highest number of new pathotypes arisen in the last 6-7 years (Vear et al., 2006). Considering population changes for virulence, a good example has recently be found in the USA by Gulya (unpublished), where 3 out of 11 pathotypes (710, 730, 770) were recorded from North and South Dakota and Minnesota in each year during the 1998 to 2007 period whereas two others (300, 772) appeared in one year and a third one (300) in two years only. Recently, Delmotte et al. (2008) analyzed the possible origin of *P. halstedii* populations existing in France using different molecular methods. Based on single nucleotide polymorphisms they assumed a multiple introduction into France of the pathogen populations exhibiting differences in virulence phenotype.

Like other biotrophic obligate parasites, *P. halstedii* has a narrow host range. In other words, though it has originally been described to occur on a number of Composites and was found to attack a few wild *Helianthus* species as well, until recently no much attention has been paid to any alternate host as potential infection sources. Recently, however, two records of the natural occurrence of this Oomycete on wild asteraceous plants appeared, on velvetleaf (*Abutilon theophrasti*) by Masirevic (2005b) in Serbia and on *Rudbeckia fulgida* by Hong (2006) in Virginia. With these records, a total of five asteraceous wild plant species (the other three being *Xanthium strumarium*, *Ambrosia artemisiifolia* and *Iva xanthifolia*) are known to be as alternative hosts of *P. halstedii*. The natural host state in each case has been proved with successful reinoculation to cultivated sunflower.

With the rapid improvement of molecular techniques and their use in plant pathology, new developments have opened new insight into research on fungal biology, detection technology, and genetics and host - pathogen interactions. For example, Hammer et al. (2007) in Germany, and Ioss et al. (2007) in France, using different approaches, were successful in detecting fungal structures from sunflower host tissues.

Furthermore, it became possible to study the genetic recombination in *P. halstedii* through parasexual events using DNA fingerprinting (Spring and Zipper, 2006). In attempts to characterize the molecular structure of this Oomycete, Thines and co-workers (2005) detected and characterized an exceptional length of ITS that was due to multiple repetitions in the ITS-2 region. Further, ITS sequence data were also used to detect possible differences between isolates differing in virulence and/or in geographic origin.

Recently, two papers dealt with the isozyme analysis of *P. halstedii* isolates in order to find out intraspecific polymorphism in sub-populations of this organism. Guchetl et al. (2007) studied eight different isoenzymes. While interracial differences on esterase were inessential, the other seven isoenzymes appeared to be monomorphous for all pathotypes studied (330, 700, 710 and 730) suggesting that downy mildew populations in the Krasnodar region had low intraspecific variability for these traits. Komjáti et al. (2008) in Hungary used cellulose-acetate gel electrophoresis to analyze sixteen isozyme systems of 10 field isolates and 35 single-spore lines representing 10 different virulence phenotypes. Apart from sunflower, isolates were also from cocklebur (*Xanthium strumarium*) and from *Helianthus x laetiflorus*. Three isozymes, isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH) and phosphoglucomutase (PGM) revealed some polymorphism among the isolates. PGM differentiated two groups among the isolates from cultivated sunflower, while the other enzymes were polymorphic between isolates from different hosts. However, polymorphisms did not relate to virulence phenotype.

To develop and release sunflower cultivars resistant to different pathotypes is of extreme importance to growers. Therefore, breeders are continuosly searching for new genes or gene clusters conferring resistance to *P. halstedii* (Sreten et al., 2007), and selecting for such genes using molecular markers. In the recent years, Radwan et al. (2004) in France and Dussle et al. (2004) in Germany achieved considerable results with PCR markers for the *Pl5*/*Pl8* locus from complete CC-NBS-LRR sequences and, with the localization of the Pl_{arg} gene using SSR markers, respectively. Furthermore, in inheritance studies of resistance to the 703 pathotype Pankovic et al. (2007) used both traditional segregation tests and PCR markers and obtained identical results related to the *Pl6* gene conferring resistance to 730.

At the Fargo conference, Felicity Vear presented an outstanding review of recent breeding work for resistance to sunflower pathogens (Vear, 2004). At that time she outlined the importance of durable resistance to combat the increasing number of new *P. halstedii* pathotypes overcoming the *Pl* gene-mediated resistance. Since then the Clermont-Ferrand group has proceeded with this work and some of their results have already been published (Tourvieille et al., 2005; Vear et al., 2006). Durable resistance was found to be independent of major gene resistance so they proposed for the future to combine both types of resistance in new cultivars for more effective and long-lasting genetic control. Partial resistance to *P. halstedii* in high oleic sunflower hybrids have been reported by Baldini et al. (2006) as well in Italy.

The hypersensitive reaction (HR), a well-known phenomenon among plant pathologists, has been the subject of investigations by Radwan et al. (2005) to characterize this mechanism in the sunflower downy mildew system. RT-PCR analysis showed that resistance was associated with the activation of a hsr203J-like gene, a molecular marker of HR in tobacco. Activation of this gene was specifically observed during the incompatible interaction and coincided with cell collapse in hypocotyls. No such HR or a significant activation of the hsr203J-like gene were observed during the compatible combination suggesting that HR failed to halt the parasite, rather it trigged a systemically-aquired resistance (SAR) taking place in the upper part of the hypocotyl and this might arrest pathogen growth.

Apart from durable resistance, induced resistance might be useful for improving downy mildew management. In the recent years, Hungarian and Spanish laboratories conducted studies to better understand this type of host defense. The plant activator benzothiadiazole (BTH) significantly depressed disease symptom appearance and pathogen growth in susceptible sunflowers treated and inoculated at the germling stage (Körösi et al., 2007). Furthermore, microscopical observations revealed a high similarity between genetic (*Pl*-gene mediated) and induced resistance responses in compatible combinations. Roldan Serrano et al. (2007) recently published a paper about chitinase and peroxidase activities in BTH-treated sunflower inoculated with *P. halstedii*. They found an increased level of activity for both enzymes in susceptible but not in resistant seedlings. In our laboratory, we compared susceptible, partially resistant and resistant interactions for various enzyme activities (unpublished) and also tested the glutathion S-transferase (GST), defensin (PDF) and catalase (CAT) gene expression. Preliminary results of this work will be presented by Körösi et al. in this conference.

Resistance or tolerance to metalaxyl has already been noted in France and the USA. Quite recent records, however, came from Spain (Molinero-Ruiz et al., 2005) and Germany (Spring et al., 2006). It is interesting to note that, as an alternative, Fernández-Ocaña et al. (2004) conducted experiments with the essential oil of *Bupluerum gibraltarium*. They found this oil acting as a host defense activator rather than directly inhibiting sporulation.

Broomrape. *Orobanche cumana* Wallr. is a parasitic plant that infects sunflower causing considerable damage. In Spain, parasitized plants exhibited lower shoot dry weight, and they were shorter (due to reduction in internode length) with smaller head diameter as compared to healthy ones (Alcántara et al., 2006). In addition, a significant decrease in the mineral composition of the leaves of affected plants could be detected.

Different pathogenic races of *O. cumana* are known to exist in various regions of Europe and in the southeastern Mediterranean where the climate is favorable for this parasite. Due to this genetic diversity a new pathogenic form, race F appeared recently in Spain (Pérez-Vich et al., 2004), in Russia (Goncharov et al., 2004), in Turkey (Kaya et al., 2004), in Israel (Eizenberg et al., 2004), and in Bulgaria (Shindrova, 2006), with the highest diversity existing in Turkey. More expanded field surveys and subsequent identification processes are required to get a better view of the incidence and distribution of pathogenic variants of this parasite. In this respect, Román et al. (2007) succeeded in developing a detection method by using cpDNA diagnostic markers and they proposed this molecular protocol for use in identification work.

In a study on the mechanism of broomrape parasitism in sunflower, Slavov et al. (2004) pointed out that seed germination of the parasite was triggered by a germination stimulant secreted by the host-plant

roots. Further, they quantified indole-3-acetic acid as early as 24 h after the seeds were exposed to the germination stimulant, suggesting the role of IAA in the germination process. When comparing different populations of this parasite for their virulence on different sunflower genotypes, Veronesi et al. (2005) found that before attachment, *Orobanche* seedlings released cell-wall-degrading enzymes such as pectin methylesterase and polygalacturonase. These enzymes' activity were very high in the most virulent, recently discovered race F. Eizenberg et al. (2005) developed a new methodology that allowed them to facilitate the in-situ study of major aspects of the host - parasite interaction.

Broomrape resistance is poorly understood and new races of the parasite evolve rapidly to overcome the resistance of newly introduced sunflowers. Labrousse et al. (2004) screened a number of recombinant inbred lines derived from interspecific crossings. A considerable variation in the characters tested showed that polygenic resistance could occur in some lines. In another experimental system Echevarría-Zomeno et al. (2006) investigated the histology of host – parasite interface. Suberization and protein cross-linking at the cell wall were seen in the resistant sunflower cells in contact with the parasite and confocal laser microscopy revealed accumulation of phenolic compounds during the incompatible reaction. Letousey et al. (2007) carried out molecular analysis of the resistance mechanism. RT-PCR and cDNA blot experiments revealed that the *Orobanche* resistant genotype exhibited a stronger overall defense response against *O. cumana* than the susceptible one. The SA-responsive gene, *def.* (defensin), appeared to be characteristic of LR1 sunflower resistance. Ha-DEF1 (a sunflower defensin) was found to induce cell death in the parasitic plants appearing as a brown symptom at the radicle apex of the parasite (de Zélicourt et al., 2007). The resistance phenomenon to broomrape in sunflower was also the subject of studies to map and characterize quantitative trait loci for resistance to race E and race F by Pérez-Vich and co-workers (2004). Their results suggested that resistance to broomrape in sunflower is controlled by a combination of qualitative, race-specific resistance affecting the presence or absence of broomrape and a quantitative, non-race specific resistance affecting their number.

In inheritance studies on the sunflower line J1 to *Orobanche* race F, Velasco et al. (2007) detected incomplete dominance of the *Or6* alleles and subsequent segregation ratios suggested the presence of a second gene, *Or7*, the expression of which was influenced by the environment. Meanwhile Spanish breeders were successful in finding sunflower germplasm resistant to race F of broomrape (Fernandez-Martinez et al., 2004) and those with quantitative resistance to the same race (Pérez-Vich et al., 2006). In addition, sunflower hybrids resistant to race F have been released in Spain (Pérez-Vich et al., 2004), in Russia (Goncharov et al., 2004), and in Turkey (Kaya et al., 2004).

For future broomrape management it might be of interest to consider to use host defense system as an alternative to genetic resistance. Buschmann et al. (2005) reported about positive results with BTH against *O. cumana* infestation, and later on Fan et al. (2007a), from the same laboratory, evaluated the efficacy of prohexadione-calcium against this parasite. Neither of these plant activators had a direct effect on the parasite, but rather induced host defense only.

An additional way of broomrape control could be by using biological antagonists. One of the candidates is *Fusarium oxysporum* f. sp. *orthoceras*, which was the subject of investigations by Dor et al. (2007). They studied the pathogenicity and toxin production of this fungus. Two main toxic metabolites caused mortality of germinating broomrape seeds and these were identified as fusaric acid and 9,10-dehydrofusaric acid. Müller-Stöver et al. (2004) also found *F. oxysporum* f. sp. *orthoceras* (Fo) as a potential of biocontrol agent and they were successful developing two granular formulations under laboratory conditions. In an other experiment Müller-Stöver and co-workers (2005) were able to increase control of *O. cumana* through integration of Fo with BTH-treatment. Under laboratory conditions no enhancing effect of BTH on virulence and growth of the fungus was observed. Fan et al. (2007b) achieved similar results when they combined the application of Fo and acibenzolar-S-methyl (ASM). The interaction between ASM and Fo was highly significant on *O. cumana* number and dry matter. ASM soil drenches combined with Fo were more effective than ASM foliar spray with Fo.

White rot. Sunflower stalk and head rot incited by *Sclerotinia sclerotiorum* (Lib.) de Bary is considered the most important disease of this crop in many parts of the world. Since cultural practices or fungicides are insufficient to control the disease, efforts are being made by breeders to develop resistant or tolerant cultivars. This may explain the dominance of publications dealing with various aspects of resistance.

Disease incidence of white rot may vary with location and season, as well as with sunflower genotype, and the symptoms appearing on sunflower stem or head are also diverse. For example, in the United States a three-year field survey (2005-2007) was made by Tom Gulya and co-workers (2008) in the main sunflower growing regions (North and South Dakota, Minnesota) regarding *Sclerotinia* stalk rot

occurrence. Both, the percentage of fields with stalk rot and the severity of affected fields varied between 16-30 %, and 4.4-6.3 %, respectively.

In the recent years, several reports have dealt with the evaluation methods of sunflower genotypes for resistance to *S. sclerotiorum*. Thus, Baldini et al. (2004) in Italy compared host reactions to basal stem and head inoculation, Pedraza et al. (2004) in Argentina examined the suitability of the length of susceptible period as a measurement of partial resistance, van Becealere (2004) in the USA described an improved screening method for assessing head rot resistance, and Castaño et al. (2005) compared the reaction of sunflower accessions to both *S. sclerotiorum* and *Albugo tragopogonis*. By looking for resistance sources among the wild *Helianthus* species, Cáceres et al. (2006) found differences in lesion length of leaves following inoculation but it was not the case with stem inoculation.

In a breeding program in France, Felicity Vear and co-workers (2007) aimed at improving the *S. sclerotiorum* head rot resistance using recurrent selection of a restorer population. In 4 cycles an 80 % reduction in diseased area was obtained and thereafter the population remained stable and homogenous for this character.

Maringolo et al. (2007) in Argentina successfully studied quantitative trait loci for sunflower capitulum resistance to head rot.

Stem canker. *Diaporthe helianthi* Muntanola-Cvetkovic, Mihaljcevic et Petrov (anamorph: *Phomopsis helianthi* Muntanola-Cvetkovic, Mihaljcevic et Petrov) has become a serious threat in sunflower production in the early 1980s in Europe and subsequently in other parts of the world, e.g. in North and South America. Relatively soon after its appearance, it became one of the most limiting factor of sunflower production in many parts of Europe, including the former Yugoslavia, Romania, Hungary, and France. However, following several years of epidemics in these countries, the disease occurrence lessened probably due to unfavorable weather conditions (dry and hot). In contrast, Huguet (2005) reported about a severe attack of this pathogen from a region of Uruguay close to the Argentinian border having an average incidence of 39%.

Walcz and Nébli (2006) investigated the persistence of this pathogen in infected stems and achenes. They found that *D. helianthi* perithecia even disposed to outdoor conditions for 3 years produced viable ascospores, as well as a few pycnidia (the latter occurring most on achenes). The fact that *D. helianthi* can be distributed with seed underlines the importance of phytosanitary measures in seed production and commerce.

Molecular studies on the intraspecific diversity of this fungus using intergenic spacer sequence analysis revealed a high homology among French/Yugoslavian and among Italian isolates (Pecchia et al. 2004). The phylogenetic tree obtained from the aligned data revealed three separate groups. The analysis also showed that all isolates originating from countries with regular and severe outbreaks of the disease (e.g. France, Yugoslavia) formed a well-defined taxon with relatively low variability compared to isolates from Italy where the disease is much seldom to occur. In another paper, Rekab et al. (2004) pointed out a polyphyletic nature of this fungus.

Besides traditional methods of resistance testing (Walser et al., 2005), Quaglia and Zazzerini (2007) reported about an *in vitro* screening for sunflower calli to *D. helianthi* fungal culture filtrate. Looking for recent publications regarding resistance breeding programs, only two reports were available. A collaborative work between Bulgaria and Germany (Encheva et al., 2004) evaluated somaclonal variation, and a study from Hungary (Csikász et al., 2006) in which selection of elite lines was described for specific resistance alleles.

Alternaria blight. The disease can be incited by two fungi *Alternaria helianthi* (Hansf.) Tubaki et Nishihara and *A. helianthinficiens* Simmons, Walcz et Roberts, but Gonorazky et al. (2005) described *A. alternata* as well as one of the seed infecting species found in Argentina. Calvet et al. (2005) determined the average decrease in the photosysnthetic rate in diseases leaves, and Leite et al. (2006) showed that disease severity could be used as an independent variable in a sunflower – *Alternaria* leaf spot management system by providing recommendations for resistance breeding or for studies on sowing date.

Madhavi et al. (2005a) compared six wild *Helianthus* species for resistance to *Alternaria* blight: *H. occidentalis* and *H. tuberosus* were found highly resistant, and *H. hirsutus* moderately resistant. Furthermore, on growth media supplemented with leaf extracts of these plant species the inhibition of fungal growth corresponded to *in vivo* responses of the particular species to inoculation. Further, the resistant *Helianthus* species possessed higher levels of constitutive as well as induced total phenols and total sugars as compared with susceptible sunflowers (Madhavi et al., 2005b).

Resistance breeding was the subject of several papers appeared in the recent years. De Oliveira et al. (2004) reported about mutation breeding from Brazil, and Murthy et al. (2005) assessed heritability of resistance using molecular markers. In India, ploidy manipulation and introgression of resistance to *A. helianthi* using wild *Helianthus* species as resistance source (Sujatha and Prabakaran, 2006), sporophytic and gametophytic recurrent selection for improving partial resistance (Rani and Ravikumar, 2006; 2007), and the description of transcripts during the necrotrophic interaction with *A. helianthi* (Anjana et al., 2007) reflected to the relative dominance of this disease in this country.

Rust. *Puccinia helianthi* Schwein. has a world-wide distribution but it has been considered as a severe pathogen causing considerable yield losses mainly in Australia and Argentina (Huguet et al. 2007). However, Zazzerini et al. (2005) reported about a considerable occurrence and spread of this disease from Mozambique as well.

The diversity of the sunflower – *P. helianthi* pathosystem has got a special attention by Sendall and co-workers (2006) describing a rapid and frequent virulence changes in the rust fungus population. Virulence data accumulated over 25 years coupled with studies on genotypic diversity and sexual reproduction permitted them to conclude that *P. helianthi* may evolve in wild sunflower populations providing a continuum of genetically heterogenous hosts on which this fungus can potentially complete its sexual cycle.

In Spain, Prats et al. (2007) carried out experiments to characterize the mechanism of resistance. Microscopical observations revealed that rust development depended on host genotype, i.e. impairment of rust spore germination and of appressorium formation associated with different excretion of coumarin on leaf surface. Mohase et al. (2006) in South Africa investigated the effect of rust infection on intercellular beta- 1,3-glucanase and chitinase activities, PAL acitivity and total salicylic acid content in relation to susceptibility vs. resistance interactions. Rust infection selectively increased the activity of pathogenesis-related proteins and other parameters studied. Treatment of susceptible plants with BTH induced intercellular glucanase activity and reduced susceptibility to rust. Induced resistance was also the subject of another paper by Amzalek and Cohen (2007) from Israel. Besides BTH, they used other inductors as DL-3-amino-n-butanoic acid (BABA), 2,6-dichloroisonicotinic acid (INA), and two enantiomers of BABA as well.

Phoma black stem. The disease is caused by *Phoma macdonaldii* Boerema (teleomorph: *Leptosphaeria lindquistii* Frezzi) appearing as black spots on stems and seldom on leaves of affected plants. Though its occurrence is quite common in several European countries, the disease is extremely severe in France where basal stem lesions often result in lodging. This could be the reason of the absolute dominance of French publications that appeared in the recent years. Darvishzadeh et al. (2007a) undertook experiments to determine the partial resistance of sunflower genotypes to seven isolates and highly significant differences were observed among genotypes, isolates and their interactions. Two genotypes exhibited specific resistance with a wide range of isolate-nonspecific partial resistance appearing as well. In addition, QTLs were also found associated with isolate specific and non-specific partial resistance (Darvishzadeh et al., 2007b). Alignan et al. (2006) developed a 1000-element cDNA microarray containing genes putatively involved in primary metabolic pathways in order to identify genes responsible for partial resistance. They were successful in identifying 38 genes differently expressed among genotypes, treatments and times. Comparative genetic analysis for the characterization of QTL involved in resistance of sunflower to *Ph. macdonaldii* has been made by Bert et al. (2004), and QTL mapping of partial resistance to stem and root necrosis in sunflower as well as inheritance studies were the subject of investigation by Abou Al Fadil et al. (2006, 2007).

Verticillium wilt (*Verticillium dahliae* Kleb.) is considered an important disease affecting sunflower in most production areas in Argentina (de la Vega et al., 2007), and is of concern also in Canada and the Unites States. Estimation of yield losses is difficult because of the absence of highly efficient chemical control (Creus et al., 2007). Therefore, host resistance is a major concern of breeders in these countries. Resistant breeding is in progress in Argentina (Maranesi and Mancuso, 2007) and in the United States (Radi and Gulya, 2007).

Charcoal rot (*Macrophomina phaseolina* [Tassi] Goidanich) may cause premature death of sunflowers grown on light, sandy soil under hot and dry climate. The disease is well-known in the Southern part of Europe, but the first occurrence in Slovakia was unexpected and probably due to the extremely warm and

dry seasons at that time (Bokor, 2007). In Hungary, Walcz and Piszker (2004) have developed an inoculation method for screening sunflower lines for resistance to this pathogen.

White blister rust (*Albugo tragopogonis*) is know to occur as a pathogen of significance only in South Africa, but it was recorded recently from Germany (Thines et al., 2006a) where the percentage of affected plants varied between 20 and 80 %. Another record is known from Belgium (Crepe et al., 2006). Thines et al. (2006b) studied the fatty acid profile, ultra structural characteristics and ITS sequencing of this fungus. Castaño et al. (2005) evaluated the reaction of a number of sunflower accessions originating from the North Central Regional Plant Introduction Office, Ames, Iowa, USA. Statistical analysis showed differential responses to white rust severity, incidence and relative incubation period among the accessions tested.

Fusarium wilt (*Fusarium* spp.) has been reported as a pathogen of concern only from Russia (Antonova, 2004) where it appeared to be harmful for sunflower production. Based on the extent of necrosis incited by the fungus on the main root and the root – hypocotyl transition zone of sunflower seedlings, some tolerance to pathogen attack could be detected among the genotypes (Antonova et al., 2005). In a breeding program a number of new breeding lines were developed exhibiting relatively good field tolerance (Goncharov et al., 2006).

Rhizopus head rot (*Rhizopus* spp.) exists under warm climatical conditions, like in North Africa, Australia and India. However, with the global warming it seems to occupy new areas, such as the Mediterraneum and southern Hungary. The most typical disease symptoms include rotting of sunflower head with a loose cover of grayish fungal spore mass. In Hungary Walcz et al. (2004) examined the effect of *Rhizopus* head rot on the oil content, oil quality and the germinability of seeds. All these parameters showed a negative tendency in the affected seeds as compared to healthy ones.

Virus (*Sunflower chlorotic mottle virus*, SuCMoV) has recently been detected in commercial hybrids and wild sunflowers in Argentina (Lenardon et al., 2005; Lenardon and Gioletti, 2007). More than two hundred lines were screened for resistance using artificial inoculations under greenhouse conditions and only three lines showed partial resistance in which virus replication was delayed and morphological traits (plant height, leaf width) were less affected. Arias et al. (2005) studied the mechanism of oxidative damage in SuCMoV infected sunflowers. Jan and Gulya (2006) in the United States reported about the registration of three virus resistant sunflower genetic stocks.

CONCLUSIONS

In this review I tried to outline the recent progress in research and development of sunflower diseases involving various aspects of each, in most cases depending on the amount of information available. Unfortunately there was no means to include all relevant experimental data, due to the page limitation for plenary papers. Similarly, I did not try to make any statistics in relation to diseases, topics or countries. Instead, I want to express my hope and feeling that the great number of references discussed in this review will give the reader an up-to-date summary of the most recent pathology research, the impact of diseases on sunflower production, and challenges remain for researchers.

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REFERENCES

Abou Al Fadil, T., G. Dechamp-Guillaume, R. Darvishzadeh, and A. Sarrafi. 2007. Genetic control of partial resistance to 'collar' and 'root' isolates of *Phoma macdonaldii* in sunflower. Eur. J. Plant Pathol. 117: 341-346.

Abou Alfadil, T., S. Poormohammad Kiani, G. Dechamp-Guillaume, L. Gentzbittel, and A. Sarrafi. 2006. QTL mapping of partial resistance to *Phoma* basal stem and root necrosis in sunflower (*Helianthus annuus* L.). Plant Sci. 172:815-823.

Alcántara, E., M. Morales-García, and J. Díaz-Sánchez. 2006. Effects of broomrape parasitism on sunflower plants: Growth, development, and mineral nutrition. J. Nutr. 29:1199-1206.

Alignan, M., T. Hewezi, M. Petitprez, G. Dechamp.Guillaume, and L. Gentzbittel. 2006. A cDNA microarray approach to decipher sunflower (*Helianthus annuus*) responses to the necrotrophic fungus *Phoma macdonaldii.* New Phytol. 170:523-536.

Amzalek, E., and Y. Cohen. 2007. Comparative efficacy of systemic acquired resistance-inducing compounds against rust infection in sunflower plants. Phytopathology 97:179-186.

Anjana, G., K.R. Kini, H.S. Shetty, and H.S. Prakash. 2007. Differential expression on sunflower peroxidase isoforms and transcripts during necrotrophic interaction with *Alternaria helianthi*. Russ. J. Plant Physiol. 54:513-517.

Antonova, T.S. 2004. Sunflower disease caused by the fungi of *Fusarium* sp. Nauka Kubani 3:33.

Antonova, T.S., S.L. Saukova, and Y.V. Klippert. 2005. The reaction of sunflower roots and stems on infection by fungi of *Fusarium* species. Nauka Kubani 4:92.

Arias, M.C., C. Luna, M. Rodríguez, S.L. Lenardon, and E. Taleisnik. 2005. Sunflower chlorotic mottle virus in compatible interactions with sunflower: ROS generation and antioxidant response. Eur. J. Plant Pathol. 111:223-232.

Baldini, M., F. Danuso, M. Turi, M. Sandra, and S. Raranciuc. 2006. Downy mildew (*Plasmopara halstedii*) infection in high oleic sunflower hybrids in Northern Italy. Helia 29:19-32.

Baldini, M., M. Vischi, M. Turi, N. Di Bernardo, S. Raranciuc, M. Echeverría, F. Castaño, G.P. Vannozzi, and A.M. Olivieri. 2004. Evaluation of genetic variability for *Sclerotinia sclerotiorum* Lib. de Bary resistance in a F2 population from a cross between susceptible and resistant sunflower. Helia 27:159-170.

Bert, P-F., G. Dechamp-Guillaume, F. Serre, I, Jouan, D. Tourvieille de Laboruhe, P. Nicolas, and F. Vear. 2004. Comparative genetic analysis of quantitative traits in sunflower (*Helianthus annuus* L.). Theor. Appl. Genet. 109:865-874.

Bokor, P. 2007. *Macrophomina phaseolina* causing a charcoal rot of sunflower through Slovakia. Biologia 62:136-138.

Buschmann, H., H. Fan, and J. Sauerborn. 2005. Effect of resistance-inducing agents on sunflower (*Helianthus annuus* L.) and its infestation with the parasitic weed *Orobanche cumana* Wallr. J. Plant Dis. Prot. 112:386-397.

Cáceres, C., F. Castaño, R. Rodríguez, A. Ridao, T. Salaberry, M. Echeverría, and M. Colabelli. 2006. Variability of *Sclerotinia* responses in *Helianthus petiolaris*. Helia 29:43-48.

Calvet, N-P., M.R.G. Ungaro, and R.F. Oliveira. 2005. Virtual lesion of *Alternaria* blight on sunflower. Helia 28:89-100.

Castaño, F., T. Gulya, J. Rué, M. Echeverria, and R. Rodríguez. 2005. Reaction of some sunflower accessions to *Albugo tragopogonis* and *Sclerotinia sclerotiorum* infections. Helia 43:25-32.

Crepe, C., S. Inghelbrecht, and S.G. Bobev. 2006. First report of white rust caused by *Albugo tragopogonis* on sunflower in Belgium. Plant Dis. 90:379.

Creus, C., M.E. Bazzalo, M. Grondona, F. Andrade, and A.J. Leon. 2007. Disease expression and ecophysiological yield components in sunflower isohybrids with and without *Verticillium dahliae* resistance. Crop Sci. 47:703-710.

Csikász, T., M. Treitz, and I. Walcz. 2006. Selection of sunflower elite lines for specific alleles for resistance to *Diaporthe helianthi*. p. 84. In: Abstr. 12th National Plant Breeding Conference, Budapest, Hungary. (in Hungarian)

Darwishzadeh,, R., G. Dechamp-Guillaume, T. Hewezi, and A. Sarrafi. 2007a. Genotype-isolate interaction for resistance to black stem in sunflower (*Helianthus annuus*). Plant Pathol. 56:654-660.

Darwishzadeh, R., S.P. Kiani, G. Dechamp-Guillaume, L. Gentzbittel, and A. Sarrafi. 2007b. Quantitative trait loci associated with isolate specific and isolate nonspecific partial resistance to *Phoma macdonaldii* in sunflower. Plant Pathol. 56:855-861.

De la Vega, A.J., I.H. DeLacy, and S.C. Chapman. 2007. Progress over 20 years of sunflower breeding in Central Argentina. Field Crops Res. 100:61-72.

Delmotte, F., X. Giresse, S. Richard-Cervera, J. M'Baya, F. Vear, J. Tourvieille, P. Walser, and D. Tourvieille de Labrouhe. 2008. Single nucleotide polymorphisms reveal multiple introductions into France of *Plasmopara halstedii*, the plant pathogen causing sunflower downy mildew. Infect. Genet. Evol. (in press).

De Oliveira, M.F., A. Tulmann Neto, R.M-V-B.C Leite, V.B.R. Castiglioni, and C.A.A. Arias. 2004. Mutation breeding in sunflower for resistance to *Alternaria* leaf spot. Helia 27:41-50.

De Zélicourt, A., P. Letousey, S. Thoiron, C. Campion, P. Simoneau, K. Elmorjani, D. Marion, P. Simier, and P. Delavault. 2007. Ha-DEF-1, a sunflower defensin, induces cell death in *Orobanche* parasitic plants. Planta 226:591-600.

Dor, E., A. Evidente, C. Amalfitano, D. Agrelli, and J. Hershenhorn. 2007. The influence of growth conditions on biomass, toxins and pathogenicity of *Fusarium oxysporum* f. sp. *orthoceras*, a potential agent for broomrape biocontrol. Weed Res. 47:345-352.

Dussle, C.M., V. Hahn, S.J. Knapp, and E. Bauer. 2004. Pl $_{Arg}$ from *Helianthus argophyllus* is unlinked to other known downy mildew resistance genes in sunflower. Theor. Appl. Genet. 109:1083-1086.

Echevarría-Zomeno, S., A. Pérez-de-Luque, J. Jorrín, and A.M. Maldonado. 2006. Pre-haustorial resistance to broomrape (*Orobanche cumana*) in sunflower (*Helianthus annuus*): cytochemical studies. J. Exp. Bot. 57:4189-4200.

Eizenberg, H., D. Plakhine, T. Landa, T. Achdari, D.M. Joel, and J. Hershenhorn. 2004. First report of a new race of sunflower broomrape (*Orobanche cumana*) in Israel. Plant Dis. 88:1284.

Eizenberg, H., D. Shtienberg, D. Silberbush, and J.E. Ephrath. 2005. A new method for in-situ monitoring of the underground development of *Orobanche cumana* in sunflower (*Helianthus annuus*) with a mini-rhizotron. Ann. Bot. 96:1137-1140.

Encheva, J., H. Köhler, W. Friedt, F. Tsvetkova, P. Ivanov, V. Encheva, and P. Shindrova. 2004. Field evaluation of somaclonal variation in sunflower (*Helianthus annuus* L.) and its application for crop improvement. Euphytica 130:167-175.

Fan, Z.W., H. Buschmann, D. Müller-Stöve, and J. Sauerborn. 2007b. Main effects and interactions among acibenzolar-S-methyl, a biocontrol fungus and sunflower cultivar on control of *Orobanche cumana* Wallr. J. Plant Dis. Prot. 114:76-81.

Fan, Z.W., H. Buschmann, and J. Sauerborn. 2007a. Prohexadione-calcium induces sunflower (*Helianthus annuus*) resistance against the root parasitic weed *Orobanche cumana*. Weed Res. 47:34-43.

Fernández-Martínez, J., B. Pérez-Vich, B. Akhtouch, L. Velasco, J. Muñoz-Ruz, and J.M. Melero-Vara. 2004. Registration of four sunflower germplasms resistant to race F of broomrape. Crop Sci. 44:1033-1034.

Fernández-Ocaña, A.M., M.V. Gómez-Rodríguez, A. Velasco-Negueruela, A.M. Camacho-Simarro, C. Fernández-López, and J. Altarejos. 2004. In vivo antifungal activity of the essential oil of *Bupleurum gibraltarium* against *Plasmopara halstedii*. J. Agric. Food Chem. 52:6414-6417.

Gontcharov, S.V., T.S. Antonova, and N.M. Araslanova. 2004. Sunflower breeding for resistance to the new broomrape race. Helia 27:193-198.

Gontcharov, S.V., T.S. Antonova, and S.L. Saukova. 2006. Sunflower breeding for resistance to *Fusarium*. Helia 29:49-54.

Gonorazky, A.G., M.C. Regente, L., and de la Canal. 2005. Stress induction and antimicrobial properties of a lipid transfer protein in germinating sunflower seeds. J. Plant Physiol. 162:618-624.

Gucherl, S.Z., T.S. Antonova, N.M. Araslanova, T.A. Chelyustnikova, M.V. Ivebor, and S.A. Ramazanova. 2007. Analysis of isoenzymes during the research of intraspecific polymorphism in sunflower downy mildew. VNIIMK Bull. p. 85.

Gulya, T.J. 2007. Distribution of *Plasmopara halstedii* races from sunflower around the world. p. 121-134. In: A. Lebeda and P.T.N. Spencer-Phillips (eds), Advances in Downy Mildew Research, Vol. 3, Proc. 2nd Int. Downy Mildew Symposium, Olomouc, Czech Republic, Palacky University and JOLA, Kostelec na Hané, Czech Republic.

Gulya, T., N. Balbyshev, and S. Radi. 2008. *Sclerotinia* stalk rot: commercial hybrids and USDA plant introductions in 2007 field trials. NSA Meeting Poster.

Hammer, T.R., M. Thines, and O. Spring. 2007. Transient expression of gfp in the obligate biotrophic oomycete *Plasmopara halstedii* using electroporation and a mechanoperforation method. Plant Pathol. 56:177-182.

Hong, C.X. 2006. Downy mildew of *Rudbeckia fulgida* ev. Goldstrum by *Plasmopara halstedii* in Virginia. Plant Dis. 90:1461.

Huguet, N.I. 2005. Occurrence of *Phomopsis helianthi* in Argentina and Uruguay. p. 6. In: Abstr. 10th FAO Consultation Meeting, Novi Sad, Serbia.

Huguet, N., J. Pérez Fernández, V. Quillehauquy, A. Escande, and F. Quiroz. 2007. Epifitia de roya negra por *Puccinia helianthi* en las principales regiones de producción de girasol. p. 381-382. In: Proc. 4to Congreso Argentino de Girasol. ASAGIR, Buenos Aires, Argentina.

Ioos, R., L. Laugustin, S. Rose, J. Tourvieille, and D. Tourvieille de Labrouhe. 2007. Development of a PCR test to detect the downy mildew causal agent *Plasmopara halstedii* in sunflower seeds. Plant Pathol. 56:209-218.

Jan, C.C., and T.J. Gulya. 2006. Registration of three virus resistant sunflower genetic stocks. Crop Sci. 46:1834-1835.

Kaya, Y., M. Demirci, and G. Evci. 2004. Sunflower (*Helianthus annuus* L.) breeding in Turkey for broomrape (*Orobanche cernua* Loeffl.) and herbicide resistance. Helia 27:199-210.

Komjáti, H., J. Bakonyi, O. Spring, and F. Virányi. 2007. Isozyme analysis of *Plasmopara halstedii* using cellulose-acetate gel electrophoresis. Plant Pathol. 57:57-63.

Körösi, K., N. Lázár, and F. Virányi. 2007. Resistance response to downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus*) activated by chemical inducers. p. 237-241. In: A. Lebeda and P.T.N. Spencer-Phillips (ed.), Advances in Downy Mildew Research, Vol. 3. Palacky University and JOLA, Kostelec na Hane, Czech Republic.

Labrousse, P., M.C. Arnaud, Y. Griveau, A. Fer, and P. Thalouarn. 2004. Analysis of resistance criteria of sunflower recombined inbred lines against *Orobanche cumana* Wallr. Crop Prot. 23:407-413.

Leite, R.M.B.V.C., L. Amorim, and A. Bergamin. 2006. Relationships of disease and leaf area variables with yield in the *Alternaria helianthi* - sunflower pathosystem. Plant Pathol. 55:73-81.

Lenardon, S.L., M.E. Bazzalo, G. Abratti, C.J. Cimmino, M.T. Galella, M. Grondona, F. Giolitti, and A.J. Leon. 2005. Screening sunflower for resistance to sunflower chlorotic mottle virus and mapping the Rcmo-1 resistance gene. Crop Sci. 45:735-739.

Lenardon, S.L., and F. Giolitti. 2007. Prevalencia e incidencia del Sunflower chlorotic mottlevirus (SuCMoV) en el sudeste de la provincia de Buenos Aires. p. 383-384. In: Proc. 4to Congreso Argentino de Girasol. ASAGIR, Buenos Aires, Argentina.

Letousey, P., A. de Zélicourt, C. Vieira dos Santos, S. Thoiron, F. Monteau, P. Simier, P. Thalouarn, and P. Delavault. 2007. Molecular analysis of resistance mechanisms to *Orobanche cumana* in sunflower. Plant Pathol. 56:536-546.

Madhavi, K.J., M. Sujatha, D. Raja Ram Reddy, and S. Chander Rao. 2005a. Culture characteristics and histological changes in leaf tissues of cultivated and wild sunflowers infected with *Alternaria helianthi*. Helia 28:1-12.

Madhavi, K.J., M. Sujatha, D. Raja Ram Reddy, and S. Chander Rao. 2005b. Biochemical characterization of resistance against *Alternaria helianthi* in cultivated and wild sunflowers. Helia 28:13-24.

Maranesi, D., and N. Mancuso. 2007. Aptitud combinatoria de 4 líneas de girasol a *Verticillium dahliae*. p. 334. In: Proc. 4to Congreso Argentino de Girasol. ASAGIR, Buenos Aires, Argentina.

Maringolo, C., C. Troglia, V. Nishinakamasu, F. Quiroz, G. Cervigni, R. Heinz, N. Paniego, and A. Escande. 2007. Podredumbre del capítulo de girasol. Identificación de QTL asociados a la resistencia a *Sclerotinia sclerotiorum*. p. 335-336. In: Proc. 4to Congreso Argentino de Girasol. ASAGIR, Buenos Aires, Argentina.

Masirevic, S. 2005a. Sunflower Diseases. Progress report for the period 2002-2005. p. 1-49. In: 10th Technical Consultation Meeting of the FAO-European Cooperative Research Network on Sunflower, Novi Sad, Serbia.

Masirevic, S. 2005b. First appearance of cross-infectivity of *Plasmopara halstedii* from velvetleaf (*Abutilon theophrasti*) to sunflower. p. 27. In: Abstr. 10th Technical Consultation Meeting of the FAO-European Cooperative Research Network on Sunflower, Novi Sad, Serbia.

Mohase, L., A.J. van der Westhuizen, and Z.A. Pretorius. 2006. Induced defense responses and rust development in sunflower. S. Afr. J. Sci. 102:144-150.

Molinero-Ruiz, M.L., J. Domínguez, T.J. Gulya, and J.M. Melero-Vara. 2005. Reaction of field populations of sunflower downy mildew (*Plasmopara halstedii*) to metalaxyl and mefenoxam. Helia 28:65-74.

Murthy, U.K., I.E. Lyngdoh, T. Gopalakrishna, M.B. Shivanna, and D. Theertha Prasad. 2005. Assessment of heritability of *Alternaria helianthi* resistance trait in sunflower using molecular markers. Helia 28:33-42.

Müller-Stöver, D., H. Buschmann, and J. Sauerborn. 2004. Increasing control reliability of *Orobanche cumana* through integration of a biocontrol agent with a resistance-inducing chemical. Eur. J. Plant Pathol. 111:193-202.

Müller-Stöver, D., H. Thomas, J. Sauerborn, and J. Kroschel. 2005. Two granular formulation of *Fusarium oxysporum* f. sp. *orthoceras* to mitigate sunflower broomrape *Orobanche cumana*. BioControl 49:595-602.

Pankovic, D., N. Radovanovic, S. Jocić, Z. Satovic, and D. Škorić. 2007. Development of co-dominant amplified polymorphic sequence markers for resistance of sunflower to downy mildew race 730. Plant Breed. 126:440-444.

Pecchia, S., E. Mercatelli, and G. Vannacci. 2004. Intraspecific diversity within *Diaporthe helianthi*: evidence from Rdna intergenic spacer (IGS) sequence analysis. Mycopathologia 157:317-326.

Pedraza, M.V., V.R. Pereyra, and A.R. Escande. 2004. Infection courts and length of susceptible period related to sunflower head rot (*Sclerotinia sclerotiorum*) resistance. Helia 27:171-183.

Pérez-Vich, B., B. Akhtouch, S.J. Knapp, A.J. Leon, L. Velasco, J.M. Fernández-Martínez, and S.T. Berry. 2004. Quantitative trait loci for broomrape (*Orobanche cumana* Wallr.) resistance in sunflower. Theor. Appl. Genet. 109:92-102.

Pérez-Vich, B., L. Velasco, J. Muñoz-Ruz, J. Domínguez, and J.M. Fernández-Martínez. 2006. Registration of three sunflower germplasm with quantitative resistance to race F of broomrape. Crop Sci. 46:1406-1407.

Prats, E., M.J. Llamas, J. Jorrin, and D. Rubiales. 2007. Constitutive coumarin accumulation on sunflower leaf surface prevents rust germ tube growth and appressorium differentiation. Crop Sci. 47:1119-1124.

Quaglia, M., and A. Zazzerini. 2007. *In vitro* screening for sunflower (*Helianthus annuus* L.) resistant calli to *Diaporthe helianthi* fungal culture filtrate. Eur. J. Plant Pathol. 118:393-340.

Radi, S.A., and T.J. Gulya. 2007. Sources of resistance to a new strain of *Verticillium dahliae* on sunflower in North America. Phytopathology 97:164.

Radwan, O., M.F. Bouzidi, P. Nicolas, and S. Mouzeyar. 2004. Development of PCR markers for the Pl5/Pl8 locus for resistance to *Plasmopara halstedii* in sunflower, *Helianthus annuus* L. from complete CC-NBS-LRR sequences. Theor. Appl. Genet. 109:176-185.

Radwan, O., S. Mouzeyar, J.S. Venisse, P. Nicolas, and M.F. Bouzidi. 2005. Resistance of sunflower to the biotrophic oomycete *Plasmopara halstedii* is associated with a delayed hypersensitive response within the hypocotyl. J. Exp. Bot. 56:2683-2693.

Rani, S.T., and R.L. Ravikumar. 2006. Saprophytic and gametophytic recurrent selection for improvement of partial resistance to *Alternaria* leaf blight in sunflower (*Helianthus annuus* L.). Euphytica 147:421-431.

Rani, S. T., and R.L. Ravikumar. 2007. Genetic enhancement of resistance to *Alternaria* leaf blight in sunflower through cyclic gametophytic and sporophytic selections. Crop Sci. 47:529-536.

Rekab, D., G. Del Sorbo, C. Reggio, A. Zoina, and G. Firrao. 2004. Polymorphisms in nuclear rDNA and mtDNA reveal the polyphyletic nature of isolates of *Phomopsis* pathogenic to sunflower and a tight monophyletic clade of defined geographic origin. Mycol. Res. 108:393-402.

Roldán Serrano, A., J. Luna del Castillo, J. Jorrín Novo, A. Fernández Ocaña, and M.V. Gómez Rodríguez. 2007. Chitinase and peroxidase activities in sunflower hypocotyls: effects of BTH and inoculation with *Plasmopara halstedii*. Biol. Plant. 51:149-152.

Román, B., C.I.G. Verdejo, Z. Satovic, M.D. Madrid, J.I. Cubero, and S. Nadal. 2007. Detecting *Orobanche* species by using cpDNA diagnostic markers. Phytoparasitica 35:129-135.

Sendall, B.C., G.A. Kong, K.C. Goulter, E.A.B. Aitken, S.M. Thompson, J.H.M. Mitchell, J.K. Kochman, W. Lawson, T. Shatte, and T.J. Gulya. 2006. Diversity in the sunflower: *Puccinia helianthi* pathosystem in Australia. Australas. Plant Pathol. 35:657-670.

Shindrova, P. 2006. Broomrape (*Orobanche cumana* Wallr.) in Bulgaria – distribution and race composition. Helia 29:111-120.

Slavov, S., H. van Onckelen, R. Batchvarova, A. Atanassov, and E. Prinsen. 2004. IAA production during germination of *Orobanche* spp. seeds. J. Plant Physiol. 161:847-853.

Spring, O., and R. Zipper. 2006. Evidence for asexual genetic recombination in sunflower downy mildew, *Plasmopara halstedii*. Mycol. Res. 110:657-663.

Spring, O., R. Zipper, and M. Heller-Dohmen. 2006. First report of metalaxyl resistant isolates of *Plasmopara halstedii* on cultivated sunflower in Germany. J. Plant Dis. Prot. 113:224.

Streten, T., D. Bosko, A. Jovanka, and S. Masirevic. 2007. Transferring of *Plasmopara* resistance from annual wild into cultivated sunflower. In: Abstr. 1st Symposium on Sunflower Industrial Uses, Udine, Italy.

Sujatha, M., and A.J. Prabakaran. 2006. Ploidy manipulation and introgression of resistance to *Alternaria helianthi* from wild hexaploid *Helianthus* species to cultivated sunflower (*H. annuus* L.) aided by anther culture. Euphytica 152:201-215.

Thines, M., H. Komjáti, and O. Spring. 2005. Exceptional length if ITS in *Plasmopara halstedii* is due to multiple repetitions in the ITS-2 region. Eur. J. Plant Pathol. 112:395-398.

Thines, M., D. Schauffele, and O. Spring. 2006b. Characteristics of *Pustula tragopogonis* (syn. *Albugo tragopogonis*) newly occurring on cultivated sunflower in Germany. J. Phytopathol. 154:88-92.

Thines, M., R. Zipper, and O. Spring. 2006a. First report of *Pustula tragopogonis*, the cause of white rust on cultivated sunflower in southern Germany. Plant Dis. 90:110.

Tourvieille de Labrouhe, D., E. Mestries, and P. Walser. 2005. Quelles perspectives pour la lutte génétique vis-a-vis du mildiou du tournesol ? OCL 12:85-92.

Van Becealere, G., and J.F. Miller. 2004. Methods of inoculation of sunflower heads with *Sclerotinia sclerotiorum*. Helia 27:137-142.

Vear, F. 2004. Breeding for durable resistance to the main diseases of sunflower. p. 15-28. In: Proc. 16th Int. Sunflower Conf., Fargo, ND, USA.

Vear, F., F. Serre, S. Roche, P. Walser, and D. Tourvieille de Labrouhe. 2006. Recent research on downy mildew resistance useful for breeding industrial – use sunflowers. In: 1st Symposium on Sunflower Industrial Uses, Udine, Italy, www.sunflowersymposium.org

Vear, F., F. Serre, S. Roche, P. Walser, and D. Tourvieille de Labrouhe. 2007. Improvement of *Sclerotinia sclerotiorum* head rot resistance in sunflower by recurrent selection of a restorer population. Helia 30:1-12.

Velasco, L., B. Pérez-Vich, C.C: Jan, and J.M. Fernández-Martínez. 2007. Inheritance of resistance to broomrape (*Orobanche cumana* Wallr.) race F in a sunflower line derived from wild sunflower species. Plant Breed. 126:67-71.

Veronesi, C., E.F. Bonnin, H. Benharrat, A. Fer, and P. Thalouarn. 2005. Are pectinolytic activities of *Orobanche cumana* seedlings related to virulence towards sunflower? Isr. J. Plant Sci. 53:19-27.

Walcz, I., E. Berkine Szili, and L. Nébli. 2004. Effect of *Rhizopus* head rot on the oil content and quality, and germinability of sunflower seeds. p. 161. In: Abstr. 50th National Plant Protection Conference, Budapest, Hungary (in Hungarian).

Walcz, I., and L. Nébli. 2006. Contribution to the knowledge of resistance of sunflower to *Diaporthe (Phomopsis) helianthi*. p. 94. In: Abstr. 52nd National Plant Protection Conference, Budapest, Hungary (in Hungarian).

Walcz, I., and Z. Piszker. 2004. Artificial inoculation technique for selecting resistance of sunflower to *Macrophomina phaseolina* (Tassi) Goidanich. p. 171. In: Abstr. 10th National Plant Breeding Conference, Budapest, Hungary (in Hungarian).

Walser, P., F. Serre, and S. Roche. 2005. Test de resistance du tournesol au phomopsis. Dépot d'un explant mycélien de *Diaporthe helianthi* sur fueille. Cahier de Techniques p. 57-60.

Zazzerini, A., L. Tosi, and A.M. Mondjana. 2005. Occurrence of *Puccinia helianthi* races on sunflower in Mozambique. J. Phytopathol. 153:733-735.

Could a crop model be useful for improving sunflower crop management?

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ABSTRACT

In France, there is a need for improved sunflower crop management, in order to meet the greater requirement for oil by increasing both seed yields and the area of this crop. The objective of this article is to review the main characteristics of sunflower crop management in France and in other countries, in order to emphasize the need for improvement, and to evaluate if the recent advances in crop modelling could help to find solutions. In France, a better adaptation of crop management to water availability is needed, as well as a more efficient control of diseases without applying more fungicides. The results of these objectives would also trigger major improvements in other countries, but there is also a need to control insects and to adapt crop management to the goals of oil quality. The main sunflower crop models are reviewed in this article, with an emphasis on the most recent ones. Their ability to contribute to improving sunflower crop management, although they do not take into account diseases and insects, is discussed. Confidence in the decisions based on simulations, and the way to evaluate it, is also examined.

Key words: crop management – crop models – management strategies – model evaluation – sunflower.

INTRODUCTION

In France, there has been a stagnation in sunflower seed yield during the past 20 years. The annual mean seed yield has ranged from 2.1 to 2.7 t ha^{-1}. The highest value was observed in 2007, but it was mainly the result of the high rainfall during summer. During that period of time, the area cultivated in sunflower has decreased by almost 50 %. Overall, there has been a decrease in seed production, although there is a need for greater quantities because of the increased demand for biodiesel. Hence, both increases in seed yield and in the area cultivated in sunflower are necessary. Improvements in crop management would probably contribute to these objectives. However, during the past few years, sustainable agriculture has become a priority in the European Union. In France, the French government decided in 2007 that the applications of pesticides should decrease by 50% within 10 years. This has to be taken into account when improving crop management. The objective of this article is to review the main characteristics of sunflower crop management in France and in other countries, in order to emphasize the need for improvement and to evaluate if the recent advances in crop modelling could help to find solutions.

DISCUSSION

1. The main problems in sunflower crop management

Sunflower crop management in France is characterized by few applications of pesticides. Most of the time, insects are not a major problem. Hence, only 40% of the sunflower area received one insecticide in 2006. The reduction of plant population due to damage by slugs, birds or game animals has been observed more often. However, only 54% of the sunflower area was sprayed against slugs in 2006, while no treatments are allowed against birds or game animals. Moreover, the application of fungicides is rare (only 7% in 2006). Fungal diseases are mainly controlled by seed treatments, long crop rotation, destruction of volunteers and of some weeds, and by the use of resistant or tolerant varieties. However, pathological premature ripening due to phoma or macrophomina is often observed, especially in dry areas. Herbicides are applied in almost 100% of the area, but between-row cultivation also contributes to weed management. This cultural operation was observed in 41% of the area in 2006. Hence, sunflower may contribute to the decrease in the application of pesticides in France, through an increase in the area of this crop to the detriment of other crops. This would be effective as long as improvements in crop protection were focused on other ways than increasing the application of pesticides. For instance, date of sowing, seeding rate and the amount of N fertiliser have an effect on several diseases, such as Phoma black stem (Debaeke and Pérès, 2003; Seassau et al., 2008). This indicates that there are possibilities for decreasing disease incidence without applying fungicides.

In France, sunflower is mainly cultivated on clay soils. Most of the time, seeds are sown after a deep tillage (72% of the area in 2006), while there is very little direct sowing (2% in 2006). There is almost no irrigation (only 4% of the area in 2006). The range of soil depth is wide, resulting in a large range of seed yields. For instance, in south-west of France in 2006, mean yields were 2.28, 2.34 and 2.73 t ha^{-1}, respectively, on shallow (13% of the area), medium (75%) and deep soils (12%). However, there is little adaptation of crop management to the expected water availability (soil field capacity, expected rainfall and irrigation). The main adaptation to reduce the effect of water shortage is earlier sowing in south of France, because severe deficits in summer are expected. In the Aude region however, where mid-summer storms are predictable, the date of sowing is delayed. The objective is to postpone the seed filling period, so that it occurs during mid-summer. The amount of N fertiliser applied is also adapted to the target yield, which results from the expected water availability. However, there are no further adaptations of the crop management to the expected water availability. For instance, the drought-tolerance of commercial cultivars, if it exists, is unknown and thus not available for farmer's decision.

The first objective of any improvement in crop management is a better adaptation to the expected water availability. It would result in more accurate date of sowing, planting density, amount of N fertiliser (more accurate target yield) and variety maturity type. The choice of the variety should also account for the differences in leaf area and in stomatal closure, which play an important role in drought tolerance (Casadebaig, 2008). The second objective is to control diseases more efficiently without foliar-applied fungicides, especially those responsible for premature ripening in dry areas.

In other countries, there are some differences in crop management, compared to France. The following section is not an exhaustive list of the main differences which have been noticed, but it is the description of three of them which could also be improved by crop modelling. Firstly, in countries other than France, crop management is sometimes more adapted to the expected water availability, especially through the target plant population. The objective of plant number per hectare in Australia is 20-25,000 in marginal dryland, 25-35,000 in favourable dryland, 35-50,000 in limited irrigation and 50-75,000 in full irrigation (Serafin et al., 2007). In the USA High Plains, plant population for irrigated sunflower should be between 42000 and 54000 final plant per hectare, while it should be lower for lower yield potentials (Meyer et al., 1999).

Secondly, the occurrence of insect problems is more acute outside Western Europe, where insect damage on sunflower is less. In North America, there is a wide pest complex because sunflower is native to this region of the world (Charlet et al., 1997). On other continents, numerous insects also attack sunflower. For example, in Africa, sunflower has been grown for a long time as an ornamental plant. Insects attacking ornamental crops later moved to commercial ones (Charlet et al., 1997). In the countries where insects cause significant yield reductions, the planting date can play a role in controlling them. For instance, in Canada, delaying planting until late May or early June has been effective in reducing densities of stem weevil larvae (The sunflower production guide, Manitoba Agriculture, 2006). It also helps to prevent the first major emergence of the overwintering sunflower midge population. On the contrary, early planting reduces seed damage of sunflower seed weevils because early planted sunflowers complete anthesis and are no longer susceptible to egg laying at the time of peak populations (Manitoba Agriculture, 2006).

Thirdly, in some countries, crop management contributes to seed quality. In France, sunflower oil is mainly used for biodiesel or for food. Oil with a high oleic content is required for biodiesel. The quality required is obtained through the cultivation of high oleic varieties, while the rest of the crop management is similar to that for other varieties. However, planting date can affect the oil quality, because warm temperatures during anthesis and the seed-filling period increase the seed content in oleic acid (Blamey et al., 1997). Hence, in Australia, for example, planting dates are grouped into an early and a late sowing window (Serafin et al., 2007). For spring sowing, high oleic acid varieties are preferred. Hence, the high temperatures occurring during seed filling for this sowing time is not a problem. In order to produce high linoleic varieties, sowing in the late plant window (December-January) is recommended so that crops fill seeds in the cooler autumn months.

In order to adapt crop management accurately to each situation, many data are needed because environmental conditions are highly variable, between years and between locations. Hence, the optimum of one cultural operation is also highly variable from one experiment to another. For instance, Robinson (1978) stated that "disagreement on the optimum plant population is common". Moreover, there are many cultural operations which interact with each other. For example, the optimum plant population density tends to be greater with irrigation (Blamey et al., 1997; Debaeke and Nolot, 2000). It is not possible to conduct the huge number of factorial experiments needed for an accurate adaptation of crop management.

In France, the recommendation of an early sowing date is mainly based on field surveys. For instance, in the south west of France, the results of 300 fields per year from 1996 to 2006 show a decrease in yield when the sowing date was delayed after 10 April (CETIOM, 2008). The difference in yield was 0.27 t ha^{-1}, between sowings before 10 April and after 10 May. However, these results are rough estimates, because they compare different fields with possible differences in other cultural operations and in soils. In order to take into account possible interactions with other factors (variety, soil depth…), and/or to give more site-specific recommendations, many more data would be necessary. There is also a need to keep adapting crop management to keep up with technical progress (new varieties…), and with changes in objectives (quality) and in environmental conditions (the possibility to irrigate or climate change). The number of years necessary to give recommendations taking into account these changes would be too great, if results from either experiments or surveys were used. All these difficulties can be overcome by using crop models, because thousands of situations can be simulated in a few hours, once these tools are validated.

2. Sunflower crop models

Villalobos (2000) reviewed sunflower crop models at the 15th International Sunflower Conference in Toulouse. At that time, several specific models of sunflower had been developed, and a few others were applicable to several crops including sunflower (generic models). These models are mathematical representations of crops and soils which take into account dynamically and on a daily basis the effects of weather and crop management on seed yield. The QSUN model was developed in the early nineties (Chapman et al., 1993). It takes into account sowing date, irrigation and variety. The OILCROP-SUN model (Villalobos et al., 1996) also considers these factors, along with fertiliser management.

Two models which have been developed since 2000 provide further possibilities. A simple model based on published relationships calculates oil quality along with seed yield (Pereyra-Irujo and Aguirrezabal, 2007). The cultural operations taken into account are the effect of sowing date, plant density and variety.

Another sunflower crop model was developed by Casadebaig (2008) to gain new insight into the way to discriminate yield build-up between varieties. Generally, in sunflower models, varieties only differ in yield components and maturity types. In this new model, varietal parameters are required for crop development, leaf area and its ability to intercept light, response of leaf expansion and stomatal closure to soil water deficit, harvest index and the maximum percentage of kernel in achenes. These parameters are easily measurable, in order to be able to account for the dozens of new varieties appearing each year on the market (Casadebaig et al., 2008). Sowing date, plant density, irrigation and N fertiliser are also considered.

However, the sunflower crop models presented above do not include diseases, insects or weeds. There has been one attempt to connect the EPIC crop model adapted to sunflower to Phomopsis stem canker (Debaeke and Chabanis, 1999). The climatic risk of contamination by ascospores was predicted from spring and summer rainfall. Then, the disease symptoms were simulated using the relationship between infected stems and the fraction of intercepted photosynthetically active radiation (IPAR), which was simulated by the EPIC crop model. Yield loss was then correlated with the symptoms, bearing in mind the period of contamination. The relationship between symptoms of Phomopsis stem canker and the IPAR or Leaf area index (LAI) was also reported by Debaeke and Estragnat (2003). Debaeke and Pérès (2003) were also able to correlate Phoma black stem damage with IPAR or LAI at anthesis.

3. The use of sunflower crop models to adapt crop management

Sunflower crop models could be used to optimize crop management, by considering crop response to long-term historical weather records. For example, simulated seed yields were compared for a range of sowing dates, in order to select the best one (Meinke et al., 1993; Rinaldi et al., 2003; Soriano et al., 2004; Casadebaig, 2008).

In Casadebaig (2008), seed yield was simulated for 5 sowing dates, 7 locations, 3 available soil water content and 25 years. In most combinations of location x soil water capacity, seed yield decreased with delaying the sowing date until the third or the fourth date, because of a greater water deficit (Fig. 1). Then, an increase in seed yield was observed between the third or fourth date and the fifth date, due to the delaying of seed filling until the period of mid-summer storms. This pattern was less marked on deep soils with high water capacities. Hence, depending on location and on soil water capacity, the greatest yield could be obtained at the early sowing date, at both the early and the late sowing dates because of mid-summer storms, or at all sowing dates because of a little water deficit due to both deep soils and humid climates.

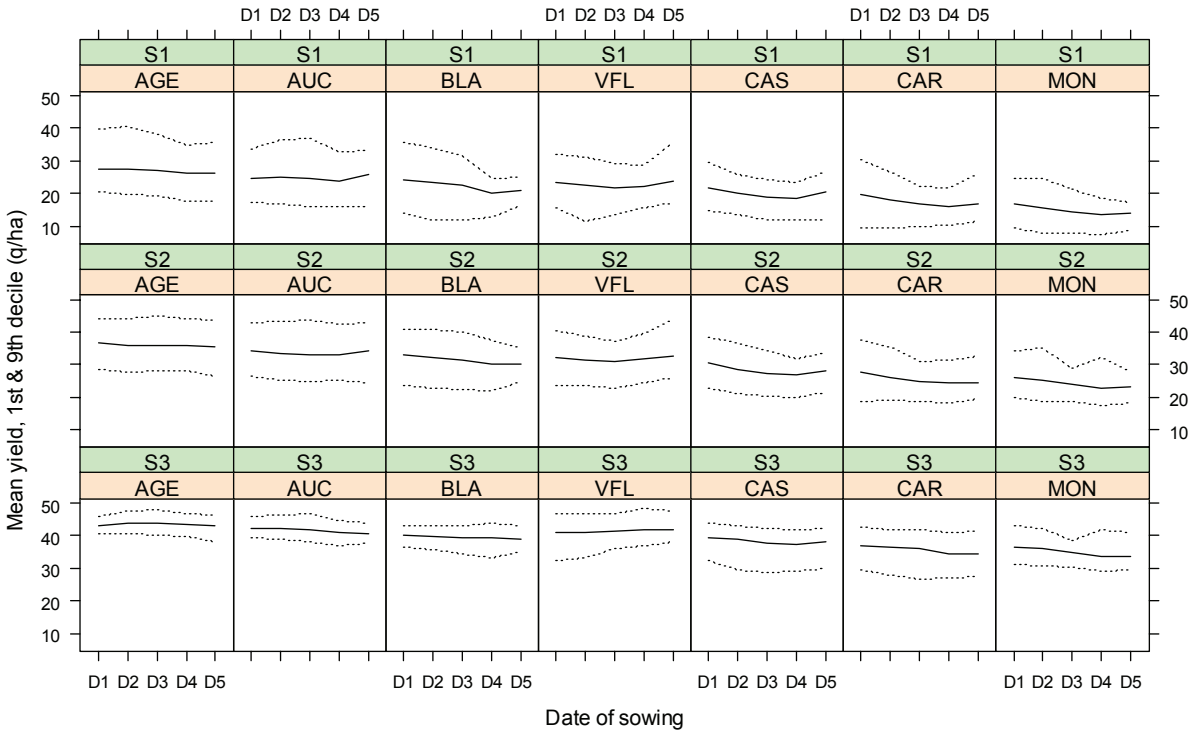

Fig. 1. Simulated seed yield versus sowing date in 7 locations and for 3 soils (Casadebaig, 2008).

Sowing dates were 1 March (D1), 25 March (D2), 15 April (D3), 10 May (D4) and 25 May (D5). Locations were representative of South of France, from the Western side (left on the Figure) to the eastern (right on the Figure): Agen (AGE), Auch (AUC), Blagnac (BLA), Villefranche de Lauragais (VFL), Castelnaudary (CAS), Carcassonne (CAR) and Montpellier (MON). Soil water capacities were 80 mm (S1), 150 mm (S2) or 250 mm (S3).

The best maturity type was similarly studied by Meinke et al. (1993). Debaeke et al. (1998) and Rinaldi et al. (2003) also compared the effect of irrigation strategies on simulated yields. Oil seed quality can also be taken into account, along with seed yield, when using a crop model to optimize crop management (Pereyra-Irujo and Aguirrezabal, 2007).

These simulations could assist farmers in making management decisions. It provides information on the effect of one or several cultural operations, in each specific soil x climate situation, which takes into account the variability between years. Experiments or surveys fail to give such precise information. However, users of crop models should be aware of 2 limits: (1) accuracy and robustness, and (2) relevance (factors not taken into account). Crop models will be powerful tools to assist farmers as long as these limits are properly managed.

Model accuracy is the ability to give simulations close to the measurements. Robustness is the capability to be accurate in other environmental conditions than those prevailing for the data set used for calibration. Both are crucial for helping farmers to make good decisions. Model accuracy is evaluated by comparing simulations and measurements not used for calibration. However, the minimum of accuracy necessary to help farmers to make the best decision is usually not discussed. This would need specific works that have never been done when using sunflower crop models. For instance, Rinaldi et al. (2003) observed a good correlation between simulated yields and independent measurements (almost perfect regression slope (0.95) and intercept (-0.07), and a fairly good R^2 value of 0.74). Observed values were obtained for several years, in locations, irrigation regimes and sowing dates similar to those prevailing for the use of the model. This evaluation of the model was encouraging. However, it was not a proof that simulations were accurate enough to make the good decision, which was to use a threshold value of 40 % of total soil water to trigger irrigation. Moreover, robustness is not usually discussed, even though the ability to give results in other situations than those prevailing in experiments is exactly the expected benefit of a crop model.

Many factors affecting seed yield or quality are not taken into account by crop models. For example, sowing date does not only have an effect on climate conditions during crop growth. Diseases and insects are also affected (Leterme, 1992; Debaeke et al., 2001; Manitoba Agriculture, 2006). Models considering these factors would be very helpful. However, this does not seem as if it will become a reality in the near future. There are numerous diseases and insects which depend on many other factors than those in the sunflower field (cropping history, spatial cropping pattern …). Moreover, their effects depend on plant tolerance or resistance, and on the application of pesticides.

However, crop models could be useful for contributing to define management strategies. Debaeke and Nolot (2000) illustrated the definition of management strategies based on a target yield (which depended on water availability), and also based on the combination of avoidance and/or tolerance of limiting factors and vegetative rationing. The limiting factors involved were both nutritional and disease ones. For each combination of soil and climate, a crop model would be useful for establishing the potential yields allowed by the water availability, solar radiation and temperature. Results of potential yields would depend on sowing date, plant number and on variety. These results could be associated with the knowledge of diseases and insects in order to define management strategies. One strategy could aim at the maximum yield. According to the hypothesis that several combinations of sowing date x plant number x variety exist, the one recommended would be that minimizing the risks of major diseases and insects. In order to minimize them further, other management strategies could aim at lower target yields. Crop models could also help to estimate the risks of diseases and insects by simulating variables correlated with them. Examples of such correlations are given in section 2 of this article (IPAR or LAI correlated with Phoma black stem or Phomopsis stem canker). Models could also be used for insect damage. For instance, they could simulate the stages of development when sunflower is more susceptible to damage from a particular insect.

CONCLUSIONS

The issue investigated in this article is the possibility of using crop models for improving sunflower crop management. In France, a better adaptation of crop management to water availability is needed, as well as a more efficient control of diseases without applying more fungicides. A huge number of experiments would be needed to reach these objectives, while surveys give only rough estimates on the effect of cultural operations. Moreover, both experiments and surveys need too many years to keep up with the continuous technical progress (new varieties…), and for the quick changes in objectives (quality) and in environmental conditions (the possibility to irrigate or climate change) that are expected in the future. Crop modelling is the only way to obtain data quickly enough. Similarly, crop models could be helpful in countries other than France, although their needs for improving crop management may be different. There have been recent advances in sunflower crop models, in simulating oil quality and in defining differences between varieties. Although diseases and insects are still not taken into account, crop models could be used to trigger management strategies. These strategies would be based on simulated potential yields and on knowledge of diseases and insects. However, the condition for using simulated results to improve crop management is the confidence in the model. Until now, models have been mainly evaluated by comparing the simulations and the measurements made in a few independent experiments, which is not enough. There is a need to evaluate the ability of models to help to make the best decision in a large range of environmental conditions.

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REFERENCES

Blamey, F.P.C., R.K. Zollinger, and A.A. Schneiter. 1997. Sunflower production and culture. p. 595-670. In: A.A. Schneiter (ed.), Sunflower Technology and Production. Agronomy Monograph 35. ASA, CSSA and SSSA, Madison, WI, USA.

Casadebaig, P. 2008. Analyse et modélisation dynamique de l'interaction génotype-environnement-conduite de culture: application au tournesol (*Helianthus annuus* L). PhD Thesis, INP Toulouse, 134p.

Casadebaig, P., P. Debaeke, and J. Lecoeur. 2008. Thresholds for leaf expansion and transpiration response to soil water deficit in a range of sunflower genotypes. Eur. J. Agron. (in press).

CETIOM. 2008. Tournesol Régions sud 2008. Brochures CETIOM, CETIOM (ed.), 26 p.

Chapman S.C., G.L. Hammer, and H. Meinke. 1993. A sunflower simulation model. I Model development. Agron. J. 85:725-735.

Charlet L.D., G.J. Brewer, and B. Franzmann. 1997. Insect pests. p. 183-261. In: A.A. Schneiter (ed.), Sunflower Technology and Production. Agronomy Monograph 35. ASA, CSSA and SSSA, Madison, WI, USA.

Debaeke P., M. Cabelguenne, A. Hilaire, and D. Rafaillac. 1998. Crop management systems for rainfed and irrigated sunflower (*Helianthus annuus* L.) in south-western France. J. Agric. Sci. 131:171-185.

Debaeke P., and J. Chabanis. 1999. An integrated simulation model of yield loss by a fungal pathogen: stem canker (*Phomosis helianthi*) in sunflower. p. 73-74. In: Proc. 1st Int. ESA Symp. on Modelling Cropping Systems, Lleida, Spain.

Debaeke P., and A. Estragnat. 2003. A simple model to interpret the effects of sunflower crop management on the occurrence and severity of a major fungal disease: Phomopsis stem canker. Field Crops Res. 83:139-155.

Debaeke P., J. Moinard, and M. Delos. 2001. Simulating the influence of sowing date on disease occurrence in sunflower. p. 123-124. In: Proc. 2nd Int. ESA Symp. on Modelling Cropping Systems, Florence, Italy.

Debaeke P., and J.M. Nolot. 2000. Testing crop management systems for sunflower in South-West France. p. 1-6. In: Proc. 15th Int. Sunflower Conf., Toulouse, France.

Debaeke, P., and A. Pérès. 2003. Influence of sunflower (*Helianthus annuus* L.) crop management on Phoma black stem (*Phoma macdonaldii* Boerema). Crop. Prot. 22:741-752.

Leterme P. 1992. *Phomopsis* et *Sclerotinia* Feuilles/bouton: des essais non spécifiques. Oléoscope 10:8-10.

Manitoba Agriculture. 2006. The sunflower production guide. MAFRI Publications, Winnipeg, MB, Canada. 40p.

Meinke H., G.L. Hammer, and S.C. Chapman. 1993. A sunflower simulation model. II. Simulating production risks in a variable sub-tropical environment. Agron. J. 85:735-742.

Meyer R., D.D. Baltensperger, A.J. Schlegel, J.M. Krall, C. Lee, and J.P. Shroyer. 1999. Agronomic practices. p. 1-3. In: R. Meyer, D. Belshe, D. O'Brien, and R Darling (eds), High Plains Sunflower Production Handbook. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, USA.

Pereyra-Irujo, G.A., and L.A.N Aguirrezabal. 2007. Sunflower yield and oil quality interactions and variability: Analysis through a simple simulation model. Agric. For. Meteor. 143:252-265.

Rinaldi M., N. Losavio, and Z. Flagella. 2003. Evaluation and application of the OILCROP-SUN model for sunflower in southern Italy. Agricultural Systems 78:17-30.

Robinson R.G. 1978. Production and culture. p. 89-143. In J.F. Carter (ed.) Sunflower science and technology. Agronomy Monograph 19. ASA, CSSA and SSSA, Madison, WI, USA.

Seassau, C., E. Mestries, P. Debaeke, and G. Dechamp-Guillaume. 2008. Effects of nitrogen and water on premature ripening caused by *Phoma macdonaldii*, a fungal pathogen of sunflower. In: Proc. 17th Int. Sunflower Conf., Cordoba, Spain.

Serafin L., S. Belfield, and D. McCaffery. 2007. Sunflower. p. 45-56. In: T. Farrell, L. Serafin, and J. Kneipp (eds), Summer Crop Production Guide. New South Wales Department of Primary Industries, Australia.

Soriano, M.A., Orgaz, F., Villalobos, F.J, and E. Fereres. 2004. Efficiency of water use of early plantings of sunflower. Eur. J. Agron. 21:465-476.

Villalobos, F.J. 2000. Principles and applications of sunflower crop simulation models. Plenary session C – Agronomy and Environment. p. 9-17. In: Proc. 15th Int. Sunflower Conf., Toulouse, France.

Villalobos, F.J., Hall, A.J., Ritchie, J.T., and F.Orgaz. 1996. OILCROP-SUN: A development, growth, and yield model of the sunflower crop. Agron. J. 88: 403-416.

Phenotypic plasticities of yield, phenological development and seed traits

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ABSTRACT

Understanding, quantifying, and exploiting the interaction between genotype and environment (G x E) is at the core of plant improvement. This paper focuses on G x E from a physiological perspective. We present a theoretical framework largely based on Bradshaw's principles of phenotypic plasticity updated to account for recent developments in physiology and genetics. Against this framework we discuss (a) associations between plasticities of different traits and (b) plasticity of seed size and composition. We show that plasticity of sunflower phenological development could be positively associated with yield plasticity under conditions when this is a desirable trait, i.e. when there is no trade-off between yield in low and high yielding environments. We propose that allometric models linking rate and duration could be useful to quantify phenotypic plasticity of agronomically important seed traits.

Key words: genetics – genotype x environment interaction – phenotypic plasticity – physiology.

INTRODUCTION

Grain and oil yield, and quality traits of sunflower depend on environmental (E), genetic (G) and G x E factors. Table 1 is a meta-analysis of 69 sunflower trials over 18 years in northern Argentina (n = 8,974), highlighting the challenge involved in breeding and selection for oil yield in sunflower where environmental and G x E sources of variation dominate. Understanding, quantifying, and exploiting G x E is at the core of plant improvement.

Table 1. Meta-analysis of 69 sunflower trials over 18 years in Argentina (n = 8,974). The partitioning of oil yield variance uses a Restricted Maximum Likelihood approach (REML) assuming all variables are random.

Random term	Variance component	s.e.
year	72692	32521
year.trial	64000	13060
year.trial.rep	4340	653
year.trial.rep.block	2623	327
genotype	2972	671
year.genotype	5704	612
residual (avg across trials)	46731	6567

Breeders are well aware of the issues involved in G x E, whereas physiologists and ecologists look at the same type of problem from the perspective of *phenotypic plasticity* or *norms of reaction* (Bradshaw, 1965; Bradshaw, 2006; De Witt et al., 1998; Pigliucci, 2001; Pigliucci et al., 1995). *Phenotypic plasticity* is "the amount by which the expressions of individual characteristics of a genotype are changed by different environments" (Bradshaw, 1965). The aim of this paper is to discuss selected aspects of phenotypic plasticity of sunflower yield and seed traits from a physiological perspective.

This article has three parts. First, we introduce some principles related to phenotypic plasticity that provide the theoretical background for the paper. Second, we explore the notion of positive associations between plasticities. Using data from sunflower trials involving a large number of hybrids and environments, we show preliminary evidence for a positive link between phenotypic plasticity of yield and phenotypic plasticity of phenological development. Third, we present a novel quantitative model to analyse seed size variation in terms of rate and duration of seed growth. For most grain species, including sunflower, we show that plasticity of seed size could be ascribed to specific allometric conditions, and that plasticity of seed size could be an important driver of yield plasticity. This allometric model could also be applied to quality related traits, e.g. oil concentration.

DISCUSSION

This paper is informed by three established principles (1-3) and a newer, less tested proposal (4):

1. "The plasticity of a character is an independent property of that character and is under its own specific genetic control" (p. 119 Bradshaw, 1965). Bradshaw (1965) insightfully formulated this proposal over forty years ago, and Reymond et al. (2003) have demonstrated unequivocally that phenotypic plasticity is a trait on its own, with its own genetic control. A corollary to this principle is that plasticity evolves (Pigliucci, 2005; Zhivotovsky et al., 1996) and therefore could be considered as a breeding aim on its own. The findings of Reymond et al. (2003) open a new, more robust opportunity to use QTLs as breeding tools, and highlight the need for appropriate quantitative models that relate traits and environmental drivers, or alternatively, establish physiologically meaningful relationships between traits.
2. Plasticity is specific for a character and is specific in relation to particular environmental influences (Bradshaw, 1965). This adds a layer of complexity to the subject, because the plasticity of a trait (e.g. kernel oil concentration) may be high or low depending on the environmental drivers.
3. There is a hierarchy of plasticities, i.e. stable traits are often associated with plastic, related traits (Bradshaw, 1965). The trade-off between seed number and size is a typical, agronomically relevant case of this principle whereby high plasticity in number is associated with low plasticity in size. Sadras (2007) has provided an evolutionary interpretation that matches the notion of a hierarchy in the plasticities of seed size and number in annual plants.
4. There are cases of *positive* associations between plasticities of certain traits. Analysis of the association between plasticity of fruit yield and plasticity of phenology in wine grape favoured the hypothesis of a positive, rather than negative (principle 3) correlation between plasticities (Sadras, Petrie, and Robinson, unpublished).

Does phenological plasticity contribute to yield plasticity?

Finlay and Wilkinson (1963) developed a method to quantify trait plasticity, that has been widely applied to the analysis of grain yield in annual crops. Calò et al. (1975) used this approach to quantify phenological plasticity of grapevine. Fig. 1 illustrates the rationale of this method applied to the analysis of plasticity of flowering time of sunflower hybrids grown in diverse environments of northern Argentina. The coefficient of phenotypic plasticity is the dimensionless slope of the linear regression between date of flowering of an individual variety in a particular environment, and the mean value of the trait across varieties in that particular environment. A variety with slope = 1 has average stability over all environments, a variety with slope > 1 has above-average plasticity, and a variety with slope < 1 has below-average plasticity.

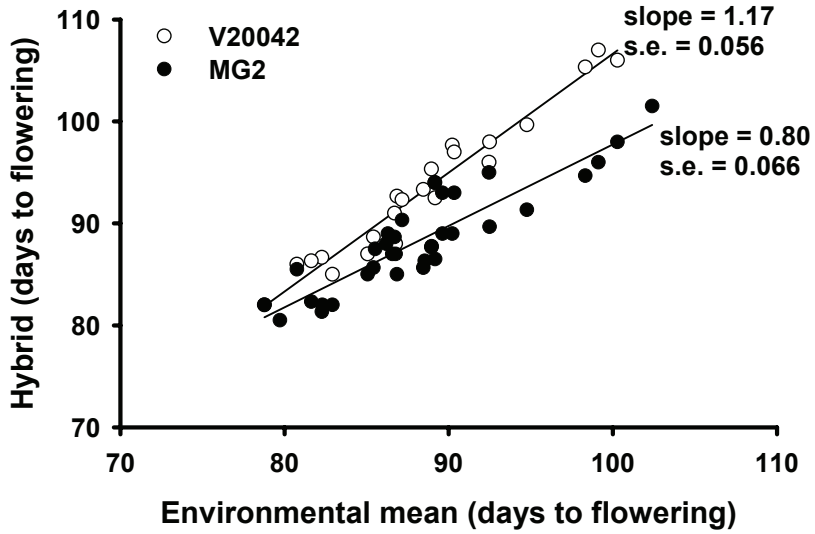

Fig. 1. Quantification of phenotypic plasticity of flowering in sunflower using the method of Finlay and Wilkinson (1963).

In the case study of this paper, environments resulted from the combination of locations and seasons, and the data set comprised 32 hybrids grown in at least 15 environments. For this data set, plasticity for yield ranged from 0.72 to 1.29 (Fig. 2). All hybrids performed similarly in the more stressful environments (i.e. the slope of the regression between minimum yield of each hybrid and its yield plasticity was not significantly different from zero, P = 0.34). Higher plasticity was associated with the ability to capture the benefits of better environments, with a rate of increase in maximum yield of 1939 kg/ha per unit increase in plasticity (P < 0.0001). A similar conclusion was reached from analysis of oil yield: oil yield plasticity ranged from 0.72 to 1.30, was correlated with plasticity of grain yield (r = 0.90, P < 0.0001) and was related to maximum (rate = 1024 kg oil/ha per unit increase in plasticity, P < 0.0001) but not with minimum oil yield (P > 0.25). High yield plasticity in this particular combination of hybrids and environments is therefore a desirable trait, as it does not involve tradeoffs between stress tolerance and yield potential.

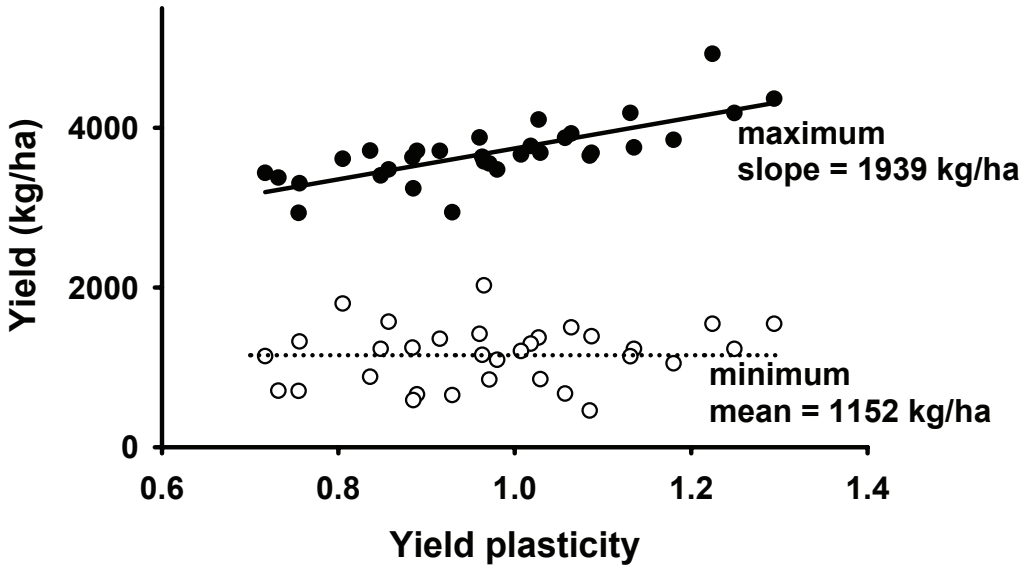

Fig. 2. Phenotypic plasticity of grain yield in sunflower hybrids was related to their ability to capture the benefits of the best environments (slope of maximum yield vs plasticity significant at P < 0.0001) and independent of their performance in the more stressful environments (slope of minimum yield vs plasticity not different from zero; P = 0.34).

In a broad sense, phenological development is recognised as the more important attribute of crop adaptation (Passioura, 1996; Passioura, 2007; Richards, 2006; Sadras and Trápani, 1999). This relates to a series of tradeoffs. Firstly, there is a trade-off between late flowering that allows for canopy and root development (Giménez and Fereres, 1986) and the decline in potential grain set generally associated with low radiation-to-temperature ratios of late flowering crops (Cantagallo et al., 1997). Secondly, in some environments, flowering date may also involve trade-offs between the risk of frost and the risk of heat stress, terminal drought, rainfall at harvest or diseases. For the combination of hybrids and environments in this analysis, we found yield plasticity was higher in late-flowering hybrids, with mean flowering date accounting for 47% of the variation in yield plasticity (Fig. 3) and 40% of the variation in oil yield plasticity (not shown). Flowering plasticity was unrelated to mean flowering date, and accounted for 20% of the variation in yield plasticity (Fig. 3) and 16% of the variation in oil yield plasticity. Maximum yield was associated with both mean flowering date (r = 0.51, P = 0.003) and flowering plasticity (r = 0.40, P = 0.02) whereas minimum yield was weakly related to mean flowering date (r = 0.34, P = 0.06) and unrelated to flowering plasticity (P = 0.34).

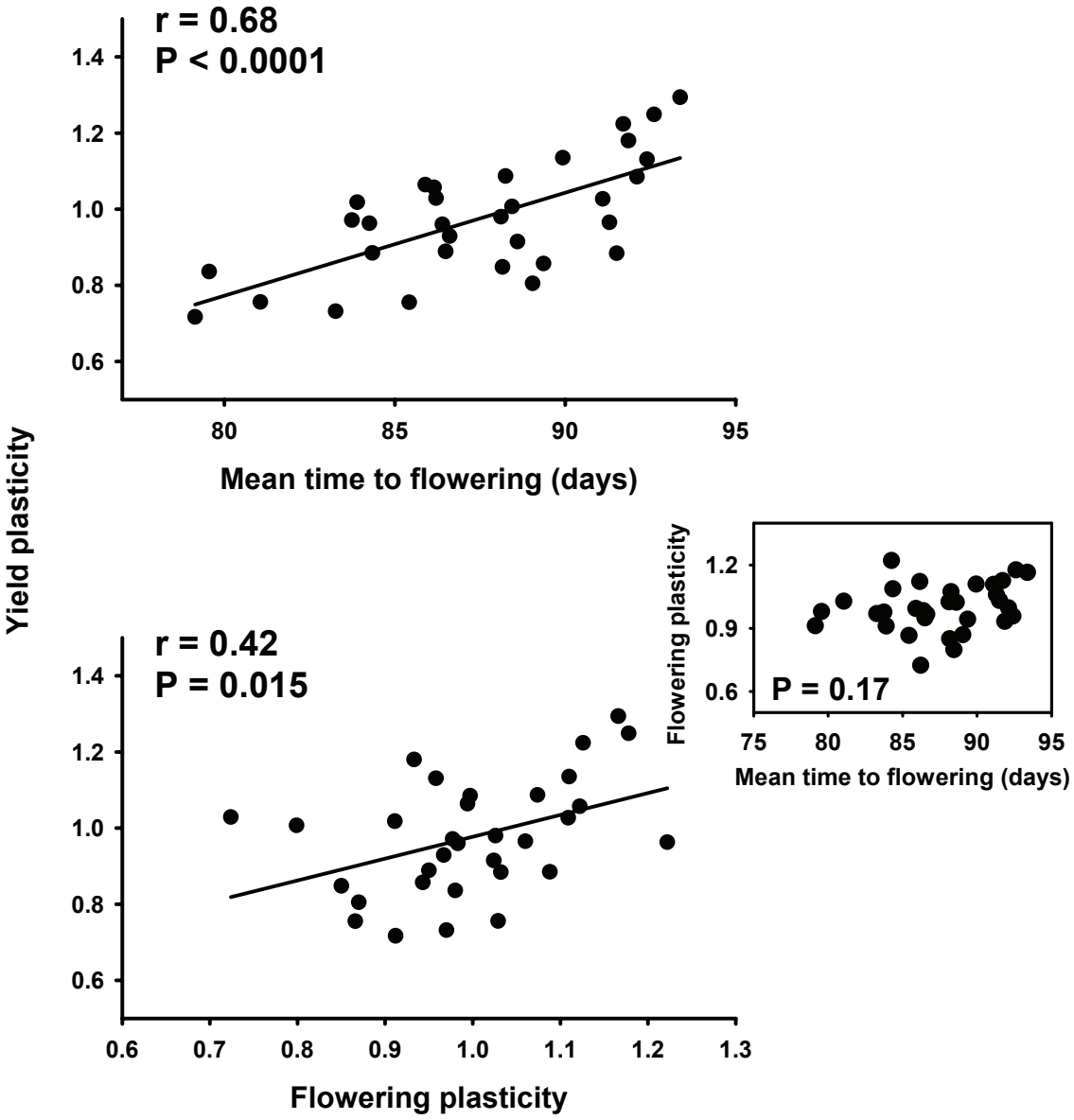

Fig. 3. Plasticity of grain yield in a collection of 32 sunflower hybrids in northern Argentina was associated with both late flowering, and flowering plasticity. Inset shows flowering plasticity was not associated with mean flowering date.

The relationships between plasticity in yield and plasticity in phenology deserve further attention. Biologically, this relationship adds a new dimension to the understanding of crop adaptation. From a breeding perspective, it would be of interest to establish the genetic basis of phenological plasticity (Principle 1), and eventually exploit this trait where plasticity in yield is a desirable trait, i.e. when performance in stressful environments does not compromise performance in better environments.

Plasticity of seed size: allometric conditions and relationship with yield plasticity

Here we explore the allometric conditions for seed size plasticity using a multi-species comparative approach, and investigate the links between seed size plasticity and yield plasticity using a limited data set of sunflower hybrids grown in contrasting environments.

Allometric conditions for plasticity of seed size and quality traits

There are many growth processes that can be approximated to sigmoidal patterns with characteristic rates and durations, including leaf expansion, seed growth, accumulation of oil in seed and accumulation of sugar and pigments in fruits. For any such process, we can express the maximum value of the trait (A) as the product of rate and duration:

$$A = \text{rate} \times \text{duration} \quad (1)$$

Sadras et al. (2007) proposed an allometric formulation of this model (Fig. 4):

$$\log \text{duration} = \log A - \alpha \log \text{rate} \quad (2)$$

The advantage of this model is that the scaling exponent α indicates three types of responses: the trait is stable as a result of full compensation between rate and duration (α = –1), the trait is variable as a result of rate (α > -1) or duration-dominated growth (α < -1). Sadras et al. (2007) used this approach to demonstrate that accumulation of anthocyanins in berries of grapevine Cabernet Sauvignon in a warm environment is highly plastic (α = -0.75 ± 0.041), in contras to sugar accumulation which is very stable (α > -1.11 ± 0.050). Fig. 5 illustrates the application of this concept to the analysis of seed size in grain crops. These particular experiments showed relatively stable seed size in soybean, with a scaling exponent correspondingly close to –1, and large variation in seed size of sunflower, with a corresponding scaling exponent significantly greater than –1 ($P < 0.05$), i.e. a flat line reflecting rate-dominated seed growth. These results cannot be considered general for these species, but particular for the combination of cultivars and environments (Principle 2).

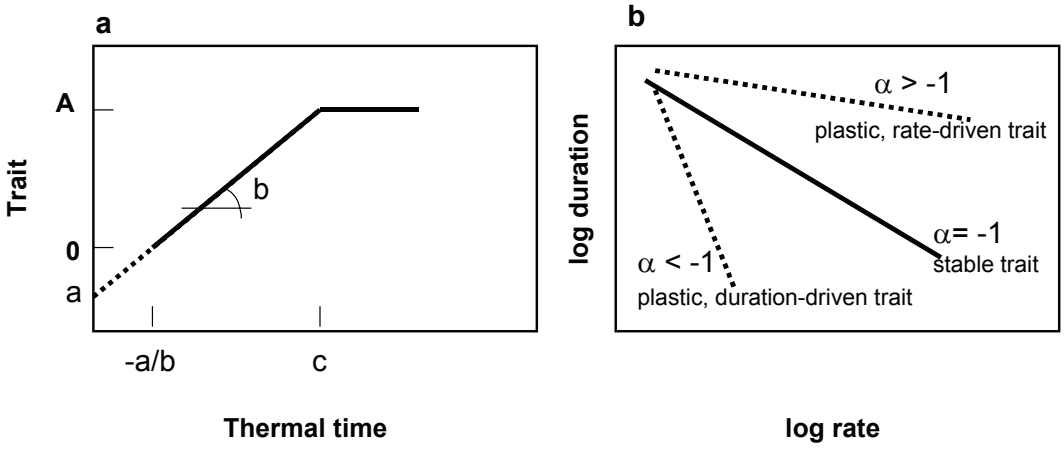

Fig. 4 (a). Many plant traits, including seed size, seed oil content and leaf area, conform to an approximate sigmoidal pattern with characteristic rates and durations. (b) The allometric relationship between duration and rate allows for a quantitative characterisation of trait plasticity. Adapted from Sadras et al. (2007).

A broader test of the concept included 45 data sets involving nine crop species, and sources of variation including genotype, environment, and their interaction (Fig 6). Relative variation in seed size ranged from 5 to 274%, and the scaling exponent was strongly concentrated in the range from 0 (large, rate-driven seed size range) to –1 (narrow seed size range due to mutually cancelled effects of rate and duration). The range of seed size declined when the scaling exponent declined from approximately 0 to –

1. An $\alpha \approx -1$ (rate and duration effects cancel each other) is necessary and sufficient for small variation in seed size, whereas $\alpha \approx 0$ is necessary but not sufficient for large seed size variation. The magnitude of seed size variation is dependent on the variation in the rate of seed growth when $\alpha \approx 0$. This double condition for seed size variability is summarised in a multiple regression model with α, and range of rate of grain filling as independent variables, which accounted for 73% of the variation in range of seed size.

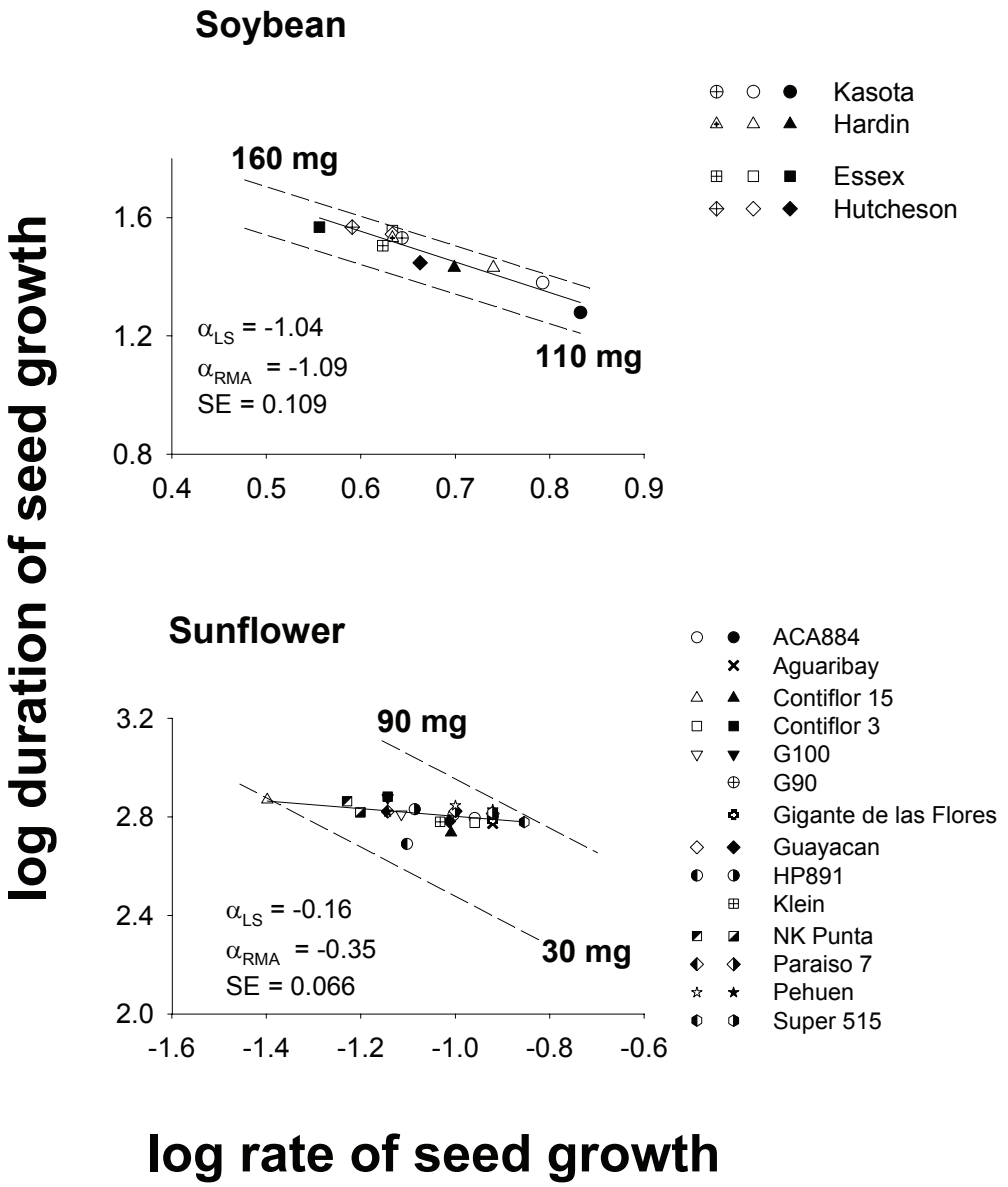

Fig. 5. Examples of intra-specific scaling relationships between rate and duration of seed growth in sunflower and soybean. Multiple symbols for a cultivar indicate different experiments or seasons. The solid line is the least squares regression, and dashed lines are isolines of seed size with $\alpha = -1$. Standard errors (SE) are common to the scaling exponents calculated with model I (α_{LS}) or model II (α_{RMA}) regression. Data sources: sunflower, López Pereira et al. (1999a); soybean (control treatment), Egli (1999). For soybean, rate is in mg seed^{-1} d^{-1} and duration in d, and for sunflower rate is in mg seed^{-1} ^{o}Cd^{-1} and duration in ^{o}Cd. Variate units do not affect the magnitude of the scaling exponent. Adapted from Sadras and Egli (2008).

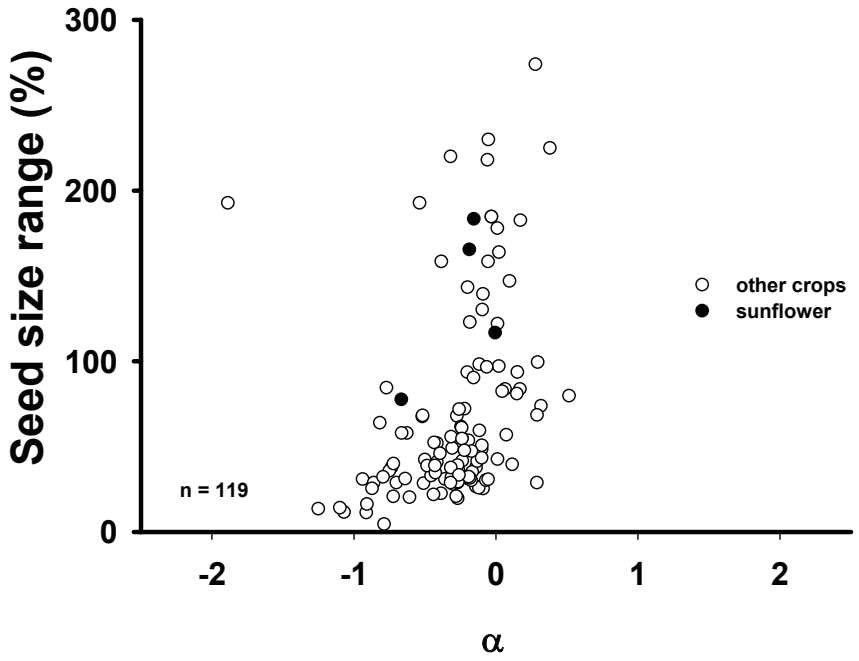

Fig. 6. Relationship between seed size range and α, the scaling exponent relating duration and rate of seed growth. Adapted from Sadras and Egli (2008).

Allometric analysis allowed for an integrated perspective on the interplay between rate and duration of seed filling, which in turn accounts for the genetic and environmental factors modulating seed size in grain crops. This allometric approach could be useful for evolutionary, agronomic and physiological analysis of seed size, and may also be used for other processes such as leaf growth or accumulation of oil or tocopherols in sunflower seed, where a framework of rates and durations is applicable. It would be of interest to consider the genetic substrate of parameter α for traits of agronomic interest (Principle 1).

Seed size plasticity and yield plasticity

The allometric relationship for sunflower in Fig. 5 was derived from crops grown under favourable conditions, i.e. hybrid grain yield ≥ 4 t/ha, oil concentration ≥ 50% (López Pereira et al., 1999a). Under these conditions, the duration of grain filling is typically around 30-35 days or about 650 ^{o}Cd (base = 4^{o}C), and differences in seed size are related to differences in rate of grain filling (de la Vega and Hall, 2002; López Pereira et al., 1999b). Relationships between rate and duration of grain filling could be different, however, in environments where excess or deficit of water supply during grain filling accelerate leaf senescence (Grassini et al., 2007; Hall et al., 1985).

Here we explore the relationships between seed size plasticity, quantified with parameter α and yield plasticity quantified with the method of Finlay and Wilkinson (1963) for a set of four sunflower hybrids grown under six environmental conditions in Argentina (for details see de la Vega and Hall 2002). The size of the data set is restricted due to the need to conciliate the time consuming sampling necessary to derive seed growth curves and α, and the relatively large number of cultivars and environments required to calculate yield plasticity. Growing conditions include a timely October sowing and a late December sowing conducive to lower yields. One of the seasons (1997/98) was "El Niño", with excessive rainfall and cloudy days detrimental to sunflower yield even for timely sown crops (Magrin et al., 1998). Yield plasticity ranked Aguará < Morgan 734 < Contiflor 15 (Fig. 7). Yield plasticity of hybrid GV25086 was similar to that of Contiflor 15 (not shown). The differences in yield stability among hybrids are partially related to their patterns of seed growth (Fig. 8). In response to late sowing, Contiflor 15 and Morgan 734 reduced both rate and duration of grain filling and Aguará slightly increased the rate of seed filing at the expense of shorter duration. Even for a set of few hybrids and growing conditions, Fig. 8 illustrates the

complex interplay of rate and duration of seed filling, and relationships between seed filing pattern and yield plasticity are not straightforward. Allometric relationships between rate and duration were loose, with large standard errors (not shown). Despite of this, the scaling coefficient α summarised the contrasting rate-duration relationships of these hybrids, and captured a substantial part of the variation in yield plasticity (Fig. 9). This reinforces the interest in the previous proposition of exploring the genetic basis of α.

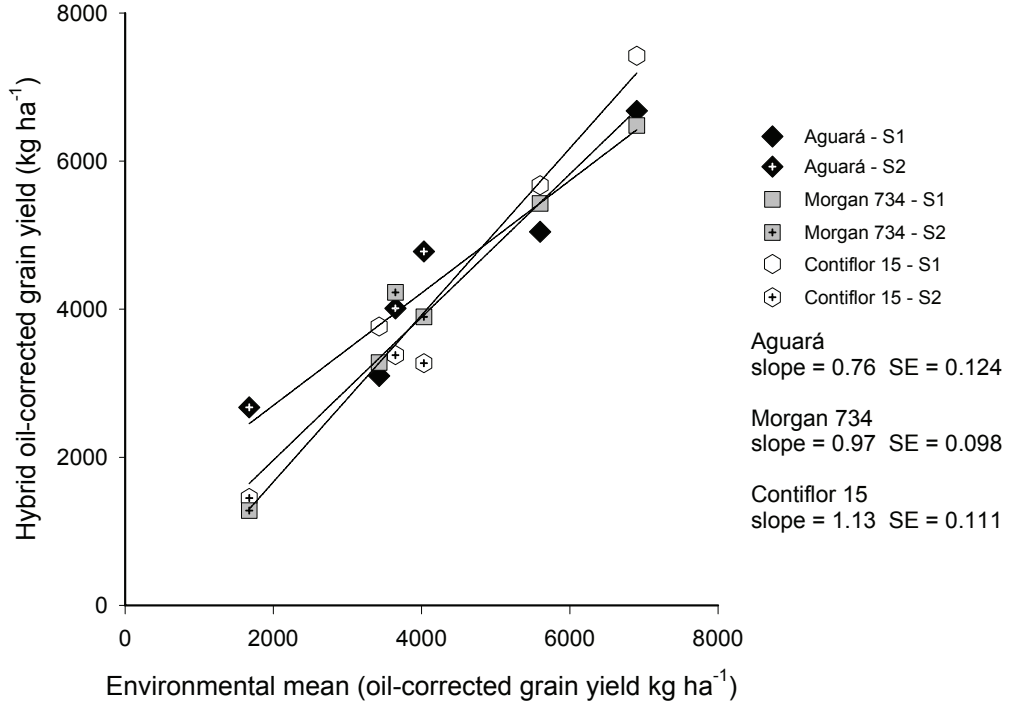

Fig. 7. Yield plasticity (slope of regressions) of three sunflower hybrids grown under six environmental conditions in Argentina. S1 is a timely October sowing, and S2 is a December sowing conducive to low yields.

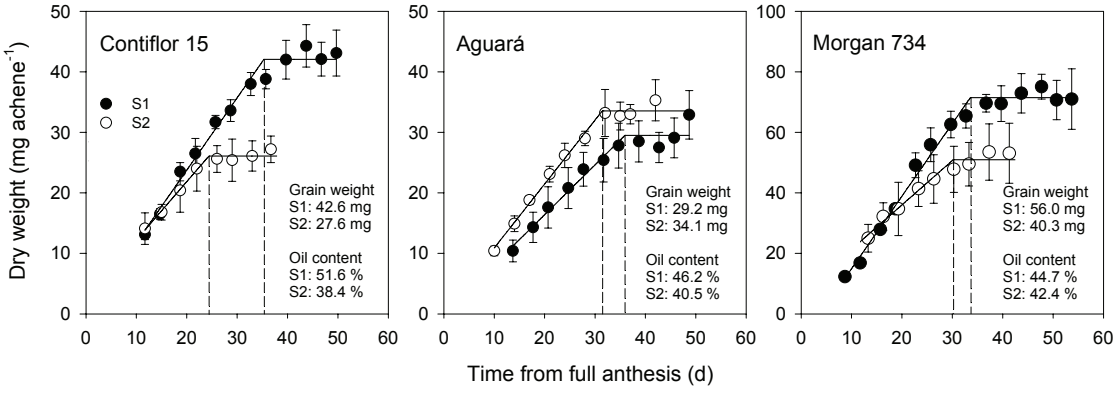

Fig. 8. Dynamics of seed growth of three sunflower hybrids sown in October (S1) or December (S2) 1996 at Venado Tuerto, Argentina. Adapted from de la Vega and Hall (2002).

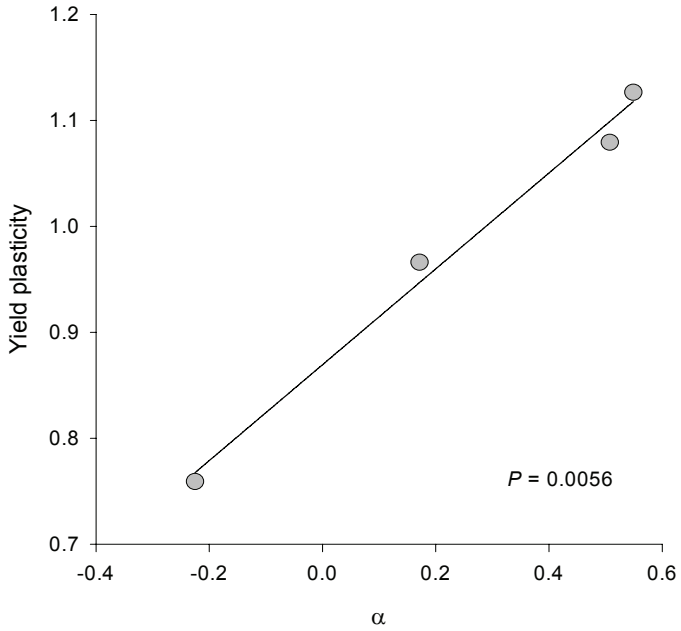

Fig. 9. Relationship between yield plasticity and α, the scaling exponent relating duration and rate of seed growth, for four sunflower hybrids grown in contrasting environmental conditions.

CONCLUDING REMARKS

The insightful vision of Bradshaw (1965), providing the contemporary definition of phenotypic plasticity and the notion that plasticity is a trait of its own, with its own genetic control acquires a new dimension when Reymond et al. (2003) demonstrate that the plasticity of certain traits could be traced back to specific QTLs. Against this conceptual framework, this paper showed that a physiological viewpoint of phenotypic plasticity can contribute to the understanding of G x E of sunflower yield. For the first time, here we showed that phenotypic plasticity of phenological development could be positively associated with yield plasticity under conditions when yield plasticity is a desirable trait, i.e. where there is no trade-off between performances in low and high yielding environments. Allometric models linking rate and duration could be useful to quantify phenotypic plasticity of agronomically important seed traits.

REFERENCES

Bradshaw, A.D. 1965. Evolutionary significance of phenotypic plasticity in plants. Adv. Genet. 13:115-155.

Bradshaw, A.D. 2006. Unravelling phenotipic plasticity - why should be bother? New Phytol. 170:644-648.

Calò, A., A. Costacurta, and C. Lorenzoni. 1975. Stabilita ambientale di alcune caratteristiche fenologiche in varieta di *Vitis vinifera*. Estr. Riv. Vitic. Enol. Conegliano 11-12:3-33.

Cantagallo, J.E., C.A. Chimenti, and A.J. Hall. 1997. Number of seeds per unit area in sunflower correlates well with a photothermal quotient. Crop Sci. 37:1780-1786.

de la Vega, A.J., and A.J. Hall. 2002. Effects of planting date, genotype, and their interactions on sunflower yield: II. Components of oil yield. Crop Sci. 42:1202-1210.

De Witt, T.J., A. Sih, and D.S. Wilson. 1998. Costs and limits of phenotypic plasticity. Trends Ecol. Evol. 13:77-82.

Egli, D.B. 1999 Variation in leaf starch and sink limitations during seed filling in soybean. Crop Sci. 39:1361-1368.

Finlay, K.W., and G.N. Wilkinson. 1963. The analysis of adaptation in a plant-breeding programme. Austr. J. Agric. Res. 14:742-754.

Giménez, C., and E. Fereres. 1986. Genetic variability in sunflower cultivars under drought. II. Growth and water relations. Aust. J. Agric. Res. 37:583-597

Grassini, P., G.V. Indaco, M.L. Pereira, A.J. Hall, and N. Trapani. 2007. Responses to short-term waterlogging during grain filling in sunflower. Field Crops Res. 101:352-363

Hall, A.J., C.A. Chimenti, F. Vilella, and G. Freier. 1985. Timing of water stress effects on yield components in sunflower. p. 131-136. In: Proc. 11th Int. Sunflower Conf., Mar del Plata, Argentina.

López-Pereira, M, V.O. Sadras, and N. Trápani. 1999a. Genetic improvement of sunflower in Argentina between 1930 and 1995. I. Yield and its components. Field Crops Res. 65:157-166.

López-Pereira, M, N. Trápani, and V.O. Sadras. 1999b. Genetic improvement of sunflower in Argentina between 1930 and 1995. II. Phenological development, growth and source-sink relationship. Field Crops Res. 63:247-254.

Magrin, G.O., M.O. Grondona, M.I. Travasso, D.R. Boullón, G.R. Rodríguez, and C.D. Messina. 1998. Impacto del fenómeno "El Niño" sobre la producción de cultivos en la Región Pampeana. INTA, Castelar, Argentina, 16 p.

Passioura, J.B. 1996. Drought and drought tolerance. Plant Growth Reg. 20:79-83.

Passioura, J.B. 2007. The drought environment: physical, biological and agricultural perspectives. J. Exp. Bot. 58:113-117.

Pigliucci, M. 2001. Phenotypic plasticity. p. 58-69. In: C.W. Fox, D.A. Roff, and D.J. Fairbairn (eds), Evolutionary Ecology. Concepts and Case Studies. Oxford University Press, New York, USA.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? Trends Ecol. Evol. 20:481-486.

Pigliucci, M., J. Whitton, and C.D. Schlichting. 1995. Reaction norms of *Arabidopsis* .1. Plasticity of characters and correlations across water, nutrient and light gradients. J. Evol. Biol. 8:421-438.

Reymond, M., B. Muller, A. Leonardi, A. Charcosset, and F. Tardieu. 2003. Combining quantitative trait loci analysis and an ecophysiological model to analyze the genetic variability of the responses of maize leaf growth to temperature and water deficit. Plant Physiol. 131:664-675.

Richards, R.A. 2006. Physiological traits used in the breeding of new cultivars for water-scarce environments. Agric. Water Manag. 80:197-211.

Sadras, V.O. 2007. Evolutionary aspects of the trade-off between seed size and number in crops. Field Crops Res. 100:125-138.

Sadras, V.O., and N. Trápani. 1999. Leaf expansion and phenologic development: key determinants of sunflower plasticity, growth yield. p. 205-232. In: D.L. Smith, and C. Hamel C (eds) Physiological control of growth and yield in field crops. Springer-Verlag, Berlin, Germany.

Sadras, V.O., and D.B. Egli. 2008. Seed size variation in grain crops: Allometric relationships between rate and duration of seed growth. Crop Sci. 48:1-8.

Sadras, V.O., R.M. Stevens, J.M. Pech, E.J. Taylor, P.R. Nicholas, and M.G. McCarthy. 2007. Quantifying phenotypic plasticity of berry traits using an allometric-type approach: A case study on anthocyanins and sugars in berries of Cabernet Sauvignon. Aust. J. Grape Wine Res. 13:72-80.

Zhivotovsky, L.A., M. Feldman, and B. Bergman. 1996. On the evolution of phenotypic plasticity in a spatially heterogeneous environment. Evolution 50:547-558.

Sunflower germplasm development utilizing wild *Helianthus* species

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ABSTRACT

The narrow genetic base of cultivated sunflower has been broadened by the infusion of genes from the wild species, which have provided a continued source of agronomic traits for crop improvement. The genus *Helianthus* comprises 51 species (14 annual and 37 perennial), all native to North America. The available genetic diversity from the wild species is continuing to be used to broaden the genetic background of the crop. Recent advances in culturing of otherwise abortive interspecific hybrid embryos have proved to be highly effective for making the difficult-to-cross wild perennial *Helianthus* species widely available for breeding purposes, either for specific major gene transfer or for the transfer of quantitative trait genes. These techniques are discussed and illustrations are shown of how they are being used to incorporate genes from several different ploidy levels of wild perennial species into cultivated sunflower for Sclerotinia stalk rot resistance and other diseases. Significant results have been reported on the germplasm development with regard to resistance to new races of downy mildew, rust, broomrape and other major diseases. In addition, new CMS and corresponding fertility restoration genes have been continuously identified and established, together with new genes helping to improve oil quality, herbicide resistance, and salt and drought tolerance. Thus far, only a small portion of the available genetic diversity of the wild *Helianthus* species has been used globally. As a whole, there is no doubt that wild *Helianthus* species will continue to provide new genetic variability to the sunflower breeding community, helping to maintain sunflower as a viable major global oilseed crop.

Key words: amphiploids – genetic diversity – genetic resources – *Helianthus* – interspecific hybridization.

INTRODUCTION

Sunflower production continues to face challenges from both abiotic and biotic factors as well as from today's ever-changing market needs. For the most part, the crop has been doing fairly well thus far. However, the limited genetic variability in cultivated sunflower has slowed the future improvement of the crop, and has placed the crop in a vulnerable position should any major shifts of disease races or pests occur. The uniform use of a single CMS PET1 cytoplasm and a few fertility restoration genes for worldwide sunflower production makes the crop extremely vulnerable. Diversity of resistance to various diseases is strategically needed. We have seen the rapid increase in the number of rust races being identified in Australia in recent years. The continuing race shift of broomrape in Spain, Turkey and the Black Sea areas since the mid-1990s has kept researchers busy for over 10 years searching for new resistance genes. For a while, the predominant rust and downy mildew races in the USA were limited to three or four, but many new races of these two diseases have been identified in the last 10 years. All the new races have the potential of becoming the predominant races in the future in response to our introduction of resistance genes. Sunflower is not always grown on prime land, but often on marginal land with minor salt and drought problems, presenting a challenge to be productive under less than ideal conditions. An early season sunflower crop has the potential to increase production by increasing the double crop potential for producers. In order to be competitive with other premium oils in the market, the reduction of saturated fatty acids in sunflower oil will play a key role in maintaining our continuing success. Evaluations of wild species have provided information about useful genes for future sunflower improvement. However, there are still numerous genes in wild sunflower species yet to be identified and introgressed into cultivated sunflower. Extensive collection efforts for wild *Helianthus* species and the regeneration of seeds at the USDA-ARS, Regional Plant Introduction Station at Ames, Iowa have greatly increased the availability of wild *Helianthus* seed for sunflower improvement. An overall advancement of our understanding of wild *Helianthus* species and improved methods of making interspecific crosses have

increased the number of useful genes available from wild *Helianthus* species, making it possible to transfer genes that were not possible three decades ago. This report will discuss the importance of wild *Helianthus* species and their utilization for sunflower improvement in the past and present, and show examples from our current wild species breeding program, and future prospects.

DISCUSSION

Helianthus collection. The USDA-ARS National Plant Germplasm System (NPGS) sunflower collection is maintained at the North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa. The collection contains 37 perennial species, 14 annual species, and the cultivated species, *Helianthus annuus* (Schilling, 2006). This NPGS sunflower collection is a diverse assemblage of 3850 accessions: 1708 cultivated *Helianthus annuus* accessions, 932 wild *Helianthus annuus* accessions, 437 accessions representing 11 other wild annual *Helianthus* species, and 773 accessions representing 37 perennial *Helianthus* species. This collection is the largest and most genetically diverse sunflower collections in the world and it is vital to the conservation of *Helianthus* germplasm. From 1976 to 1996, 10,000 samples of wild sunflower were distributed to 300 researchers in 30 countries. These accessions have become the basis of wild species research programs in Argentina, France, Italy, Spain, Germany, Bulgaria, Romania, Czechoslovakia, Hungary, Russia, Yugoslavia, India, China, and Mexico.

Notable is the collection at the Institute of Field and Vegetable Crops, Novi Sad, Serbia, which contains 39 of the 51 wild species (IBPGR, 1984; Cuk and Seiler, 1985). The wild species collection of the Dobroudja Agricultural Institute (DAI) at General Toshevo, Bulgaria, is also notable, containing 428 accessions representing 37 of the 51 species of *Helianthus* (Christov et al., 2001). The wild species collection maintained at INRA, Montpellier, France has more than 600 accessions of 45 of the 51 wild sunflower species (Serieys, 1992). The Instituto de Agricultura Sostenible (CSIC) Cordoba, Spain maintains 44 annual and perennial accessions of *Helianthus* (Ruso et. al., 1996).

***Interspecific hybridization*: *The early years*.** Prior to the embryo culture method developed by Chandler and Beard (1983), nearly all the interspecific crosses were conducted in a classical fashion. All the annual *Helianthus* species, except *H. agrestis*, can be hybridized and F_1s backcrossed with cultivated lines using classical breeding methods. Direct crosses of cultivated lines with many perennial *Helianthus* species are also possible using conventional methods. Hybrids of *H. mollis* Lam. x *H. annuus* L. and *H. strumosus* L. x *H. annuus* (Heiser and Smith, 1964) and of *H. decapetalus* L. x *H. annuus* (Heiser et al., 1969; Georgieva-Todorova, 1984) have been reported. Hybrids of *H. tuberosus* L. x *H. annuus* (Heiser et al., 1969; Atlagić et al., 1993), *H. annuus* x *H. hirsutus* Raf. (Georgieva-Todorova, 1984), and *H. rigidus* (= *pauciflorus*) Nutt. x *H. annuus* (Vrânceanu and Iuoras, 1988) have also been successful. Atlagić (1990) summarized five interspecific hybrids involving crosses of perennial species *H. hirsutus*, *H. laevigatus* T. and G., *H. rigidus* (= *pauciflorus*), *H. tuberosus*, *H. maximiliani* Schrad., and *H. nuttallii* T. and G. with cultivated sunflower. Whelan (1978) used wild *H. annuus* as an "intermediate" parent or "bridge" to produce the first hybrids obtained between cultivated sunflower and *H. giganteus* L. and *H. maximiliani*.

***Interspecific hybridization*: *Utilizing embryo rescue*.** The development of a two-step embryo culture procedure by Chandler and Beard (1983) greatly facilitated interspecific hybridization. They successfully produced 53 interspecific cross combinations without the exhaustive effort of endless pollination, and 21 of these combinations had not been previously produced. Jan and Chandler (unpublished data) further modified the original procedure for culturing difficult hybrid embryos of wild perennial *Helianthus* species with cultivated *H. annuus* by adding vitamins, increasing sucrose to 20 g/kg, and the conversion from liquid to a solid medium with 0.7% agar. In addition, both growth and germination media were adjusted to pH 5.5 with 2-[N-morpholino]ethanesulfonic acid (MES) buffer. Using these modified media, 18 perennial species x *H. annuus* hybrids were established in one season, and many of them represented the first hybrid combinations ever produced (Jan, 1988).

Kräuter et al. (1991) cultured 0.2 to 1.5-mm small embryos on B5 medium with 90 g/kg sucrose, and embryos >1.5 mm on a modified MS (Murashige and Skoog, 1962) medium with 10 g/kg sucrose. When these embryos reached the size of 2 to 3 mm, they were transferred to MS medium for germination. Using this method, they obtained 33 interspecific hybrid combinations with an overall success rate of 41%. Using cultivated sunflower embryos of varying sizes, Espinasse et al. (1985) concluded that a high sucrose concentration of 90 g/kg and low nitrogen content were required for culturing small young embryos less than 2 mm in size.

As suggested by Dewey (1980), induced polyploidy could also serve as a bridge for interspecific gene transfer in sunflower. Jan and Chandler (1989) successfully doubled chromosomes of P21 x *H. bolanderi* F_1 hybrids, and increased seed set on doubled heads. Jan (1988) reported the success of a modified colchicine chromosome-doubling technique on 19 embryo-cultured wild x cultivated interspecific hybrids, and its positive effect on backcross seed set. Chromosome doubling of each head was verified by pollen grain size and stainability (Alexander, 1969). Chromosome doubling increased pollen grain size and stainability of interspecific hybrids. The increased pollen grain size directly reflected chromosome doubling and provided a reliable criterion for classifying treated plants.

Chromosome doubling restores normal fertility of amphiploids by providing an identical pairing partner for each chromosome. However, this increased fertility is likely to reduce the enforced interspecific chromosome pairing and gene exchanges during meiosis when an F_1 head is not chromosomally doubled. It would be helpful if the researchers could backcross onto both doubled and nondoubled heads, and at the same time intercross doubled heads for amphiploid production. More cytological evaluations are needed to compare the efficiency of interspecific gene transfer with or without the assistance of chromosome doubling of F_1s. Without chromosome doubling, we expect very low number of BC_1F_1 seeds and a high frequency of weak BC_1F_1 plants. With chromosome doubling, due to preferential pairing of *H. annuus* chromosomes during meiosis, we expect a reduced pairing of *H. annuus* chromosomes with chromosomes of wild *Helianthus* species.

***Interspecific hybridization*: *Introgression of genes into cultivated lines*.** In recent years, interest in interspecific hybridization has been greater for transferring useful genes from wild species into cultivated lines to develop pre-breeding germplasms for sunflower improvement. Characteristics such as disease and insect resistance, salt tolerance, drought tolerance, fatty acid variation, CMS, and fertility-restoration diversity have been emphasized.

By successful hybridization between *H. petiolaris* and *H. annuus* and backcrossing with *H. annuus*, Leclercq (1969) transferred the *H. annuus* genome into cytoplasm of *H. petiolaris* Nutt. and obtained the first cytoplasmic male sterile plants. Whelan (1980; 1981) and Whelan and Dorrell (1980) used the same technique to obtain cytoplasmic male sterility conditioned by the cytoplasm of three species, *H. petiolaris*, *H. giganteus*, and *H. maximiliani*.

Due to the use of a single male-sterile cytoplasm for worldwide hybrid sunflower production and its consequence of genetic vulnerability, a large portion of the interspecific hybridization in sunflower has focused on the identification of new CMS sources and their fertility restoration genes. Of the total 70 CMS sources resulting from interspecific hybridization, 39 were derived from wild *H. annuus* and 23 from other wild annual species, and only eight from wild perennial species. Extensive research is now focused on the identification of fertility restoration genes using both cultivated and wild species, and evaluation of their inheritance.

Rapid improvement of interspecific F_1 meiotic abnormality and low fertility was demonstrated by Whelan (1978; 1979) when he discovered CMS-PET2, G1G1, and MAX1. The differences of the parents were shown as translocations and a paracentric inversion as indicated in F_1 meiosis, which can quickly be eliminated after one or more backcrosses with cultivated lines (Whelan, 1982).

Helianthus tuberosus x *H. annuus* hybrids have been used widely in the Former Soviet Union (FSU) as a source of disease resistance. Hybrids of *H. annuus* x *H. resinosus* Small (2n=102) had stainable pollen from 0 to 50%, and meiotic diakinesis had 28 to 36 bivalents with 1 to 6 univalents (Georgieva-Todorova, 1983). The high number of bivalents suggests a high homology between the chromosomes from *H. resinosus* and those from *H. annuus*. In general, good pollen stainability is expected in the F_1s of hexaploid *Helianthus* species crossed with *H. annuus*. Atlagić (1990) reported average pollen stainability of 49.8%, 40.9%, and 64.6, respectively, for the hybrids of *H. annuus* with *H. pauciflorus*, *H. tuberosus* and *H. laevigatus.* Seiler's (1991a; 1993) release of 12 interspecific germplasm lines derived from perennial accessions of *H. hirsutus*, *H. resinosus*, and *H. tuberosus* also supports the reasonably good fertility of *H. annuus* x hexaploid accessions and some selected *H. annuus* x tetraploid accessions.

An unusual cytoplasmic-nuclear interaction causing plants with reduced vigor has been observed, and a single dominant gene was needed to restore normal plant growth (Jan, 1992). With continuous backcrossing with HA 89 as the recurrent parent into the cytoplasms of five diploid perennial species, *H. mollis*, *H. maximiliani*, *H. grosseserratus* Martens, *H. divaricatus* L., and *H. angustifolius* L. and selection for normal segregants, Jan (1992) discovered the vigor-reducing effects of these cytoplasms and a single nuclear vigor-restoration gene was needed to restore the vigor. The vigor-reducing cytoplasmic effects also have been observed in progenies when backcrossing HA 89 into cytoplasms of *H. hirsutus*, *H. occidentalis* Riddell, and *H. giganteus*. A considerable number of cultivated lines were found to possess

the same vigor restoration gene, and it was suspected to have been derived from *H. tuberosus* because of that species' popular use in early breeding programs in the FSU. Our recent discovery of a different vigor restoration gene derived from *H. giganteus* suggested the existence of different vigor restoration genes in varying perennial *Helianthus* species compensating for specific cytoplasmic effects causing reduced vigor (Jan, 2003).

Transferring genes from wild annual species into cultivated lines can be accomplished rather easily with conventional crossing and backcrossing. Seiler (1991b, c) released 15 interspecific germplasm lines having genes from wild annual species, and 13 tolerant to sunflower downy mildew, using the conventional method of crossing and backcrossing. Jan and Chandler (1985a) transferred resistance genes for powdery mildew (*Erysiphe cichoracearum* DC.) from *H. debilis* Nutt. and rust (*Puccinia helianthi* Schwein.) and downy mildew resistance genes from wild *H. annuus* into cultivated sunflower (Quresh et al., 1993; Quresh and Jan, 1993; Tan et al., 1992).

Crossing cultivated sunflower with wild perennial *Helianthus* species often results in serious problems of early hybrid embryo abortion, as well as high levels of sterility in the F_1 or BC_1F_1 generation. However, utilizing an embryo-culturing technique, 26 interspecific hybrids of wild perennials x cultivated line P21 were produced. Subsequent chromosome doubling of the F_1s of diploid and tetraploid wild accessions crossed with P21 improved backcross and sib-pollinated seed set drastically (Jan, 1988). Amphiploids of wild species utilizing *H. gracilentus* A. Gray, *H. pumilus* Nutt., *H. hirsutus*, *H. strumosus*, *H. maximiliani*, *H. nuttallii*, *H. mollis*, and *H. grosseserratus* crossed with cultivar P21 have been produced by sib-pollination of chromosomally doubled heads of each cross. These amphiploids can be maintained by sib-pollination, have improved pollen stainability and larger pollen grains, and have improved backcross seed set (Jan and Fernández-Martínez, 2002).

Interspecific gene transfer facilitated by the chromosome doubling of extremely difficult diploid perennials x *H. annuus* and tetraploid x *H. annuus* crosses has been demonstrated. Positive results of gene transfer from *H. hirsutus* into cultivated sunflower have been obtained (Jan and Zhang, 1995). By monitoring the rust resistance genes of *H. hirsutus*, which is immune to the four North American (NA) rust races, the hexaploid amphiploid was backcrossed with *H. annuus* twice. The resulting triploid BC_2F_1s had a complete set of 34 chromosomes of *H. annuus*, plus 17 chromosomes from *H. hirsutus*, and were all resistant to the four NA rust races. Several BC_3F_1 plants had 2n=36 or 37 chromosomes and were resistant to NA rust races 1 and 2, and further backcrossing resulted in many BC_4F_1 race 1- and 2-resistant plants with 2n=34. More recently, Jan et al. (2002) produced four sunflower germplasms with resistance to broomrape (*Orobanche cumana* Wallr.) race F, with resistance genes transferred from wild perennial *Helianthus* via interspecific amphiploids. In addition, interspecific amphiploids of perennial x cultivated have provided fertility restoration genes for the new CMS cytoplasms derived from *H. giganteus* (Jan, 2004) while no *Rf* genes were identified in cultivated lines. Surprisingly, *Rf* genes for this CMS were identified in four out of the seven amphiploids tested.

Chandler (1991) reviewed sunflower genomic relationships and came to the conclusion that there is little evidence of the existence of distinct genomes in *Helianthus*. The author's observation of many interspecific hybrids agrees with Chandler's statements. Even the most sterile interspecific hybrids involving diploid perennial species and cultivated *H. annuus* had satisfactory chromosome pairing (Jan and Chandler, 1985b). In order to utilize this high degree of chromosome similarity between cultivated lines and wild *Helianthus* species for interspecific gene transfer, the best approach would be to backcross without F_1 chromosome doubling. Without chromosome doubling, maximum chromosome pairing between cultivated lines and the wild species will be achieved. With chromosome doubling, preferential chromosome pairing of identical chromosomes in each parent will reduce the interspecific chromosome pairing and gene exchanges. However, the latter approach may have the advantage of having improved backcross fertility, and the reduced degree of gene exchange will enhance the quick recovery of a recurrent parent genotype carrying the specific selected gene. This was demonstrated with the rust resistance gene transfer from *H. hirsutus* into cultivated line HA 89 via amphiploidization, where chromosomes from *H. hirsutus* demonstrated their ability to challenge the perfect pairing of *H. annuus* chromosomes and to incorporate the resistance genes into the *H. annuus* genome (Jan and Zhang, 1995).

***Interspecific hybridization*: *Amphiploids*.** Colchicine treatment of interspecific F_1 hybrids resulted in high frequencies of chromosome doubling and the production of amphiploids (Jan and Fernández-Martínez, 2002). The tetraploid amphiploids produced included crosses of P21 x *H. bolanderi* (Jan and Chandler, 1989), *H. gracilentus* x P21, *H. grosseserratus* x P21, *H. cusickii* A. Gray x P21, *H. mollis* x P21, *H. maximiliani* x P21, and *H. nuttallii* x P21. These amphiploids have restored fertility, and provide easily available genetic diversity for the improvement of cultivated sunflower. The first hexaploid

amphiploids in sunflower have also been produced from crosses of *H. hirsutus* x P21 and *H. strumosus* x P21.

The interspecific amphiploids will enable the establishment of a number of chromosome addition lines for genetic studies of specific chromosomes of both cultivated and wild *Helianthus* species. With the available amphiploids and some specific interspecific crosses, the potential exists to establish additional lines with HA 89 chromosome pairs in *H. californicus*, and the chromosome pairs of *H. hirsutus*, *H. angustifolius*, *H. cusickii*, *H. gracilentus*, *H. grosseserratus*, *H. nuttallii, H. strumosus*, and *H. giganteus* in HA 89.

Male sterility. A single male-sterile cytoplasm, PET1, derived from *H. petiolaris* subsp. *petiolaris* (Leclercq, 1969) and the identification of dominant fertility restoration genes (Enns et al., 1970; Kinman, 1970; Vrânceanu and Stoenescu, 1971) advanced sunflower production from the use of open-pollinated cultivars to hybrid production 40 years ago. This source of cytoplasmic male sterility and a few fertility restoration genes, including the widely used Rf_1 and Rf_2 genes, have been used exclusively for sunflower hybrid production worldwide (Fick and Miller, 1997).

A total of 70 CMS sources have been identified from progenies of crosses between wild *Helianthus* accessions and cultivated lines, from wild accessions grown in observation nurseries, or from induced mutation. Fertility restoration genes have been reported for 34 CMS sources, and detailed inheritance studies have been conducted for only 19 of the CMS sources (Serieys, 2002). In general, it is relatively easy to isolate stable CMS cytoplasms, but the identification of simple and completely dominant fertility restoration genes has been far less successful.

Many CMS sources from wild *H. annuus* (ANN1 through ANN9) were discovered in field-grown populations. All these CMS lines except ANN8 were completely male-sterile with degenerated anthers. Restoration genes were found for ANN2, 3, 4, and 7 using a set of 20 fertility-restoration testers, plus male-fertile plants of each respective wild species accession. Inheritance studies of fertility restoration of ANN2 and ANN3 indicated complete fertility restoration by single dominant genes (Jan, 1991). Serieys (1994) also reported complete male sterility and full fertility restoration by single dominant genes for CMS-ANO1, CMS-NEG1, and CMS-PRP1. The utilization of these CMS sources for potential hybrid production should be pursued.

Diseases. Diseases limit production in a majority of sunflower producing countries. Sunflower is a host to a wide array of diseases that can cause serious economic damage in terms of yield and quality, with the fungal diseases the most numerous and economically serious. In the USA, the major diseases of concern are downy mildew, rust, Sclerotinia head and stalk rot, and Phoma black stem. Verticillium wilt, Phomopsis stem canker, Alternaria leaf spot, Septoria leaf spot, charcoal stem rot, and Rhizopus head rot occur to a lesser degree. In Europe and adjacent Mediterranean countries, downy mildew, Sclerotinia head rot, Phomopsis, Botrytis gray rot, and charcoal rot are considered the most important diseases. Some diseases are important in only a few countries, such as Verticillium wilt in Argentina and white rust (*Albugo*) in South Africa. Genetic resistance to the prevailing North American races of rust has been identified in three wild annual species, *H. annuus*, *H. petiolaris*, and *H. argophyllus* T. and G. (Jan et al., 2004a). Genes for rust resistance are frequent in the wild progenitors of the cultivated sunflower (Quresh et al., 1993). In most cases rust resistance appears to be conditioned by single dominant genes.

Downy mildew can be controlled by single, race-specific dominant resistance genes. Multi-race resistant germplasm and single-race resistant germplasms have been developed from wild sunflower species (Miller and Gulya, 1988; Tan et al., 1992; Jan et al., 2004b). Wild *Helianthus annuus, H. petiolaris* and *H. praecox* Engelm. and A. Gray are sources of single dominant genes for single race resistance, while *H. argophyllus* is the source of dominant genes for all known races of the fungus (Miller and Gulya, 1988; Miller et al., 2002).

Sclerotinia wilt (white mold) causes the greatest losses to sunflower on a global basis. This is in part due to the wide host range of *Sclerotinia sclerotiorum* (Lib.) de Bary being a facultative parasite that attacks 360 species of plants. It appears that Sclerotinia resistance is complex and controlled polygenically involving many genes, each with small effects. This means that the breeding strategy using wild species as a source of resistance needs to be quite different than for other diseases. A detailed approach and strategy for developing Sclerotinia stalk rot resistance will be discussed later in this section.

There are reports of identification of cultivated sunflower genotypes with low susceptibility or moderate resistance to Sclerotinia white mold. Wild species have also been identified as a potential source of genes for Sclerotinia tolerance. Interspecific hybrids with perennial *H. maximiliani* (Maximilian's sunflower) exhibited higher levels of resistance than head rot resistant inbred lines

(Cerboncini et al., 2002; Ronicke et al., 2004). Rashid and Seiler (2004) identified potential sources of Sclerotinia head and stem rot resistance in populations of perennial *H. maximiliani* and *H. nuttallii* from Canada. Perennial *H. resinosus* has been identified as a good source for resistance to Sclerotinia head rot by Mondolot-Casson and Andary (1994). The Sclerotinia disease complex appears to be very complicated. The prospect of finding a single dominant gene for resistance does not look promising, but progress is being made in the development of germplasm with increased tolerance to Sclerotinia head rot. Currently there are no commercial hybrids which possess a satisfactory level of resistance to Sclerotinia rot.

Some progress has been made in increasing the resistance to midstalk Sclerotinia rot in cultivated sunflower. Kohler and Friedt (1999) indicated that progenies of interspecific crosses involving *H. mollis* and *H. tuberosus* had increased levels of tolerance to midstalk white mold infection. Miller and Gulya (1999) developed four maintainer and four restorer oilseed lines with improved tolerance to midstalk Sclerotinia rot.

Sclerotinia sclerotiorum generates substantial quantities of oxalic acid, which has been identified as one of the key components in the infection process. One strategy for resistance is to obtain plants that are resistant to free oxalic acid by engineering them to degrade it. A wheat (*Triticum aestivum* L.) oxalate oxidase gene (OXO) has been identified and transferred into sunflower via transformation (Scelonge et al., 2000). A transgenic sunflower line, *H. annuus* cv. SMF3, constitutively expressed the wheat OXO gene (Hu et al., 2003) and exhibited enhanced resistance against the oxalic acid-generating fungus Sclerotinia. This approach to white mold resistance in sunflower awaits further testing and commercialization.

Phomopsis brown stem canker was first discovered in sunflower in Yugoslavia in 1980 and now is considered a serious problem in much of Europe (Mihaljcevic et al., 1982; Acimovic, 1984; Škorić, 1985). Cuk (1982) reported that wild *H. debilis* and *H. pauciflorus* are potential sources of resistance to *Phomopsis helianthi* Munt-Cvet. et al. Kurnik and Walcz (1985) reported resistance to stem canker in *H. argophyllus*, tolerance in two other wild species, and susceptibility in local populations of *H. tuberosus.* Dozet (1990) observed a high degree of resistance in two populations of *H. tuberosus*. Cultivated hybrids developed from *H. tuberosus* and *H. argophyllus* have high field tolerance to Phomopsis brown stem canker (Škorić, 1985). Škorić (1985) hypothesized that the resistance may be controlled by two or more complementary genes.

Alternaria leaf spot causes losses in cultivated sunflower in the USA and other parts of the world. In warm climates with high rainfalls, it causes defoliation and reduces yield significantly (Sackston, 1981). All 21 annual taxa and 18 of 21 perennial species evaluated were susceptible to *A. helianthii* (Hansf.) Tub. and Nish. spores applied in a suspension. Perennial species *H. hirsutus, H. pauciflorus* subsp. *subrhomboideus*, and *H. tuberosus* appear to resist infection by *Alternaria helianthi* (Morris et al., 1983). Lipps and Herr (1986) showed that 13 accessions of *H. tuberosus* had significantly less Alternaria leaf spot than commercial hybrids and concluded that the species is a potential source of resistance to leaf spot. Several wild annual species, *H. praecox, H.* x *laetiflorus* Pers., *H. debilis* subsp. *cucumerifolius*, and *H. debilis* subsp. *silvestris*, had high levels of resistance to Alternaria and *Septoria helianthi* Ellis and Kellerm. in field evaluations (Block, 1992). Although potential sources of resistance to *Alternaria* have been identified, resistance genes have not been transferred to cultivated lines.

Powdery mildew is a widely distributed pathogen of cultivated sunflower in warmer regions of the world (Zimmer and Hoes, 1978). This foliar disease is found mostly on senescing leaves, and is generally not of major economic concern. *Helianthus debilis* subsp. *silvestris, H. praecox* subsp. *praecox*, *H. bolanderi* A. Gray and 14 perennial species exhibited powdery mildew tolerance in both field and greenhouse tests (Saliman et al., 1982). Not all populations of some perennial species are resistant; populations of *H. grosseserratus* and *H. maximiliani* showed differential reactions. Jan and Chandler (1985a) characterized resistance to powdery mildew from *H. debilis* subsp. *debilis* as incompletely dominant. They incorporated genes from this species into a cultivated background and have released a germplasm pool PM1 having the resistance genes (Jan and Chandler, 1988).

Currently, cultivated sunflower does not possess resistance to Rhizopus head rot. Yang et al. (1980) reported that four out of 32 wild species and subspecies tested were resistant when inoculated with *R. arrhizus* A. Fischer and *R. oryzae* Went. The resistant sources were: *H. divaricatus*, *H. hirsutus*, *H.* x *laetiflorus*, and *H. resinosus*. Further breeding will be needed to transfer the identified sources of resistance into cultivated sunflower.

So far, most genotypes of sunflower have exhibited susceptibility to the pathogen *Phoma macdonaldii* Boerema. Under natural infection, wild sunflower species *H. maximiliani*, *H. argophyllus*, *H. tuberosus*, and *H. pauciflorus* possess excellent resistance to Phoma black spot (Škorić, 1992).

Interspecific lines based on *H. tuberosus* have resistance to charcoal rot. Wild species *H. mollis*, *H. maximiliani*, *H. resinosus*, *H. tuberosus*, and *H. pauciflorus* have also shown resistance. The number of genes and the inheritance of resistance to the pathogen have not been ascertained, although resistance appears to be dominant.

Broomrape (*Orobanche cumana*) is a parasitic weed that infects sunflower roots causing severe crop losses in Southern Europe and the Black Sea region. It has also been observed in Australia, Mongolia, and China and is generally associated with drier climates. Five resistance genes (Or_1 through Or_5) have been used successfully for broomrape control following the progression of races A through E. Since broomrape is a highly variable pathogen, the breakdown of resistance is a frequent phenomenon, and multiple sources of resistance are needed. Ruso et al. (1996) evaluated wild annual and perennial sunflower species reaction to Spanish races and found two annual species, *H. anomalus* Blake and *H. exilis* Gray that had resistance and all 26 perennial species were resistant.

Recent studies indicated the development of a new broomrape race in Spain, designated race F, which attacks all commercial sunflower hybrids, overcoming the previously effective resistance genes (Domínguez et al., 1996). High levels of resistance to race F have been observed in populations of wild perennial sunflower (Fernández-Martínez et al., 2000). Jan et al. (2002) released four race F resistant germplasms, BR1 through BR4, which were derived from wild perennial sunflowers *H. maximiliani*, *H. grossesserratus*, and *H. divaricatus*. Fernández-Martínez et al. (2004) released four sunflower germplasms, K-96, L-86, P-96 and R-96, with resistance to race F based on cultivated sunflower from Eastern Europe. Resistance to race F appears to be controlled by dominant-recessive epistasis, complicating the breeding by requiring the genes to be incorporated into both parental lines of a resistant hybrid (Akhtouch et al., 2002). Other germplasms have been released which have resistance to various races (other than race F) of broomrape including seven germplasms based on cultivated sunflower from the FSU, Romania, and Turkey (Miller and Domínguez, 2000).

Diseases*: *Current progresses in developing Sclerotinia stalk rot resistant germplasm utilizing wild perennial Helianthus. Sclerotinia stalk and head rot caused serious economic loss for more than 50 percent of the seed yield. Cultivated sunflower lacks resistance to *Sclerotinia,* although some differences in susceptibility exist. However, the over 51 species of *Helianthus*, consisting of diploid, tetraploid and hexaploid, represent a diverse potential source of Sclerotinia resistance genes. Evaluation of wild germplasm indicated that several wild perennial species possess high levels of resistance to Sclerotinia head rot and stalk rot.

Since 2005, a program focusing on the transfer of Sclerotinia stalk rot resistance from wild *Helianthus* species of different ploidy levels (2x, 4x, 6x) into adapted sunflower germplasm via interspecific hybridization was started at the Sunflower Research Unit in Fargo. In our initial experiment, hexaploid perennial *H. californicus* DC. was identified to be highly resistant to Sclerotinia stalk rot, and was crossed with the moderately tolerant line HA 410 (Miller and Gulya, 1999) followed by continuously backcrossing with HA 410 until BC_4F_1 (Feng et al., 2006).

At the same time, to expand the diversity of the resistance gene sources, interspecific amphiploids were identified that segregated for high levels of resistance to Sclerotinia stalk rot. These amphiploids had high crossability and played a critical role as bridges for interspecific gene transfer, avoiding the direct crossing of HA 410 with those wild *Helianthus* species known to cross with extreme difficulty. Thus, in 2006, amphiploids involving six wild diploid or tetraploid species were crossed with HA 410 and further backcrossed twice to transfer stalk rot resistance (Jan et al., 2006). Furthermore, based on two years of information, an additional project was started to transfer Sclerotinia stalk rot resistance from three diploid perennial species to HA 410 in 2007.

Hexaploid *H. californicus* was crossed with HA 410 in 2005 resulting in F_1 plants with 2n=68 chromosomes, which were backcrossed with HA 410 from BC_1F_1 through BC_4F_1. The chromosome numbers of the BC progeny were gradually reduced to 2n=34 (Table 1). As a result, their pollen fertility increased from 4.6, 31.3, and 38.5, to 73.9% in the BC_4F_1 generation, suggesting the continuing improvement of fertility as more *H. californicus* chromosomes were eliminated. Consistent with the improvement of pollen fertility, seed sets increased from 0.05% in BC_1F_1 up to 35.3% in BC_4F_1. It was noticed that the variation of pollen fertility was high among the BC progenies, for example, 4.6 to 62.1% in BC_2F_1, 5.0 to 95.6% in BC_3F_1, and 10 to 96.9% in BC_4F_1. This wide range of pollen fertility was expected primarily due to the variation in chromosome numbers of the individual BC progenies. Currently, of the 79 BC_4F_1 plants, 14 plants with 2n=34 have produced sufficient seed for field testing. Also, progenies derived from advanced backcross generations (BC_4) would be ideal genetic stocks for identifying chromosome segments of wild species in the cultivated background.

Table 1. Chromosome number, pollen fertility and seed set of F_1 and backcrossed progenies of *H. californicus* with HA 410 in 2005-2007.

	F_1	BC_1F_1	BC_2F_1	BC_3F_1	BC_4F_1
2n	68	50-53	40-49	35-44	34-40
Fertile pollen %	37.8	4.6 (0.9-10.2)	31.3 (4.6-62.1)	38.5 (5.0-95.6)	73.9 (10-96.9)
Seed set % (Seeds/florets)	2.71	0.05 (48/99,900)	3.35 (183/5,460)	11.9	35.3

However, the BC_1F_1 generation with 2n=51 had the most unbalanced genome relationship, obviously corresponding to the low backcross seed set. Since we only started to observe 2n=34 plants in the BC_4F_1 generation, it is obvious that deriving genes from hexaploid species often takes a long time, but the resulting germplasm will be much more of the cultivated type than that resulting from using other faster approaches. The disadvantage of this approach is less genetic variability at the 2n=34 stage for the selection of QTL as in the case for Sclerotinia resistance.

For interspecific amphiploids, a sufficient number of F_1 hybrids between the five amphiploids and HA 410 were produced in 2006 (Jan et al., 2006), which was followed with two more cycles of backcrosses with HA 410. The chromosome number, pollen fertility and seed set of crosses of interspecific amphiploids crossed with HA 410 and the backcrossed progenies are summarized in Table 2.

Table 2. Chromosome number, pollen fertility and seed set of F_1 and backcrossed progenies of interspecific amphiploids with HA 410 in 2006 and 2007.

	F_1			BC_1F_1			BC_2F_1
Parentage	2n	Fertile pollen (%)	×HA 410 seed set (%) (seeds/florets)	2n	Fertile pollen (%)	×HA 410 seed set (%) (seeds/florets)	2n
H. strumosus × P21 2n=102	68	89.4 (74.3-97.9)	19.7 (755/3,800)	49-51	26.0 (6.6-42.5)	1.9 (282/11,900)	34-41
H. grosseserratus × P21 2n=68	51	43.3 (2.4-72.8)	9.1 (165/1,818)	37-44	35.3 (6.2-84.6)	3.5 (77/4,240)	34-38
H. maximiliani × P21 2n=68	51	49.9 (2.4-66.3)	13.7 (711/5,190)	37-47	29.9 (2.4-70.9)	2.0 (97/7,920)	34-37
H. nuttallii 730 × P21 2n=68	51	29.7 (3.7-57.1)	1.1 (32/2,800)	36-43	41.0 (1.3-84.1)	8.3 (460/7,610)	34-37
H. divaricatus × P21)× (*H. grosseserratus* × P21) 2n=68	51	27.3 (1.0-48.4)	18.1 (835/4,620)	36-46	21.3 (1.4-85.1)	6.0 (434/6,020)	34-37

A total of 145 BC_2F_1 plants from five crosses between selected amphiploids and HA 410 were obtained. Because the amphiploids had a full set of 2n chromosomes from the cultivated sunflower, the elimination of the wild species chromosomes after each backcross was faster than that of the backcrosses of *H. californicus* x HA 410, and the 2n=34 progenies also had slightly higher pollen fertility and seed set (Table 1). After two backcross cycles, of the 145 BC_2F_1 plants, 47 plants had 2n=34 chromosomes and have produced sufficient seed for field testing. With continuous selection of target traits, amphiploids are expected to be extremely efficient in selecting the trait while eliminating the other undesirable wild species genes. As for the use of hexaploid wild species, the rapid elimination of wild species genes may prove amphiploids less efficient for transferring QTL.

For the diploid resistance source, *H. maximiliani*, *H. giganteus* and *H. grosseserratus* were used to pollinate NMS HA 89, and the resulting F_1 hybrids were obtained by rescuing the 5-day-old immature embryos on artificial medium as described by Feng et al. (2006). For the crosses of diploid perennials and HA 410, a total of 181 embryos were obtained from the interspecific crosses of NMS HA 89 with *H.*

maximiliani, *H. giganteus*, and *H. grosseserratus*, respectively (Table. 3). By using embryo rescue, 67 hybrid seedlings were established in the greenhouse, suggesting that the interspecific hybridization using wild species as the pollen donor was successful. Pollen fertility of the F_1 hybrids from NMS HA 89 crossed by diploid wild perennials was very low (around 1%) (Table 3). Consequently, only 155 BC_1F_1seeds were produced from 64,618 florets pollinated with HA 410. This result was consistent with the conclusion that diploid perennial species could be crossed with cultivated sunflower, but the frequency of successful crosses was low (Atlagić et al., 1995). The extremely low backcross seed set of the F_1 plants is the most limiting stage for transferring genes from diploid perennials. However, since the F_1 plants are generally perennial, sufficient BC_1F_1 seeds can be obtained by repeated pollination. The forced chromosome pairing between the cultivated and the wild diploid perennials will promote chromosome recombination and result in BC_1F_1 plants with a large number of wild species traits for the selection of QTL.

Table 3. Pollen fertility of F_1s between NMS HA 89 and wild diploid *H. maximiliani*, *H. giganteus* and *H. grosseserratus,* and backcross seed set with HA 410 in 2007.

Parentage	No. F_1 embryo/florets	No. seedlings	Fertile pollen %	BC seeds/florets
NMS HA 89 × *H. maximiliani*	10/8083	9	1.7 (0-1.7)	21/11408
NMS HA 89 × *H. giganteus*	23/5480	15	0.6 (0.3-0.8)	26/6750
NMSHA89 × *H. grosseserratus*	148/14200	43	1.0 (0-1.6)	108/46460
Total	181/ 27763	67	--	155/ 64618

In conclusion, potential interspecific pre-breeding Sclerotinia resistance lines from diploid, tetraploid and hexaploid germplasm have been produced during the past three years. Evaluation of these pre-breeding lines for their reaction to Sclerotinia stalk rot will verify the effectiveness of each approach for the selection of QTLs. The effectiveness of using each of the above approaches will also be verified by tracking of the wild species' specific molecular markers in progeny plants when they first reach the 2n=34 stage and are ready for seed increase for the field evaluation. Ultimately, we expect to identify and release germplasms with improved resistance to Sclerotinia stalk rot within the shortest time period possible.

***Insects*.** North America has the greatest problems with insect pests because the insect pests of sunflower have co-evolved with their native sunflower hosts in natural communities. In the major production area of North America, there are about 15 principal insect pests of cultivated sunflower, and of this total about six are considered of major importance as potential economic pests from year to year (Charlet and Brewer, 1997). The insects of main concern include: the sunflower beetle, the sunflower stem weevil, the red and gray seed weevils [*Smicronyx fulvus* (LeConte), and *S. sordidus* (LeConte)], the banded sunflower moth, *Cochylis hospes* Walsingham, the sunflower moth, *Homoeosoma electellum* (Hulst), and the sunflower midge, *Contarinia schulzi* Gagne.

Host-plant resistance is a pest management method that utilizes the plant's own defense mechanisms against the insect. Since wild sunflower are native to North America where their associated herbivores and entomophages co-evolved, there is an opportunity to search for insect resistance genes in the diverse wild species. Sunflower moth tolerance was observed in annual *H. petiolaris* and perennials *H. maximiliani*, *H. ciliaris* DC., *H. strumosus*, and *H. tuberosus* (Rogers et al., 1984). Stem weevil tolerance was found in perennials *H. grosseserratus*, *H. hirsutus*, *H. maximiliani*, *H. pauciflorus*, *H. salicifolius* Dietr., and *H. tuberosus* (Rogers and Seiler, 1985). Sunflower beetle tolerance was observed in annuals *H. agrestis* Pollard and *H. praecox,* and in perennials *H. grosseserratus*, *H. pauciflorus*, *H. salicifolius*, and *H. tuberosus* (Rogers and Thompson, 1978; 1980). Charlet and Seiler (1994) found indications of resistance to the red sunflower seed weevil in several native *Helianthus* species.

Interspecific germplasm using wild species as resistance sources have been created. In preliminary testing, Charlet et al. (2004) noted that germplasm derived from *H. petiolaris* had the lowest number of stem weevils. Among material tested in a banded sunflower moth evaluation nursery, germplasm derived from *H. praecox* subsp. *hirtus* had less than 2% damage. Germplasm that incorporated *H. strumosus* and *H. tuberosus* had very little red sunflower seed weevil damage in test plots. Breeding populations of promising germplasms are being developed for further testing.

Oil and oil quality. Variability for oil concentration exists in the wild species. Annual *H. anomalus* has the highest oil concentration of 460 g/kg, the highest ever observed in a wild sunflower species, followed by *H. niveus* (Benth.) Brandegee subsp. *canescens* with 402 g/kg, *H. petiolaris* with 377 g/kg, and *H. deserticola* Heiser with 343 g/kg (Seiler, 2007). Perennial *H. salicifolius* had a concentration of 370 g/kg (Seiler, 1985; Seiler and Brothers, 2003). Cultivated sunflower generally contains 450 to 470 g/kg. Reduced concentrations of saturated palmitic and stearic fatty acids have been observed in a population of wild *H. annuus* that had a combined palmitic and stearic acid concentration of 58 g/kg (Seiler, 1998). This is 50% lower than in oil of cultivated sunflower. A combined palmitic and stearic acid concentration of 65 g/kg was observed in a wild perennial species, *H. giganteus* L. (Seiler, 1998).

Salt and drought tolerance. Several species of *Helianthus* are native to salt-impacted habitats. Interspecific germplasm derived from *H. paradoxus* Heiser has been identified with high salt tolerance, withstanding salt concentrations up to EC 24.7 d/Sm. It appears that one major gene controls salt tolerance, although a modifier gene may also be present, possibly recessive in control (Miller, 1995). Two salt-tolerant parental oilseed maintainer lines, HA 429 and HA 430, have been released (Miller and Seiler, 2003). Blanchet and Gelfi (1980) evaluated stomatal resistance, leaf-water potential, photosynthetic activity, leaf structure, and number of stomata. They concluded that *H. argophyllus* is the best candidate source for drought tolerance genes because its pubescent leaves reflect sunlight, reduce water loss, and exhibit low transpiration rates. *Helianthus niveus* subsp. *canescens* was their second choice.

Herbicide tolerance. A wild population of annual *H. annuus* from a soybean field in Kansas that had been repeatedly treated with imazethapyr for seven consecutive years developed resistance to the imidazolinone and sulfonylurea herbicides (Al-Khatib et al., 1998). Resistance to imazethapyr and imazamox herbicides has great potential for producers in all regions of the world for controlling several broadleaf weeds. Several populations of wild sunflower (*H. annuus* and *H. petiolaris*) from the USA and Canada have been screened for resistance to these two herbicides. Eight percent of 50 wild sunflower populations had some resistance to imazamox and 57% had some resistance to tribenuron in the central U.S (Olson et. al., 2004). In Canada, 52% of 23 wild *H. annuus* populations had some resistance to tribenuron (Miller and Seiler, 2005). Genetic stocks IMISUN-1 (oil maintainer), IMISUN-2 (oil restorer), and IMISUN-3 (confection maintainer) have been developed and released (Al-Khatib and Miller, 2000). Miller and Al-Khatib (2002) also released one oilseed maintainer and two fertility restorer breeding lines with imidazolinone herbicide resistance. Genetic stocks SURES-1 and SURES-2 with resistance to the sulfonylurea herbicide tribenuron have been developed and released by Miller and Al-Khatib (2004). Additionally, two oilseed germplasm lines, HA 442 and RHA 443 have been released with imidazolinone resistance (Miller et al., 2006). The imidazolinone and sulfonylurea herbicides may control broomrape in areas of the world where this parasitic weed attacks sunflower (Alonso et al., 1998).

CONCLUSIONS AND PROSPECTS

Significant progress has been made in increasing the number of accessions in the wild sunflower species collection to preserve the wild species and increase the available genetic diversity for improvement of the crop. Interspecific gene transfer for sunflower improvement has been practiced since the very early years by breeders in the FSU and it has continued to play a key role as the crop developed into a major global oilseed crop. Recent advances in culturing of otherwise abortive interspecific hybrid embryos have proved to be highly effective for making the difficult-to-cross wild perennial *Helianthus* species crosses widely available for breeding purposes, either for specific major gene transfer or for the transfer of quantitative trait genes. Significant results have been reported on the germplasm development with regard to resistance to new races of downy mildew, rust, broomrape and other major diseases. In addition, new CMS and corresponding fertility restoration genes have been continuously identified and established, together with new genes helping to improve oil quality, herbicide resistance, and salt and drought tolerance. Thus far, only a small portion of the available genetic diversity of the wild *Helianthus* species

has been used globally. As a whole, there is no doubt that wild *Helianthus* species will continue to provide new genetic variability to the sunflower breeding community, helping to maintain sunflower as a viable major global oilseed crop.

REFERENCES

Acimovic, M. 1984. Sunflower diseases in Europe, the United States, and Australia, 1981-1983. Helia 7:45-54.

Akhtouch, B., J. Muñoz-Ruz, J.M. Melero-Vara, J.M. Fernández-Martínez, and J. Domínguez. 2002. Inheritance to race F of broomrape in sunflower lines of different origins. Plant Breed. 121:266-268.

Alexander, P. 1969. Differential staining of aborted and non-aborted pollen. Stain Technol. 44:117-122.

Al-Khatib, K., and J.F. Miller. 2000. Registration of four genetic stocks of sunflower resistant to imidazolinone herbicides. Crop Sci. 40:869-870.

Al-Khatib, K., J.R. Baumgartner, D.E. Peterson, and R.S. Currie. 1998. Imazethapyr resistance in common sunflower (*Helianthus annuus*). Weed Sci. 46:403-407.

Alonso, L.C., M.I. Rodríguez-Ojeda, J. Fernández-Escobar, and G. Lopez-Calero. 1998. Chemical control of broomrape (*Orobanche cernua* Loefl.) in sunflower (*Helianthus annuus* L.) resistant to imazethapyr herbicide. Helia 21:45-54.

Atlagić, J. 1990. Pollen fertility in some *Helianthus* wild species and their F_1 hybrids with the cultivated sunflower. Helia 13:47-54.

Atlagić, J., B. Dozet, and D. Škorić. 1993. Meiosis and pollen viability in *Helianthus tuberosus* L. and its hybrids with cultivated sunflower. Plant Breed. 111:318-324.

Atlagić, J., B. Dozet, and D. Škorić. 1995. Meiosis and pollen grain viability in *Helianthus mollis*, *Helianthus salicifolius*, *Helianthus maximiliani* and their F_1 hybrids with cultivated sunflower. Euphytica 81:259-263.

Blanchet, R., and N. Gelfi. 1980. Physiologie végétale caracteres xérophytiques de quelques especes d'*Hélianthus* susceptibles d'etre utilisés pour ameliorer l'adaptation aux conditions seches du tournesol cultivé (*Helianthus annuus* L.). CR. Acad. S.C. Paris 290:279-282.

Block, C. 1992. Screening for Alternaria head blight resistance in the USDA germplasm collection. p. 64-66. In: Proc. 14th Sunflower Res. Workshop. Nat. Sunfl. Assoc., Bismarck, ND, USA.

Cerboncini, C., G. Beine, P.C. Binsfeld, B. Dresen, H. Peisker, A. Zerwas, and H. Schnabl. 2002. Sources of resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary in the natural *Helianthus* gene pool. Helia 25:167-176.

Chandler, J.M. 1991. Chromosome evolution in sunflower. p. 229-249. In: T. Tsuchiya and P. K. Gupta (eds), Chromosome Engineering in Plants: Genetics, Breeding, Evolution, Part B. Elsevier Science Publishers B.V., Amsterdam, The Netherlands.

Chandler, J.M., and B.H. Beard. 1983. Embryo culture of *Helianthus* hybrids. Crop Sci. 23:1004-1007.

Charlet, L.D., and G.J. Brewer. 1997. Management strategies for insect pests of sunflower in North America. Recent Res. Devel. Entomol. 1:215-229.

Charlet, L.D., and G.J. Seiler. 1994. Sunflower seed weevils (Coleoptera:Curculionidae) and their parasitoids from native sunflowers (*Helianthus* spp.) in the Northern Great Plains. Ann. Entomol. Soc. Am. 87:831-835.

Charlet, L.D., J.F. Miller, and G.J. Seiler. 2004. Evaluation of sunflower for resistance to stem and seed insect pests in North America. p. 861-866. In: G.J. Seiler (ed.), Proc. 16th Int. Sunflower Conf., Fargo, ND, USA.

Christov, M., L. Nikolova, and T. Djambasova. 2001. Evaluation and use of wild *Helianthus* species grown in the collection of Dobroudja Agricultural Institute, General Toshevo, Bulgaria for the period 1999-2000. p. 30-31. In: G. J. Seiler (ed.), FAO Sunflower Subnetwork Progress Report 1999-2000. FAO, Rome, Italy.

Cuk, L. 1982. The uses of wild species in sunflower breeding. J. Edible Oil Industry 1:23-27.

Cuk, L., and G.J. Seiler. 1985. Collection of wild sunflower species. A collection trip in the USA. Zbornik-Radova 15:283-289.

Dewey, D.R. 1980. Some applications and misapplications of induced polyploidy to plant breeding. p. 445-470. In: W.H. Lewis (ed.), Polyploid: Biological Relevance. Plenum Pub. Corp., New York, USA.

Domínguez, J., J.M. Melero-Vara, J. Ruso, J.F. Miller, and J.M. Fernández-Martínez. 1996. Short communication: Screening for resistance to broomrape (*Orobanche cernua*) in cultivated sunflower. Plant Breed. 115:201-202.

Dozet, B.M. 1990. Resistance to *Diaporthe/Phomopsis helianthi* Munt.-Cvet. et al. in wild sunflower species. p. 86-88. In: Proc. 12th Sunflower Res. Workshop, Nat. Sunfl. Assoc., Bismarck, ND, USA.

Enns, H., D.G. Dorrell, J.A. Hoes, and W.O. Chubb. 1970. Sunflower research, a progress report. p. 162-167. In: Proc. 4th Int. Sunflower Conf., Memphis, TN, USA.

Espinasse A., C. Lay, and C.D. Dybing. 1985. Factors controlling in vitro development of sunflower embryos. Agronomie 5:825-832.

Feng J.H., G.J. Seiler, T.J. Gulya, and C.C. Jan. 2006. Development of Sclerotinia stem rot resistant germplasm utilizing hexaploid *Helianthus* species. In: Proc. 28th Sunflower Res. Workshop, Fargo, ND, USA. Nat. Sunfl. Assoc., Bismarck, ND, USA. http://www.sunflowernsa.com/research/research-workshop/ documents/ Feng_Sclerotinia_06.pdf.

Fernández-Martínez, J.M., J.M. Melero-Vara, J. Muñoz-Ruz, J. Ruso, and J. Domínguez. 2000. Selection of wild and cultivated sunflower for resistance to a new broomrape race that overcomes resistance to *Or5* gene. Crop Sci. 40:550-555.

Fernández-Martínez, J.M., B. Pérez-Vich, B. Akhtouch, L. Velasco, J. Muñoz-Ruz, J.M. Melero-Vara, and J. Domínguez. 2004. Registration of four sunflower germplasms resistant to race F of broomrape. Crop Sci. 44:1033-1034.

Fick. G.N., and J.F. Miller. 1997. Sunflower Breeding. p. 395-439. In: A. Schneiter (ed.), Sunflower Technology and Production. ASA, CSSA, and SSSA, Madison, WI, USA.

Georgieva-Todorova, Y. 1983. Hybridization between *Helianthus annuus* L. (2*n*=34) and *Helianthus resinosus* Small (2*n*=102). C. R. Acad. Bulg. Sci. 36:963-966.

Georgieva-Todorova, J. 1984. Interspecific hybridization in the genus *Helianthus* L. Z. Pflanzenzucht. 93:265-279.

Heiser, C.B., and D.M. Smith. 1964. Species crosses in *Helianthus*: II. Polyploid species. Rhodora 66:344-358.

Heiser, C.B., D.M. Smith, S.B. Clevenger, and W.C. Martin. 1969. The North American sunflowers (*Helianthus*) Mem. Torr. Bot. Club 22:1-219.

Hu, X., D. Bidney, N. Yalpani, J.P. Duvick, O. Crasta, O.P. Folkerts, and G. Lu. 2003. Over expression of a gene encoding hydrogen peroxide-generating oxalate oxidase evokes defense responses in sunflower. Plant Physiol. 133:170-181.

International Board for Plant Genetic Resources (IBPGR). 1984. Report of a Working Group on Sunflowers (First Meeting) Int. Board for Plant Genetic Resources, Rome, Italy.

Jan, C.C. 1988. Chromosome doubling of wild x cultivated sunflower interspecific hybrids and its direct effect on backcross success. p. 287-292. In: Proc. 12th Int. Sunflower Conf., Novi Sad, Yugoslavia.

Jan, C.C. 1991. Cytoplasmic male sterility and fertility restoration of two wild *Helianthus annuus* accessions. p. 100. Agron. Abstr., ASA, Madison, WI, USA.

Jan, C.C. 1992. Cytoplasmic-nuclear gene interaction for plant vigor in *Helianthus* species. Crop Sci. 32:320-323.

Jan, C.C. 2003. Transfer of vigor restoration gene from *Helianthus giganteus* into cultivated background and its implication on gene transfer utilizing interspecific amphiploids. p. S202. In: 6th European Conf. Sunflower Biotech., Seville, Spain.

Jan, C.C. 2004. A new CMS source from *Helianthus giganteus* and its fertility restoration genes from interspecific amphiploids. p. 709-712. In: G.J. Seiler (ed.), Proc. 16th Int. Sunflower Conf., Fargo, ND, USA.

Jan, C.C., and J.M. Chandler. 1985a. Transfer of powdery mildew resistance from *Helianthus debilis* Nutt. into cultivated sunflower (*H. annuus* L.). Crop Sci. 25:664-666.

Jan, C.C., and J.M. Chandler. 1985b. Interspecific hybrids of diploid perennials x cultivated *Helianthus annuus* L. p. 58. Agron. Abstr., ASA, Madison, WI, USA.

Jan, C.C., and J.M. Chandler. 1988. Registration of powdery mildew resistant sunflower germplasm pool, PM 1. Crop Sci. 28:1040.

Jan, C.C., and J.M. Chandler. 1989. Interspecific hybrids and amphiploids of *Helianthus annuus* x *H. bolanderi*. Crop Sci. 29:643-646.

Jan, C.C., and J.M. Fernández-Martinez. 2002. Interspecific hybridization, gene transfer, and the development of resistance to broomrape race F in Spain. Helia 25:123-136.

Jan, C.C., and T.X. Zhang. 1995. Interspecific gene transfer from tetraploid *Helianthus hirsutus* into cultivated sunflower. p. 48-49. In: Proc. 17th Sunflower Res. Workshop, Natl. Sunfl. Assoc., Bismarck, ND, USA.

Jan, C.C., J.M. Fernández-Martínez, J. Ruso, and J. Muñoz-Ruz. 2002. Registration of four sunflower germplasms with resistance to *Orobanche cumana* race F. Crop Sci. 42:2217-2218.

Jan, C.C., Z. Quresh, and T.J. Gulya. 2004a. Registration of seven rust resistant sunflower germplasms Crop Sci. 44:1887-1888.

Jan, C.C., A.S. Tan, and T.J. Gulya. 2004b. Registration of four downy mildew resistant sunflower germplasms. Crop Sci. 44:1887.

Jan C.C., J.H. Feng, G.J. Seiler, and T.J. Gulya. 2006. Amphiploids of perennial *Helianthus* species x cultivated sunflower possess valuable genes for resistance to Sclerotinia stem and head rot. In: Proc. 28th Sunflower Res. Workshop, Fargo, ND, USA. Nat. Sunfl. Assoc., Bismarck, ND, USA. http://www.sunflowernsa.com/ research/research-workshop/documents/Jan amphiploids_06.pdf

Kinman, M.L. 1970. New developments in the USDA and state experiment station sunflower breeding programs. p. 181-183. In: Proc. 4th Int. Sunflower Conf., Memphis, TN, USA.

Köhler, R.H., and W. Friedt. 1999. Genetic variability as identified by AP-PCR and reaction to mid-stem infection of *Sclerotinia sclerotiorum* among interspecific sunflower (*Helianthus annuus* L.) hybrid progenies. Crop Sci. 39:1456:1463.

Kräuter, R., A. Steimetz, and W. Friedt. 1991. Efficient interspecific hybridization in the genus *Helianthus* via "embryo rescue" and characterization of the hybrids. Theor. Appl. Genet. 82:521-525.

Kurnik, E., and I. Walcz. 1985. *Diaporthe helianthi* on sunflower in Hungary and the result of breeding for resistance. p. 603. In: Proc. 11th Int. Sunflower Conf., Mar del Plata, Argentina.

Leclercq, P. 1969. Cytoplasmic male sterility in sunflower. Ann. Amelior. Plant 19:99-106.

Lipps, P.E., and L.L. Herr. 1986. Reactions of *Helianthus annuus* and *H. tuberosus* plant introductions to *Alternaria helianthi*. Plant Dis. 70:831-835.

Mihaljcevic, M., M. Muntanola-Cvetkovic, and M. Petrov. 1982. Further studies on the sunflower disease caused by Diaporthe (*Phomopsis helianthi*) and possibilities of breeding for resistance. p. 57. In: Proc. 10th Int. Sunflower Conf., Surfer's Paradise, Australia.

Miller, J.F. 1995. Inheritance of salt tolerance in sunflower. Helia 18:9-16.

Miller, J.F., and K. Al-Khatib. 2002. Registration of imidazolinone herbicide-resistant sunflower maintainer (HA 425) and fertility restorer (RHA 426 and RHA 427) germplasms. Crop Sci. 42:988-989.

Miller, J.F., and K. Al-Khatib. 2004. Registration of two oilseed sunflower genetic stocks, SURES-1 and SURES-2 resistant to tribenuron herbicide. Crop Sci. 44:1037.

Miller, J.F., and J. Domínguez. 2000. Registration of seven *Orobanche* resistant sunflower germplasms. Crop Sci. 40:588.

Miller, J. F., and T.J. Gulya. 1988. Registration of six downy mildew resistant sunflower germplasm lines. Crop Sci. 28:1040-1041.

Miller, J.F., and T.J. Gulya. 1999. Registration of eight Sclerotinia-tolerant sunflower germplasm lines. Crop Sci. 39:301-302.

Miller, J.F., and G.J. Seiler. 2003. Registration of five oilseed maintainer (HA 429-HA 433) sunflower germplasm lines. Crop Sci. 43:2313-2314.

Miller, J.F., and G.J. Seiler. 2005. Tribenuron resistance in accessions of wild sunflower collected in Canada. In: Proc. 27th Sunflower Res. Workshop, Nat. Sunfl. Assoc., Bismarck, ND, USA. http://www.sunflowernsa.com/research/research-workshop/documents/miller_tribenuron_ 05.pdf.

Miller, J.F., T.J. Gulya, and G.J. Seiler. 2002. Registration of five fertility restorer sunflower germplasms. Crop Sci. 42:989-991.

Miller, J.F., T.J. Gulya, and B.A. Vick. 2006. Registration of imidazolinone herbicide-resistant maintainer (HA 442) and fertility restorer (RHA 443) oilseed sunflower germplasm. Crop Sci. 46:483

Mondolot-Cosson, L., and C. Andary. 1994. Resistance factors of wild species of sunflower, *Helianthus resinosus* to *Sclerotinia sclerotiorum*. Acta Hort. 381:642-645.

Morris, J.B., S.M. Yang, and L. Wilson. 1983. Reaction of *Helianthus* species to *Alternaria helianthi*. Plant Dis. 67:539-540.

Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant 15:473-497.

Olson, B., K. Al-Khatib, and R.M. Aiken. 2004. Distribution of resistance to imazamox and tribenuron-methyl in native sunflowers. In: Proc. 26th Sunflower Res. Workshop, Nat. Sunfl. Assoc., Bismarck, ND, USA. http://www.sunflowernsa.com/research /research -workshop/ documents/ 158.pdf.

Quresh, Z., and C.C. Jan. 1993. Allelic relationships among genes for resistance to sunflower rust. Crop Sci. 33: 235-238.

Quresh, Z., C.C. Jan, and T.J. Gulya. 1993. Resistance of sunflower rust and its inheritance in wild sunflower species. Plant Breed. 110:297-306.

Rashid, K.Y., and G.J. Seiler. 2004. Epidemiology and resistance to Sclerotinia head rot in wild sunflower species. p. 751-754. In: G.J. Seiler (ed.), In: Proc. 16th Int. Sunflower Conf., Fargo, ND, USA.

Rogers, C.E., and G.J. Seiler. 1985. Sunflower (*Helianthus*) resistance to stem weevil (*Cylindrocopturus adspersus*). Environ. Entomol. 14:624-628.

Rogers, C.E., and T.E. Thompson. 1978. Resistance in wild *Helianthus* to the sunflower beetle. J. Econ. Entomol. 71:622-623.

Rogers, C.E., and T.E. Thompson. 1980. *Helianthus* resistance to the sunflower beetle. J. Kansas Entomol. Soc. 53:727-730.

Rogers, C.E., T.E. Thompson, and G.J. Seiler. 1984. Registration of three *Helianthus* germplasms for resistance to the sunflower moth. Crop Sci. 24:212-213.

Ronicke, S., V. Hahn, R. Horn, I. Grone, L. Brahn, H. Schnabl, and W. Freidt. 2004. Interspecific hybrids of sunflower as sources of Sclerotinia resistance. Plant Breed. 123:152-157.

Ruso, J., S. Sukno, J. Domínguez -Giménez, J.M. Melero-Vara, and J.M. Fernández-Martínez. 1996. Screening wild *Helianthus* species and derived lines for resistance to several populations of *Orobanche cernua*. Plant Dis. 80:1165-1169.

Sackston, W.E. 1981. The sunflower crop and disease: Progress, problems, prospects. Plant Dis. 65:643-648.

Saliman, M., S.M. Yang, and L. Wilson. 1982. Reaction of *Helianthus* species to *Erysiphe cichoracearum*. Plant Dis. 66:572-573.

Schilling, E.E. 2006. *Helianthus*. Vol. 21:141-169. In: Flora of North America Editorial Committee (eds), Flora of North America North of Mexico. Oxford University Press, New York and Oxford.

Scelonge, C., L. Wang, D. Bidney, G. Lu, C. Hastings, G. Cole, M. Mancl, J-L. D'Hautefeuille, C. Sosa-Domínguez, and S. Coughlan. 2000. Transgenic Sclerotinia resistance in sunflower (*Helianthus annuus*). p. K66-K71. In: Proc. 15th Int. Sunflower Conf., Toulouse, France.

Seiler, G.J. 1985. Evaluation of seeds of sunflower species for several chemical and morphological characteristics. Crop Sci. 25:183-187.

Seiler, G.J. 1991a. Registration of six interspecific sunflower germplasm lines derived from wild perennial species. Crop Sci. 31:1097-1098.

Seiler, G.J. 1991b. Registration of 15 interspecific sunflower germplasm lines derived from wild annual species. Crop Sci. 31:1389-1390.

Seiler, G.J. 1991c. Registration of 13 downy mildew tolerant interspecific sunflower germplasm lines derived from wild annual species. Crop Sci. 31:1714-1716.

Seiler, G.J. 1993. Registration of six interspecific germplasm lines derived from wild perennial sunflower. Crop Sci. 33:1110-1111.

Seiler, G.J. 1998. The potential use of wild *Helianthus* species for selection of low saturated fatty acids in sunflower oil. p. 109-110. In: A.M. de Ron (ed.), International Symposium on Breeding of Protein and Oil Crops. EUCARPIA, Pontevedra, Spain.

Seiler, G.J. 2007. Wild annual *Helianthus anomalus* and *H. deserticola* for improving oil content and quality in sunflower. Ind. Crop. Prod. 25:95-100.

Seiler, G.J., and M.E. Brothers. 2003. Exploration for wild *Helianthus anomalus* and *H. deserticola* in the desert southwest USA. In: Proc. 25th Sunflower Res. Workshop, Nat. Sunfl. Assoc., Bismarck, ND, USA. http://www.sunflowernsa.com/research/research_workshop/ documents /90.pdf.

Serieys, H. 1992. Sunflower: A catalogue of the wild species of the genus *Helianthus*, ENSAM and INRA, Montpellier, France.

Serieys, H. 1994. Report on the past activities of the FAO working group "Identification, study and utilization in breeding programs of new CMS sources for the period 1991-1993." Helia 17:93-102.

Serieys, H. 2002. Report on the past activities of the FAO Working Group "Identification, Study and Utilization in Breeding Programs of New CMS Sources" for the Period 1999-2001. p. 1-54. FAO, Rome.

Škorić, D. 1985. Sunflower breeding for resistance to *Diaporthe/Phomopsis helianthi* Munt.-Cvet. et al. Helia 8: 21-23.

Škorić, D. 1992. Achievements and future directions of sunflower breeding. Field Crop. Res. 30:195-230.

Tan, A.S., C.C. Jan, and T.J. Gulya. 1992. Inheritance of resistance to race 4 of sunflower downy mildew in wild sunflower accessions. Crop. Sci. 32:949-952.

Vrânceanu, A.V., and M. Iuoras. 1988. Hibridul interspecific dintre *Helianthus rigidus* Desf. si floarea-soarelui cultivata (*H. annuus* L.). Probl. Genet. Teor. Aplic. 2:109-119.

Vrânceanu, A.V., and F.M. Stoenescu. 1971. Pollen fertility restorer genes from cultivated sunflower. Euphytica 20:536-541.
Whelan, E.D.P. 1978. Hybridization between annual and perennial diploid species of *Helianthus*. Can. J. Genet. Cytol. 20:523-530.
Whelan, E.D.P. 1979. Interspecific hybrids between *Helianthus petiolaris* Nutt. and *H. annuus* L.: Effect of backcrossing on meiosis. Euphytica 28:297-308.
Whelan, E.D.P. 1980. A new source of cytoplasmic male sterility in sunflower. Euphytica 29:33-46.
Whelan, E.D.P. 1981. Cytoplasmic male sterility in *Helianthus giganteus* L. X *H. annuus* L. interspecific hybrids. Crop Sci. 21:855-858.
Whelan, E.D.P. 1982. Trisomic progeny from interspecific hybrids between *Helianthus maximiliani* and *H. annuus*. Can. J. Genet. Cytol. 24:375-384.
Whelan, E.D.P., and D.G. Dorrell. 1980. Interspecific hybrids between *Helianthus maximiliani* Schrad. and *H. annuus* L.: Effect of backcrossing on meiosis, anther morphology, and seed characteristics. Crop Sci. 20:29-34.
Yang, S.M., J.B. Morris, and T.E. Thompson. 1980. Evaluation of *Helianthus* spp. for resistance to *Rhizopus* head rot. p.147-151. In: Proc. 9th Int. Sunflower Conf., Torremolinos, Spain.
Zimmer, D. E., and J.A. Hoes. 1978. Diseases. p. 225-262. In: J.F. Carter (ed.), Sunflower Science and Technology. Agron. Monogr. 19. CSSA, ASA, and SSSA, Madison, WI.

Current advances in sunflower oil applications

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ABSTRACT

The fatty acid and triglyceride molecular species of a given oil determine its physical, chemical, and nutritional properties. Thus, applications for a specific oil depend mainly on its fatty acid composition and the way in which the fatty acids are arranged in the glycerol backbone. Minor components, such as tocopherols, could also modify oil properties, such as thermo-oxidative resistance. Sunflower seed commodity oils predominantly contain linoleic and oleic fatty acids, with lower contents of palmitic and stearic acids. High-oleic sunflower oil, which could actually be considered as a commodity oil, contains oleic acid of up to around 90%. New sunflower varieties with different fatty acids and tocopherols compositions have been selected. Due to these modifications, they possess new properties and are much better adapted for direct home consumption, food industry and other applications such as biolubricants and biodiesel production.

Key words: biodiesel – fatty acids – oil quality – oxidative stability – tocopherols – triglycerides.

INTRODUCTION

Oils are mainly constituted by triglycerides, but also contain small quantities of diglycerides, polar lipids, tocopherols, free fatty acids, etc. Triglycerides, which account for more than 95% of total oil, consist of a glycerol molecule with three fatty acids esterified in the hydroxyl residues, one in the central position of the glycerol molecule and the other two at the terminal positions. The most common fatty acids forming these triglycerides in sunflower are: saturated palmitic and stearic acids, monounsaturated oleic acid and polyunsaturated linoleic acid. The final use of each type of oil is defined by both its physical and chemical characteristics, which depend on its fatty acids and triglyceride composition. For instance, the difference between oils and fats is due to the amount of their saturated fatty acids. Their thermo-oxidative stability depends mainly on the amount of polyunsaturated fatty acids they contain (oils with a high content of these unsaturated fatty acids are more unstable), and their content and type of tocopherols. Therefore, the performance of an oil for a specific use will depend on these characteristics. Considerable research efforts are being put into the following aspects. On the one hand, more stable sunflower oils are being obtained by increasing their content in monounsaturated fatty acids (oleic acid) and decreasing their content in polyunsaturated fatty acids (linoleic acid). These oils are also suitable for biolubricants. Their stability could also be increased by modifying their tocopherol content. On the other hand, healthy substitutes for animal, tropical or hydrogenated fats required by the food industry are being obtained by increasing their content in saturated fatty acids, mostly stearic, which does not modify the plasma cholesterol content.

DISCUSSION

Sunflower oils

Depending on their particular use, oils or fats must have a specific composition to fulfill the requirements of each application. Deep-frying, and other industrial processes for food preparation, require fats and oils with a high thermo-oxidative stability. In these applications, due to easy storage and pouring, oils are better than fats. For margarine, spreads, confectionery, and related products, fats with a certain degree of plasticity are required. For biolubricant production, oil liquid at temperatures below 0ºC with a good thermo-oxidative stability is required. Biodiesel production only requires a minimal stability and standard sunflower oils are equally as good as canola or other vegetable oils, but, probably for this application, palm oil is even better.

By lowering the content of unsaturated fatty acids or modifying minor components, such as tocopherols, the stability of oils could be enhanced, making them suitable for deep frying and biolubricant uses. Increasing the saturated fatty acids content will increase the proportion of solid fat and, therefore, its melting temperature. With the exception of animal fats, palm oil fractions and lauric oils, natural fats hardly fulfill the requirements of most industrial processes. Nevertheless, the above mentioned fats are considered unhealthy by many authors and by the World Health Organization (WHO, 2003) because of

their high content in palmitic, myristic and lauric fatty acids, so they have been substituted by hydrogenated vegetable oils. However, the hydrogenation process generates *trans* isomers of unsaturated fatty acids, which are also considered to be nutritionally undesirable. In general, dietary recommendations encourage the intake of unsaturated fatty acids, such as oleic and linoleic, and stearic as a saturated fatty acid (Kelly et al., 2001; Mensink, 2005; WHO, 2003).

Different sunflower lines with modifications in the fatty acid composition of their oils have been obtained (Table 1). Since the selection of the high-oleic mutant by Soldatov (1976), several new fatty acid mutants have been obtained by ionization, radiation or chemical mutagenesis, among them three independent high-palmitic lines, with around 30% of palmitic acid in their oils, two in standard high-linoleic background and another in high-oleic background (Ivanov et al., 1988; Osorio et al., 1995; Fernández-Martínez et al., 1997), and some high-stearic acid in high-linoleic background (Osorio et al., 1995; Fernández-Moya et al., 2005) have been obtained. Lines with high-stearic in high-oleic background were obtained later by recombination (Fernández-Moya et al., 2005). In spite of their higher saturated acid content, these sunflower oils have a low content of saturated fatty acid in the middle position of the triglyceride (Alvarez-Ortega et al., 1997), differentiating them completely from animal, palm and hydrogenated fats.

Table 1. Fatty acid composition of several sunflower oil mutant lines with modifications in their oils, compared to the standard sunflower oil.

Sunflower	Oil	Fatty acid composition (%)				
line	phenotype	16:0	16:1	18:0	18:1	18:2
Standard	Normal	7		6	29	58
HA-OL9[a]	High oleic	5		3	**90**	2
CAS-4[b]	Medium stearic	6		**12**	28	53
CAS-3[b]	High stearic	5		**26**	15	53
CAS-30[c]	High stearic	6		**30**	10	50
CAS-15[c]	High stearic-oleic	6		**24**	**62**	5
CAS-5[b, d]	High palmitic	**31**	5	3	12	48
CAS-12[e]	High palmitic-oleic	**32**	6	4	**54**	3

[a]Soldatov, 1976; Fernández-Martinez et al. 1993.
[b]Osorio et al. 1997.
[c]Fernández-Moya et al. 2005.
[d]Ivanov et al. 1988.
[e]Fernández-Martínez et al. 1997.

New research has been carried out to obtain fractions with improved properties from these oils. Thus, high stearic and oleic sunflower oils have been cold-fractionated to obtain stearin and olein fractions (Table 2). In this case, because of the unimpaired distribution of triglycerides species between the fractions, fatty acid composition analysis is not a satisfactory method to characterize them, and, instead, the triglyceride composition has to be determined. Table 2 shows the triglyceride subclasses of these fractions; the liquid olein fraction has mostly triunsaturated triglycerides, mainly OOO, (see Table 2 for abbreviations) and OOL, and monosaturated triglycerides, mainly EOO, and in a lesser amount POO and EOL, whereas the stearin has a higher content of disaturated triglycerides than the olein fraction, EOE and POE being the principal triglycerides. The melting properties of the stearin, measured as the solid content at different temperatures by differential scanning calorimetry are similar to cocoa butter.

Table 2. Triglyceride subclasses composition of the liquid fraction (olein) and solid fraction (stearin) obtained from high stearic and oleic sunflower oil[1].

Sunflower	Oil Fraction	TAG Types (%)		
		SUS	SUU	UUU
HEHO	Olein	3.1	56.7	40.2
HEHO	Stearin	73.3	17.9	8.8

[1]SUS, disaturated triglycerides; SUU, monosaturated triglycerides; and UUU, triunsaturated triglycerides. S, saturated; U, unsaturated; HE, high stearic acid; HO, high oleic acid.

Tocopherols, good antioxidant molecules, are one of the minor components of sunflower oil, with α-tocopherol, or vitamin E being the standard in commodity sunflower oil, new lines with modified profiles of tocopherols have been obtained (Table 3). These new lines have been obtained from germplasm of

wild and cultivated sunflower (Demurin, 1993; Velasco et al., 2004). The tocopherols accumulated in these lines mainly depend on modifications on the genes which control the biosynthetic pathway. The oils containing γ-tocopherol and δ-tocopherol have the advantage of a higher oxidative stability, but a reduced vitamin E content.

Table 3. Tocopherol composition of oils extracted from modified sunflower lines.

Oil Type	Tocopherol composition (%)			
	α-T	β-T	γ-T	δ-T
Standard α-T	95	4	1	0
Medium β-T[a]	50	50	0	0
High β-T[b]	75	25	0	0
High γ-T[a]	5	0	95	0
High δ-T[b]	5	0	30	65

[a]Demurin, 1993
[b]Velasco et al., 2004

Sunflower oil applications

Standard sunflower oil possesses good properties for low temperature and general food applications (salad dressings, emulsions, etc), but for high temperature applications and deep frying, oils with a lower content of polyunsaturated fatty acids are required, and these are the high-oleic oils. The oil properties at a high temperature also depend on the tocopherols, oils with a higher content of γ and δ-tocopherols being more stable than oils with α and β-tocopherols. Margarine and plastic fat production demands oils with high contents of saturated fatty acids such as palmitic or stearic acids, preferably stearic because of the unhealthy effect of palmitic acid, as stated in Kelly et al. (2001): "The food industry might wish to consider the enrichment of foods with stearic acid in place of palmitic acid and trans fatty acids".

Thermo-oxidative treatments to test oil stability are usually carried out at 180 °C for 10 h monitorizing the formation of polar and polymer compounds. TAG polymerization in the different oils increased with time (Fig. 1). In this regard, oils could be classified into three groups; standard oils, with a high content of polymerised TAGs, up to around 17% after 10 h treatment; high-oleic sunflower and palm olein oils with around 10% of polymerised TAGs after the same treatment; and the high-palmitic and high-oleic sunflower oil with only 6% of polymerised TAGs at the same time. This indicates that oils with the higher content of oleic and palmitic acids are the best for high temperature applications. Rejection levels of 12% of polymers have been recommended in current regulations for discarding used frying fats for human consumption (GFSR, 2000). As a result, commodity oils, soybean, canola and standard sunflower oils must be rejected after 8 h at 180ºC, while high-oleic sunflower could still be used after 10 h and the high-palmitic and high-oleic oil would be even further from rejection.

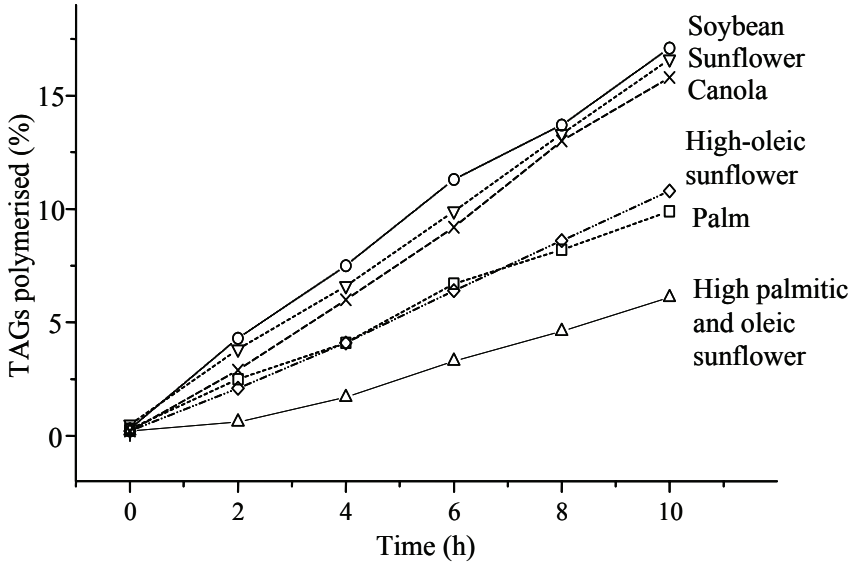

Fig. 1. TAG polymerization at 180ºC of oils of vegetal origin. Soybean, canola and sunflower are the standard commodity oils, palm is a commercial palm olein, and high-oleic and high-palmitic high-oleic sunflower oils are genetically modified sunflower oils (Marquez et al., 1999).

As stated above, tocopherols could also modify the thermo-oxidative stability of the oils. Fig. 2 shows the polymerised TAGs at 180ºC of genetically modify sunflower oils. Oils tested in this experiment were standard, high-oleic containing α-tocopherol, high-oleic and high-palmitic containing α-tocopherol and high-oleic and high-palmitic containing γ-tocopherol.

After 10 h at 180ºC, standard and high-oleic sunflower oils have 17.4% and 8.2% of polymerised TAGs, while the high-palmitic and oleic oils have only 2.3% and 1.4%. Furthermore, after 25 h of experiment, the polymerised TAGs were only 8.7 and 4% of polymerized TAGs and had less than 12% of polymers and were therefore still suitable for human consumption (Marmesat et al., 2008). These two high-palmitic oils have a very high oxidative stability and the oil with γ-tocopherol is the best as it always has less than half of the polymerised TAGs than the same oil with α-tocopherol and, even more, after 25 h it was less polymerised than the standard sunflower after 2 h, making this oil extremely stable.

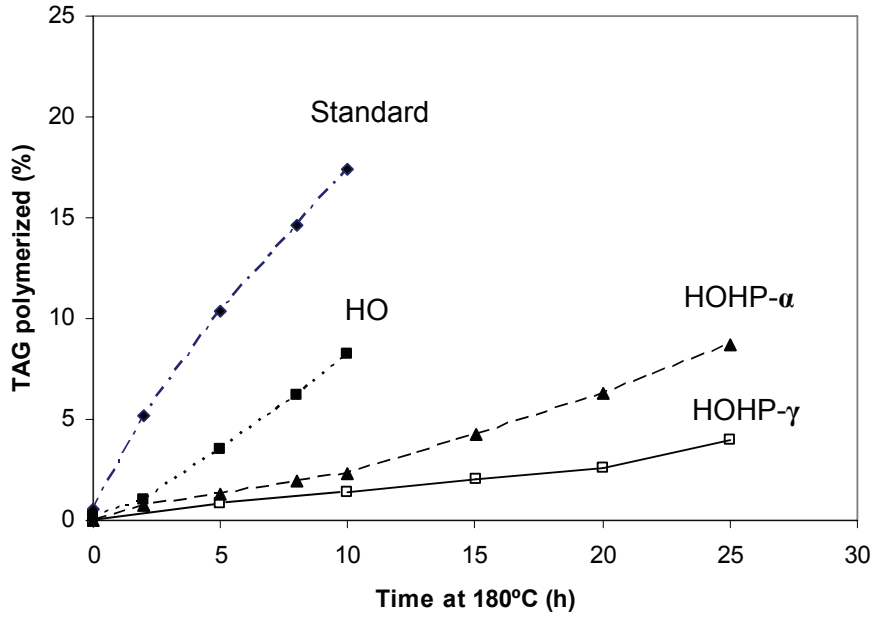

Fig. 2. TAG polymerization at 180ºC of standard sunflower oils compared with high-oleic oil (HO), high-oleic and high-palmitic oil containing α-tocopherol (HOHP-α), and high-oleic and high-palmitic oil containing γ-tocopherol (HOHP-γ).

High-stearic high-oleic sunflower oils, and also the liquid fraction obtained from them by cold fractionation, have good thermo-oxidative stabilities. These oils have a reduced content of polyunsaturated fatty acids, high content of oleic and some stearic acid. Experiments made to determine their oxidative stability have shown the total modified TAGs after 10 h at 180ºC of these oils in comparison with high-oleic and high-oleic and palmitic with different tocopherol contents (Table 4). Due probably to the different origin of oils and authors, differences were found in the data for standardising the results. The data presented here were corrected according to the results obtained with respect to the high-oleic oil present in all experiments, 1 being the value assigned to the high-oleic oil.

For oils with a high saturated content, which could be solid at relatively high temperatures, a new parameter must be defined, i.e. the cloud point which is the temperature at which the liquid became turbid. Oils with a cloud point of above 0ºC are difficult to transport and need special factory requirements. In winter during transport storage oil could become solid, and so good deep frying oils must have a good oxidative stability and be liquid, at least up to 0ºC. The high-stearic and high-oleic oils are very stable, but they are solid at room temperature, and the high-palmitic oils are also quite solid at temperatures of between 0 and 10ºC. High-oleic sunflower oil and olein fractions from HSHO sunflower are probably the best oils for deep frying, mainly if they contain γ-tocopherol instead of α-tocopherol.

Table 4. Total modified TAGs and cloud point of sunflower oils with different fatty acid and tocopherol compositions.

Fatty acids Phenotype	Total modified TAG High oleic = 1	Cloud point (ºC)
Standard	1.29	-8
High oleic	1.00	-8
High oleic and palmitic α tocopherol	0.61	6
High oleic and palmitic γ tocopherol	0.48	
High oleic and stearic	0.59	24
Oleine from HSHO[1]	0.76	-4

[1]High saturated, high oleic acid

For the elaboration of some food products the industry needs solid or semisolid fats, whose traditional sources have been animal and some tropical fats, such as palm and lauric oils (palm kernel and coconut). Studies in human health have demonstrated that these fats are unhealthy due to their elevated contents of medium and long chain saturated fatty acids (mainly myristic and palmitic acids). Their intake increases the plasma levels of LDL-cholesterol (bad cholesterol), which generates an increment in the risk of suffering cardiovascular diseases. The effect of fats on cholesterol levels depends on their fatty acid composition (Mensink et al., 2003). The relationship between plasma cholesterol levels and cardiovascular diseases is well-known. The ingested fatty acids modulate the lipoprotein levels (and therefore the type of cholesterol). In general, unsaturated fatty acids (oleic, linoleic, and linolenic acids) increase the HDL and diminish the LDL, and for that reason they are considered as being healthy. On the other hand, saturated fatty acids (lauric, myristic and palmitic) increase both the LDL and the HDL and therefore the ratio LDL/HDL. But stearic acid, in spite of being saturated, does not have any effect on the cholesterol content (Kelly et al., 2001; WHO, 2003; Mensink, 2005). In conclusion, the ingestion of stearic, oleic or linoleic acid does not modify the profile of lipoproteins (Thijssen and Mensink, 2005). The main reason for this is that stearic acid is transformed very quickly into oleic acid in the liver (Pearson, 1994).

To solve the problem regarding the use of hydrogenated vegetable fat, animal fat or tropical fats, a research project has been carried out with the aim of obtaining natural sunflower oils that could be used directly in the food industry for the production of margarine and similar products without the need of any chemical manipulation. New lines have been selected by classic methods, without the application of genetic engineering techniques, just the same as the high-oleic sunflower mutant. Sunflower lines with a high-stearic acid content together with oleic or linoleic acids are a healthy alternative to these unhealthy fats. In Table 4, the clouding point of some sunflower oils is shown. Among these, setting a good example, the high-stearic and oleic fat from sunflower has a clouding point of 24ºC, making it suitable for the manufacture of margarine, spreads, bakery and other products where a plastic fat is needed.

The triglyceride composition of these new oils is different to those of the standard sunflower oil, making them appropriate for industry demands (Table 5; Fig. 3). High-stearic lines contain a considerable percentage of triglycerides with two saturated fatty acid molecules, EOE and POE being the most abundant species in high-stearic high-oleic oils, and ELE and PLE those in the lines with a high-linoleic background. These triglycerides have linoleic or oleic acids in the central position of the triglyceride, which makes them appropriate for the production of margarines. With fats constituted by these types of triglycerides and keeping in mind the effect on the levels of cholesterol of these fatty acids, besides the fact that they do not contain saturated acids in the central position of the triglyceride, we can guarantee that a fully vegetable and healthy margarine can be manufactured for the first time with the healthy stearic acid (WHO, 2003) and no saturated fatty acids in the middle position of triglycerides (Renaud et al., 1995).

Table 5. Triglyceride subclasses composition of standard sunflower (RHA-274), high-oleic (CAS-9), high-stearic and high-linoleic (CAS-30) and high-stearic and high-oleic (CAS-15) oils. SUS, disaturated triglycerides; SUU, monosaturated triglycerides; and UUU, triunsaturated triglycerides. S, saturated; U, unsaturated fatty acids.

TAG type	RHA-274	CAS-30	CAS-9	CAS-15
SUS	1.8	29.0	0.9	18.4
SUU	30.7	57.0	21.5	61.9
UUU	67.5	13.8	77.5	19.1

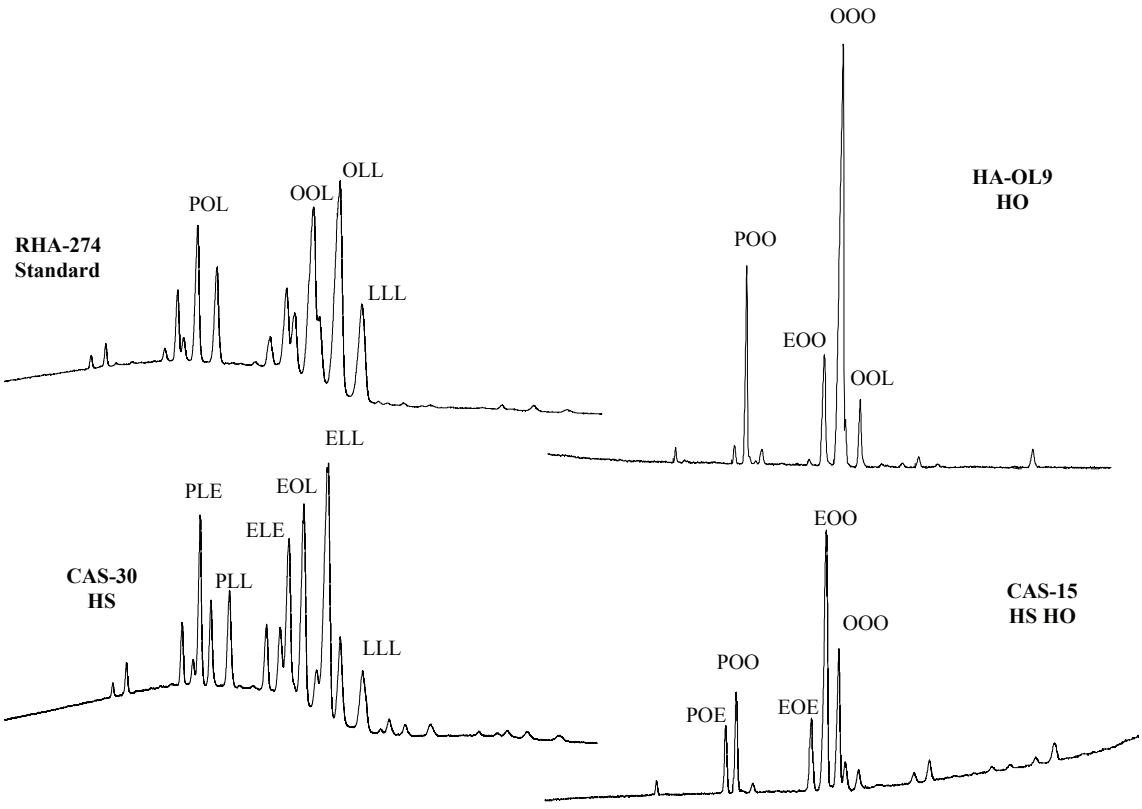

Fig. 3. Triglyceride chromatographs of standard RHA-274, high-oleic HA-OL9, high-stearic CAS-3 and high-stearic and oleic CAS-15 sunflower oils, showing the main triglycerides molecular species of each oil. P, palmitic; E, stearic;, O, oleic; and L, linoleic fatty acids.

To sum up, these new sunflower oils, with modified tocopherols and fatty acid composition, which were developed as a feedback for the food industry requirements to offer healthier products, together with the two others available nowadays (normal and high-oleic) could cover the requirements of the food industry without any chemical manipulation, with the aim of increasing the consumers' quality of life.

REFERENCES

Álvarez-Ortega, R., S. Cantisán, E. Martínez-Force, and R. Garcés. 1997. Characterization of polar and nonpolar seed lipid classes from highly saturated fatty acid sunflower mutants. Lipids 32:833-837.

Demurin, Y. 1993. Genetic variability of tocopherol composition in sunflower seeds. Helia 16:59–62.

Fernández-Martínez, J.M., J. Muñoz, and J. Gómez-Arnau. 1993. Performance of near-isogenic high and low oleic acid hybrids of sunflower. Crop Sci. 33:1158-1163.

Fernández-Martínez, J.M., M. Mancha, J. Osório, and R. Garcés. 1997. Sunflower mutant containing high levels of palmitic acid in high oleic background. Euphytica 97:113–116.

Fernandez-Moya, V., E. Martínez-Force, and R. Garcés. 2005. Oils from improved high stearic acid sunflower seeds. J. Agric. Food Chem. 53:532-533.
GSFR. 2000. German Society for Fat Research. Proceedings of the 3rd International Symposium of Deep-Fat Frying - Final Recommendations. Eur. J. Lipid Sci. Technol. 102:594.
Ivanov, P., D. Petakov, V. Nikolova, and E. Petchec. 1988. Sunflower breeding for high palmitic acid content in the oil. p. 463–465. In: Proc. 12th Int. Sunflower Conference, Novi Sad, Yugoslavia.
Kelly, F.D., A.J. Sinclair, N.J. Mann, A.H. Turner, L. Abedin, and D. Li. 2001. A stearic acid-rich diet improves thrombogenic and atherogenic risk factor profiles in healthy males. Eur. J. Clin. Nut. 55:88-96.
Marmesat, S., L. Velasco, M.V. Ruiz-Méndez, J.M. Fernández-Martínez, and C. Dobarganes. 2008. Thermostability of genetically modified sunflower oils differing in fatty acid and tocopherol compositions. Eur. J. Lipid Sci. Technol. (In press).
Marquez-Ruiz, G, R. Garcés, M. Leon-Carnacho, and M. Mancha. 1999. Thermoxidative stability of triacylglycerols from mutant sunflower seeds. J. Am. Oil Chem. Soc 76:1169-1 174.
Mensink, R.P., P.L. Zock, A.D.M. Kester, and M.B. Katan. 2003. Effect of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am. J. Clin. Nut. 77:1146-1155.
Mensink, R.P. 2005. Effect of stearic acid on plasma lipid and lipoproteins in humans. Lipids 40:12501-1205.
Osorio, J., J.M. Fernández-Martínez, M. Mancha, and R. Garcés. 1995. Mutant sunflower with high concentration of saturated fatty acids in the oil. Crop Sci. 35:739–742.
Pearson, T. A. 1994. Stearic acid and cardiovascular disease: answers and questions Am. J. Clin. Nutr. 60(S):1071S-1072S.
Renaud, S.C., J.C. Ruf, and D. Petithory. 1995. The positional distribution of fatty acids in palm oil and lard influences their biologic effects in rats. J. Nutr. 125:229–237.
Soldatov, K.I. 1976. Chemical mutagenesis in sunflower breeding. p. 352-357. In: Proc. 7th Int. Sunflower Conf., Krasnodar, USSR.
Thijssen M.A., and R.P. Mensink. 2005. Small differences in the effects of stearic acid, oleic acid, and linoleic acid on the serum lipoprotein profile of humans. Am. J. Clin. Nutr. 82:510–516.
Velasco, L., B. Pérez-Vich, and J.M. Fernández-Martínez. 2004. Novel variation for tocopherol profile in a sunflower created by mutagenesis and recombination. Plant Breed. 123:490–492.
WHO, 2003. Technical Report Series 916. Diet, Nutrition and the Prevention of Chronic Diseases.

Sunflower in Spain: Past and present trends in an international context

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ABSTRACT

Despite sunflowers having been brought to Spain at the beginning of the 16th century, and in-shell sunflower seed production having been traditionally grown in this country, the oilseed type was not introduced into Spanish agriculture until the end of the 1960's. The development of the oilseed sunflower in Spain has been through several stages marked in many cases by national and EU political decisions. The present trend points towards a recovery of the sunflower area lost in Spain during the last decade and the demand of vegetable oils for biodiesel production or for specific food uses may also serve to secure a plateau price and keep the oilseed oil demand higher than ever. A binding target of 10% for biofuels has been set in the EU for 2020. One output and one input trait are segmenting the sunflower market. The high oleic (HO) sunflowers, including mid oleic (NuSun), have continued to grow, NuSun representing over 96% of the total high oil sunflowers in the US, while in Europe in some countries such as France, Spain and Hungary, the HO area is expanding very quickly. The recent development of herbicide-tolerant sunflowers solved one of the historical deficits in sunflower crop management: i.e., post emergence weed control and may also serve for the chemical control of broomrape and contribute to increase seed yield. The combination of high oil value and potential yield increase makes sunflowers a competitive choice option for farmers.

Key words: herbicide-tolerant – high oleic – market trends – mid oleic – Spain

INTRODUCTION

Sunflowers were introduced into Europe via Spain at the beginning of the 16th century (Putt, 1978). After this, it moved in an eastward direction in Europe, in the beginning as an ornamental plant, and, in a second phase, becoming a food. The earliest record of using sunflower seeds as a source of oil was found in an English patent of 1716. Although this patent refers to the use of sunflower oil for wool, paint, leather, etc. manufacture, most of the crop was used for food. The commercial manufacture of sunflower oil started in Russia between 1830 and 1840.

Although "in shell" sunflower seeds have been traditionally produced in Spain, the oilseed type was not introduced until the second half of the last century. There are different stages in the history of oilseed sunflower production in Spain (Fig. 1). These have been influenced in many cases by political decisions, which have had a great impact on the area planted. These influences and other factors having an effect on sunflower evolution and present situation in Spain are analyzed in this review.

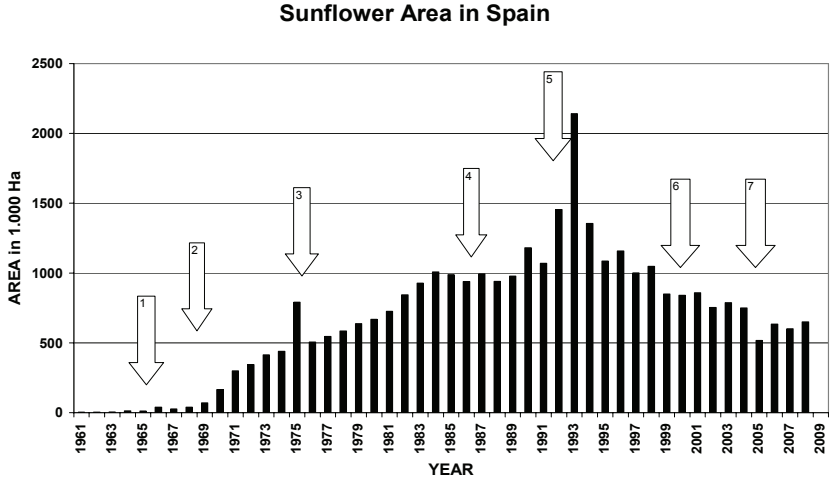

Fig. 1. Historical Oilseed sunflower planning area in Spain Source (MAPA, 2007)

DISCUSSION

Stages in the history of sunflower development in Spain

First Stage: 1965 to 1969. During this period, Spain was deficient in edible oil production and had to rely on imports for consumption. The first sowing of oilseed sunflowers with a black shell opened up a unique opportunity for Spanish farmers and industry. For farmers, to plant the traditional fallow land after cereals´ with sunflower, and for the industry to develop a crushing industry that pushed the crop area up.

Second Stage: 1969 to 1975. This period corresponds to the rapid expansion of the sunflower crop in Spain. At the end of this period the sunflower area planted in Spain was of 781,800 ha. The crushing industry played a key role in the development of sunflower crop as it provided:

- Certified seed for planting.
- Technical staff and mechanical planters to spread crop management techniques.
- Local agents who contracted and collected the crop.
- Financing of the crop, anticipating in many cases payments before harvesting

Also, by the year 1971, the World Bank had launched a program to assist in the development of sunflower in Spain, establishing a research centre for oilseed research in Córdoba in CRIDA 10 within the National Institute for Agricultural Research (INIA). Some of the young researchers from that time have made their scientific careers in working in sunflowers in Spain and are now relevant scientists in sunflower research.

Third Stage: 1975 to 1986. During this period, Spain's vegetable oil imports were restricted to state commerce. Only the Government could import both oilseed for crushing, and oil. Imports were made in the event of any shortage of oil. Additionally, the Government fixed a contract price for the crop that the industry had to pay the farmer. The Ministry of Agriculture assured itself the first bid for the oil. With this intervention, the period was marked by a notable increase in sunflower in Spain. The sunflower-planted area grew in over a million hectares in 1984. The period ends with the entry of Spain into the European Economic Community (EEC) in 1986.

Fourth Stage: 1986 to 1992. The EEC protection system was based on a *price support mechanism,* as a means of increasing agricultural output and productivity and ensuring agricultural income. The most important feature of this mechanism was to ensure that Community farmer prices *were higher* than the world average. The EEC set reference prices for a guaranteed maximum production (GMP) of oilseeds. The farmers received these prices. The crushing industry received aid for the oil extraction, which was fixed by the difference between the reference price and the international market price at the time of extraction. During the transitional period of ten years set for Spain in 1986, the Spanish Ministry of Agriculture established an Intervention price for the farmer at the intervention centres. This price would rise 1/10, to reach the annual Community price. It also established a target price for the local industries to calculate the aid for oil extraction. There was also an aid scheme for the export of surplus oil. The sunflower-planted area in Spain rose to 1,454,500 ha in 1992.

Fifth Stage: 1993 to 2000. The early development of the Common Agricultural Policy (CAP) allowed the EEC to move quite rapidly from a complete deficit to a surplus of production in the main products, and, therefore, to transform the EU from being a net importer to a net exporter on the world market. In 1992, the MacSharry reforms (named after the European Commissioner for Agriculture, Ray MacSharry) were proposed to pacify the EU's external trade partners at the Uruguay round of the GATT trade talks with regard to agricultural subsidies and to cope with EU budgetary difficulties. The 1992 reform, implemented in 1994, took a decisive step towards market orientation by gradually changing the basic mechanisms of the CAP from a *price support system* towards *direct income support.*

In 1993, Spain renounced the last years of the transitional period to become fully integrated into the new CAP. In the absence of any limitation of surface and with sunflowers taking advantage of a particularly high per hectare grant, its cultivation grew in over 2,000,000 ha. Then, the Spanish government imposed a series of restrictions on sunflower planting to limit the undesirable presence of "Premium hunters." These measures stabilized sunflower area to around 1,100,000 hectares. Two systems limited the oilseed area planted. One reduced the aid in proportion to the exceeding of the Maximum Guaranteed Area imposed by the EU/USA Blair-House agreement. The other was a reduction in the aid per hectare if the local oilseed prices exceeded the international reference prices fixed for the period to calculate the amount of aid per hectare. This last calculation could have also led to bonuses in the case of a fall in international reference prices for oilseed by fifteen percent below the reference prices.

The free import/export of oilseeds and oil in Spain, coupled with the no tariff barriers in the EU, created a complex situation for many local crushing industries and collectors. Losses were frequent in those taking long sunflower positions during the harvest due to fluctuations in international prices. Meanwhile, cereals continued to enjoy tariff protection and an intervention price in the EU.

Sixth Stage: From 2000 to 2005. The EU's total budget on agricultural spending fell substantially from 1992 to 1999. However, almost 50% of this budget was still being spent on agriculture in a declining economic sector, which did not create new jobs. Therefore, criticism has focused more and more on the fact that agriculture absorbs huge amounts of money, depriving other policies and tasks of the EU of their potential to create new jobs due to a lack of appropriate financial resources necessary for their development. Agenda 2000 seems therefore to be the Commission's attempt to define new, or, rather, additional, objectives of the CAP, by extending it to function as a rural development strategy. The 'Agenda 2000' reforms divided the CAP into two pillars: production support and rural development. Several rural development measures were introduced including diversification, setting up producer groups and support for young farmers. Agri-environment schemes became compulsory for every Member State.

In Spain, as in other EU member states, there was a progressive lowering of the sunflower planting area as per hectare aid was fixed as being the same for all crops as well as fallow. Fallow is an attractive cereal rotation in countries with periodic droughts. The intervention price for cereals was also lowered during this period. The sunflower area fall was inevitable in Spain despite the environmental fixed aid of 60€/Ha allowed by the EU to Spain for sunflowers. This aid, in many cases, was not implemented as there was no extra budget for it. Many Regional Governments in Spain were devoting all their rural development funds to other five year programs thus limiting their access to it.

In 1997, I reviewed what was then the draft of "Agenda 2000" (Alonso, 1997). Its optimistic estimate indicated that the reduction in Spain's sunflower sowing area could reach from 30 to 40%. The sunflower area planted in 2002 was 753,893 ha. i.e., a 32% reduction in the average area planted during the 1994-1998 period. The sunflower area fell further to 517,125 Ha in 2005 due to the EU policy and to the local drought conditions.

Seventh Stage: After 2005. On 26th June 2003, the EU farming ministers adopted a fundamental reform of the CAP, through the Council Regulation (EC) No 1782/2003, (Official Journal of the European Union, 2003a) based on "decoupling" subsidies from particular crops (though, as Spain did, the Member States could choose to maintain a limited amount of a specific subsidy). The new "single farm payment" is subject to 'cross-compliance' conditions relating to environmental, food safety and animal welfare standards. Many of these were already either good practice recommendations or separate legal requirements regulating farm activities. The aim was to make more money available for environment, quality, or animal welfare programmes.

The purpose of the single farm payment is to ensure income stability for farmers who are able to decide what they produce according to supply and demand. Thus, the EU has opted for less government influence and more of a market place drive for farmer's crop choice. These reforms came into force in 2004-2005. The Member States had the choice of applying for a transitional period delaying the reform in their country to 2007 and phasing the reforms up to 2012.

In 2004, after the expansion of the EU, the new EU member states had immediate access to price support measures (export refunds and intervention buying). However, direct payments will be phased over 10 years (2004-2013), starting at 25% of the rate paid to existing countries in 2004. The EU provided the 2004 entrants with access to a rural development fund for early retirement, environmental issues, poorer areas, technical assistance. The EU states agreed in 2002 that agricultural expenditure up to 2013 should not increase in real terms. This will require a cut in subsidies to the original states of around 5% to finance payments to the new members. With Romania and Bulgaria joining in 2007, the required cut will increase to 8%.

The sunflower area planted in Spain recovered in 2006 and 2007 from the 2005 lower yield. The 2007 recovery was moderated by the international increase in cereal prices at the end of 2006, when the oilseeds had relatively low prices. However, the price increase boost for vegetable oils in 2007 placed sunflowers as a very competitive alternative to cereals. This favourable situation is becoming better for sunflowers as the input prices for farming are also increasing very quickly, particularly fertilizers, and diesel. Thus, it bodes well for sunflower planting area recovery in Spain and the EU in the coming years.

Outlook on the international sunflower and oilseed situation.
During the second half of 2007, and up to the beginning of the new marketing year, prices in the oilseed complex have continued their pronounced rise, and vegetable oil prices have reached record high values (See Fig. 2).

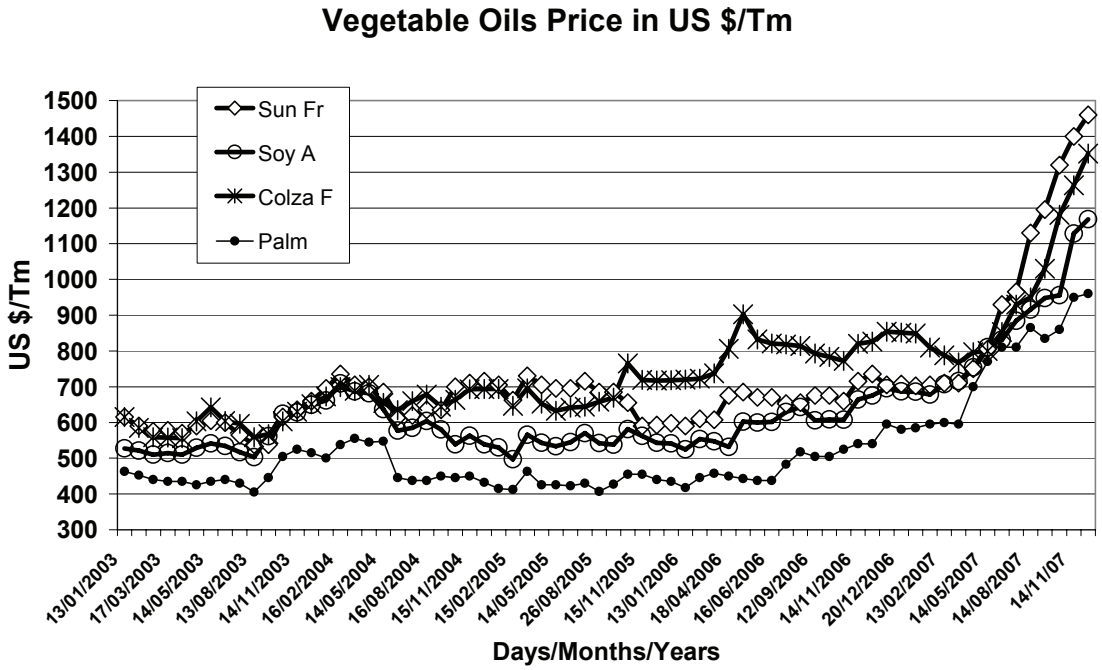

Fig. 2. Vegetable oil prices since 2003. Source: SOS. Elaborated from Oil World (2007)

According to the FAO outlook global market analysis (FAO, 2007), a key factor behind this price rise is that oil-crop markets have come under the direct influence of developments in the related feed grain market. The unprecedented rise in international maize prices has spilled over to the oilseeds and meal market, and, in particular, to the soybean complex.

World stocks and stock-to-use ratios of both oil and meals are falling to critical levels, because of a significant drop in oilseed production in 2007 (see Table 1), coinciding with a steady expansion in global demand for food, feed and energy use, thus calling for a steep reduction in inventories (See Table 2). The two main factors behind the drop in total oilseed output are: first, increased competition from grains, notably in the United States but also in China and CIS (Commonwealth of Independent States) countries, which has interrupted the steady expansion in the world oilseed area. Second, unfavourable weather conditions which have affected oilseed production in several key growing areas or countries, including the European Union, CIS, Australia, Canada, China, Turkey and the United States. The decline in production of soybeans and sunflowers has been responsible for the drop in oilseed production in 2007. Sunflower production in 2007/08 was 8.2% lower than in 2006/07. Sunflower production went down by 26.3% in the EU, 20.7% in Ukraine and 16.2% in Russia (see Table 3).

The present forecasts for global supply/demand in 2008 point towards a continued firmness in international prices for oilseeds and oilseed products. Furthermore, the growing biodiesel requirements have led to an increased demand for vegetable oils. This trend, combined with a constant rise in the consumption of vegetable oil as a food, has led to a gradual tightening in global supplies, thus explaining the recent rise in vegetable oil prices, which may continue during 2008.

Table 1. World production of major oilseeds

	2005/06	2006/07 Prel.	2007/08 Proj
	Million Tm		
Soybeans	220.59	237.27	219.85
Cottonseed	43.95	45.82	45.37
Rapeseed	48.74	46.80	47.62
Groundnuts (unshelled)	33.04	32.41	33.11
Sunflower	30.02	30.15	27.67
Palm kernels	9.98	10.27	11.11
Copra	5.50	5.28	5.37
Total	**391.82**	**408.00**	**390.10**

Source: USDA Marc 2008 (USDA;2008)

Note: The split years bring together northern hemisphere annual crops harvested in the latter part of the first year shown, with southern hemisphere annual crops harvested in the early part of the second year shown, For tree crops, which are produced throughout the year, calendar year production for the second year shown is used

In USA, the USDA January 2008 report (USDA, 2008) showed an unexpected sharp decline in corn ending supplies. In order to compensate for the corn shortage, 2.4 million acres of corn would be needed in the new plantings. On the other hand, soybean needs to gain between 8 and 10 million acres in 2008 to prevent ending supplies from dropping to dangerously low levels. But it was not just the USDA reports that gave markets a lift to new highs. There was also a continuous flow of fund money into all commodities, and this will continue during 2008. Poor weather conditions in South America's soybean growing areas, such as the warm, dry summer in Argentina, also had an effect on oilseed prices during February and March 2008.

Table 2. World oilseeds and product markets at a glance

	2005/06	2006/07 *estim.*	2007/08 *f'cast*
	Million Tm		
Total oilseeds			
Production	404	417	403
Vegetable Oils and Animal Fats			
Production	149	151	154
Supply (Production + Opening Stocks)	168	172	174
Utilization[1]	146	152	157
Trade[2]	72	76	79
Stock-to-utilization ratio (%)	14	13	11
Oil meals and cakes[3]			
Production	101	106	102
Supply (Production + Opening Stocks)	113	121	119
Utilization *	98	102	108
Trade *	55	59	62
Stock-to-utilization ratio (%)	15	17	11

Source: (FAO, 2007)

[1]Residual of the balance.

[2]Trade data refer to exports based on a common October/September marketing season.

[3]All meal figures are expressed in protein equivalent; meals include all meals and cakes derived from oilcrops as well as fish meal and other meals from animal origin.

Table 3. Sunflower seed; World supply and disappearance in 1000 t

	2002/03	2003/04	2004/05	2005/06	2006/07	2007/08F
Seed Production						
Argentina	3,700	3,240	3,600	3,800	3,500	4,500
Other Europe	749	876	867	830	850	490
European Union 27	5,183	6,155	6,463	5,958	6,483	4,772
Peoples' Republic of China	1,946	1,743	1,552	1,927	1,900	1,800
Russian Federation	3,685	4,850	4,800	6,450	6,750	5,650
Ukraine	3,270	4,252	3,050	4,700	5,300	4,200
United States	1,112	1,209	930	1,823	972	1,310
India	1,625	1,700	1,224	1,550	1,280	1,650
Turkey	820	600	650	750	850	700
Other	1,897	2,266	2,270	2,290	2,294	2,599
TOTAL	**23,987**	**26,891**	**25,406**	**30,078**	**30,179**	**27,671**
Seed Import						
Turkey	229	660	529	345	408	380
European Union 27	705	1,066	413	627	572	400
Other	451	442	192	316	663	294
TOTAL	**1,385**	**2,168**	**1,134**	**1,288**	**1,643**	**1,074**
Seed Export						
Argentina	213	46	107	45	70	200
United States	166	170	141	178	181	166
Russia/Ukraine	517	1239	57	616	484	365
Other	571	852	918	783	1,219	752
TOTAL	**1,467**	**2,307**	**1,223**	**1,622**	**1,954**	**1,483**
Area Harvest (1000 Ha)	**20,202**	**23,287**	**21,369**	**23,117**	**23,841**	**22,790**
Yield Tm/Ha	**1.19**	**1.15**	**1.19**	**1.30**	**1.27**	**1.21**

The US has passed a new energy bill adding a more bullish sentiment to the corn and vegetable oil markets. The new energy bill also raises the corn-based ethanol mandate.

Reduced growth in global oils/fats supplies and an unprecedented fall in meal supplies, because of a significant drop in oilseeds production in 2007, are expected to coincide with a steady expansion in global demand for food, feed and energy use, thus calling for a severe reduction in inventories. World stocks and stock-to-use ratios of both oil and meals have fallen to critical levels.

The biofuel impact

Another favourable situation for oilseeds in general, was the impact when the European Commission brought forward the legislative proposals that were adopted in 2003 in the form of the bio fuels directive (Directive 2003/30/EC of the European Parliament and of the council of 8 May 2003) (Official Journal of the European Union, 2003b) and article 16 of the energy taxation directive of the Council Directive 2003/96/EC of 27 October 2003, (Official Journal of the European Union, 2003c).

The biofuels directive expressed the clear intention of *"promoting the use of biofuels… in each Member State, with a view to contributing to objectives such as meeting climate change commitments, environmentally friendly security of supply and promoting renewable energy sources"*. It included an interim target for 2005 and a target for 2010 of a 2% and 5.75%, respectively, share of the market for petrol and diesel. These indicative targets, once adopted, were not mandatory, but they constituted a moral commitment on behalf of Member States.

During the 2007 Spring EU Council, Europe's Heads of State agreed on the 3 targets for 2020: A binding target of 20% renewable energy (RES) by 2020 and a separate binding target of 10% for biofuels; A 20% energy efficiency target (EE) and 30% greenhouse gas (GHG) reduction target. These three targets have laid the foundations for renewable energy sources to become a major pillar of the EU future energy supply.

Table 4. European Biodiesel production capacity growth 2003-2007.

Country	Production Capacity in 1000 t				
	2003 EU15	**2004 EU 15**	**2005 EU 15**	**2006 EU 25**	**2007 EU 27**
Germany	1,025	1,088	1,903	2,681	4,361
Italy	420	419	827	857	1,366
France	500	502	532	775	780
UK	5	15	129	445	657
Spain		70	100	224	508
Greece			35	75	440
Belgium			55	85	335
Austria	50	100	125	134	326
Poland			100	150	250
Portugal			6	146	246
Sweden	8	8	12	52	212
Czech Republic			188	203	203
The Netherlands			0	0	115
Slovakia			89	89	99
Denmark	41	44	81	81	90
Romania					81
Bulgaria					65
Lithuania			10	10	42
Estonia			10	20	35
Hungary			0	12	21
Latvia			5	8	20
Slovenia			17	17	17
Malta			2	3	8
Ireland			0	0	6
Cyprus			2	2	6
Total EU	**2,049**	**2,246**	**4,228**	**6,069**	**10,289**

Source EBB, Situation at 01/07/2007 (EBB,2007)

The new 10% minimum target in 2020 (The impact of a minimum 10% obligation for biofuel use in the EU-27 in 2020 on agricultural markets) has also been seen to be relative to the existing legislation which put the target at 5.75% in 2010. The current directive may fail to produce the incorporation of 5.75% in 2010 due to the market and technologies having little time to react.

Since 2003, biodiesel has proved to be a significant demand shifter in the overall vegetable oil industry. The confluence of environmental concerns, high energy prices and government incentives has led to a significant increase in the biodiesel production capacity in the EU and worldwide. In 2007, there were 185 fully operational biodiesel plants, in Europe and another 58 plants were under construction. Thus, the EU biodiesel production capacity reached 10.2 million tonnes (see Table 4). In Spain in 2007 there were 23 fully operational biodiesel plants and 26 plants under construction, with a joint production capacity of 921,000 and 2,961,200 Tm/year, respectively. Furthermore, there were another 24 projects for a joint production capacity of 2,692,000 Tm/year (See Table 5).

This huge EU biodiesel production capacity risks remaining idle and the production stagnating or declining, the same as during the 2nd half of 2007. The record high vegetable oil prices at the end of 2007 and beginning of 2008 is making many operations unviable. To make things worse, the US Federal subsidies since 2004 (up to \$264/m^3 i.e., \$ USA 300/Tm ~ €200/tm) biodiesel blends tend towards increasing biodiesel imports in the EU. The US "B99" blend (a blend of a small amount of mineral diesel with biodiesel) exported to the EU, benefits the blender's credit as this is not restricted to biodiesel produced and consumed on the US territory. Thus, "B99" exports to the EU were boosted in 2007 as in most cases B99 blends are sold in the European market as "pure biodiesel" and at a substantial discount

(over €120-180/tonne), in some cases at a lower price than that of the raw materials purchased by the EU industry for producing biodiesel.

Table 5. Biodiesel number of plants in Spain in 2007 and their production capacity in 1000 Tm

	Operational		**In Construction**		**In Project**	
Province	**Number**	**Capacity**	**Number**	**Capacity**	**Number**	**Capacity**
A Coruña			1	200	1	103
Alava	1	30				
Alicante	1	20			1	200
Almeria	1	6	1	6		
Asturias	1	4			2	270
Badajoz			2	360		
Baleares	1	16				
Barcelona	1	31				
Burgos	1	8	1	49		
Cadiz			1	200	3	330
Cantabria			1	155		
Ceuta					1	250
Ciudad Real	1	32	1	100	1	110
Cordoba			2	7		
Cuenca	2	122	1	50		
Girona	1	5				
Granada					1	80
Huelva			2	400		
Huesca	1	50	3	102		
Jaen	1	100	1	200		
La Rioja			1	250		
Las Palmas			1			
León					3	310
Lleida					1	110
Lugo					1	20
Madrid	1		1	45		
Murcia					1	140
Navarra	1	70			2	124
Pontevedra			1	300		
Sevilla	2	86	1	60	1	300
Tarragona	1	50			2	80
Teruel					2	115
Toledo	3	156				
Valencia	1	110				
Valladolid			1	70		
Vizcaya			2	400	1	150
Zamora	1	20	1	7		
Total Spain	**23**	**921**	**26**	**2,961**	**24**	**2,692**

Source (Biodieselspain,2007): Situation 31/12/2007

The new US biodiesel mandate could eventually require as much as one-third of total US vegetable oil production, assuming no vegetable oil imports to the US. Eventually, all the biodiesel produced in the States may remain there, leaving more room for the EU biodiesel factories to produce EU biodesel needs.

The Latin American biofuel industry is headed by Brazil's mature ethanol industry. Brazil will also have included mandatory B2 and B5 by 2008 and 2013, respectively, including tax breaks. Argentina also has a B5 project for 2010.

In Asia, in several southeastern countries, indicative B2 or B5 targets are gradually moving to mandatory targets. Malaysia has a B5 mandatory by 2010; Indonesia B5 by 2025; Thailand B10 by 2012 and Philippines B2 by 2009. China has no concrete biodiesel policy but is targeting 15% use of biofuels by 2020; South Korea has a mandatory B5 blending implemented in 2006 and India is preparing legislation on biodiesel to support cultivation and commercial activities of *Jatropha*-based biodiesel.

According to Rabobank reports on biodiesel (Hansen, 2006; Tan, 2007), the biodiesel production could have a considerable impact on global vegetable oil demand in 2010. Considering the 2005 vegetable oil production of 96 million tonnes, the vegetable oil demand for biodiesel production and the extra food demand may require another 18 million tonnes and 13 million tonnes, respectively, in 2010.

As was recently affirmed by Miriann Fischer Boel, the European Commissioner responsible for Agriculture and Rural development in a speech during the 2008 World Biofuels Market Congress in Brussels (Fischer Boel, 2008) the EU policy on this subject, despite being controversial, has a solid justification and everyone in the sector can be confident that no policy u-turns lie ahead. Biofuels must be a part of the future of sustainable energy production in the EU for two reasons: the fight against climate change, where biofuels are an important weapon, and energy security against future supply problems. In this speech, Ms. Fischer Boel, gave the answer to various objections raised recently against biofuels and in these answers we can find the clue to the next direction of EU policy. The first objection is that using first-generation biofuels in many cases supposedly does not cut down greenhouse gas emissions. It is true that some biofuels do not show clear benefits, but biodiesel made from European-grown rapeseed makes a greenhouse gas saving of 44 per cent compared to fossil fuels. The typical figure for ethanol made from sugar beet is 48 per cent. Under the rules proposed by the Commission, a given biofuel would count towards a Member State's target only if it made a greenhouse gas saving of at least 35 per cent compared to fossil fuels, which is a very healthy difference. And the standard applies both to domestic production and to imports. When calculating this saving, the EU proposes to take into account the value of by-products such as animal feed. The second objection to the 10 per cent target is that it will mean destructive land conversion. The Commission recognises these dangers and has proposed the following: no biofuel would count towards a Member State's usage target if it does not meet strict sustainability criteria. For example, this would exclude biofuel coming from either land with a high biodiversity value or land with high carbon stocks.

As regards the basic argument, that more biofuel means painfully high prices, and, therefore, less food for the poor, Ms Fischer Boel says, that it is not fair to make biofuel a scapegoat for the extreme market movements of recent times. According to the OECD, cereal use for ethanol in Europe, North America and Asia increased by 17 million tonnes in 2006. But, in the same year, the combined cereals supply shortage in these countries was 60 million tonnes – nearly four times as much. Clearly, this was not just a "biofuel story".

In a 2006 study on agricultural market growth impacts on the production of biofuel, two scenarios were considered. In one of them, referred to as "High oil price scenario" with sustained crude oil prices at US $ 60/barrel, the summary conclusion was:

With sustained higher crude oil prices, there are two main forces at play that affect world markets for agricultural commodities. First, due to higher agricultural production costs that lead to lower quantities of production, commodity prices increase. At the same time, higher oil prices – and higher oil-based fuel pump prices – increase incentives to produce more biofuels (even though partially dampened by higher feedstock prices), which creates an additional demand for feedstock products. Again, this causes prices of agricultural commodities to increase.

Whatever the reasons behind the current very high vegetable oil prices, it is clear that these are limiting the potential use of biodiesel for economic reasons. On the other hand, the present crude oil prices above US $100/barrel, and the equally high values for protein meals are improving their profitability, in particular for integrated industrial facilities that can produce either vegetable oils for food or for biodiesel.

With the large demand for vegetable oils for biodiesel production and keeping in mind the mandatory uses of biofuels in many countries, we can expect that this industry will serve to prevent the oilseed market prices falling below the threshold prices at which the production cost of biodiesel is equal to the domestic tax-free diesel prices.

Sunflower oil quality traits and food and non-food market trends

High oleic sunflowers have been developed from the sunflower variety Pervenets obtained by Soldatov in 1976 (Soldatov, 1976). High oleic sunflower oil is specialty oil with high oleic acid (monounsaturated)

levels. It must be at least 77% monounsaturated to meet product descriptions but often a level of above 80% is required by the industry.

Most of the world's vegetable oils and fats show a mixed fatty acid composition and their physical-chemical properties, as well as their physiological-medical benefits, are fixed in the fatty acid composition (Table 6). In triglycerides, the hydroxyl groups of a glycerol molecule are chemically bound to different fatty acids, varying in chain length and/or in number and position of C-C double-bonds, which cause bends in the C-C chain. In high-oleic oils, such as high-oleic sunflower oil, all properties are strongly dominated by its high content of oleic acid (C18:1) and low polyunsaturated fatty acid (PUFA).

Table 6. Differences in physicochemical characteristics between the two types of sunflower oil

Characteristic	High oleic sunflower	Conventional Sunflower
Fluidity	Liquid +	Liquid +
Resistance to heat	Good +	Average -
Resistance to oxidation	Good +	Average -
Effect on Cholesterol; LDL (Bad)	Reduces +	Reduces +
Effect on Cholesterol; HDL (Good)	No Change +	Reduces -

LDL is atherogenic; HDL is antiatherogenic

This combination of fatty acid content offers many advantages:

- For nutritional purposes: no *trans* fatty acids; low saturated fatty acid content; reduction in "bad cholesterol"; GMO free.
- For all uses: high oxidative stability, reduced rancidity; extended shelf life
- In processing: It is liquid ant thus easy to transport and to store.

High oleic sunflower oil offers advantages not only for the food industry but also for the biodiesel industry. The European Standard requirements set out in regulation EN 14214 (European Standard prEN 14214, 2002) defined 25 parameters for fatty acid methyl esters for bio-diesel. Among them, the iodine value is set at 120. While conventional sunflower oil has an iodine value of about 137, high oleic sunflower has about 87. This allows to reduce the iodine values in mixtures with other vegetable oils such as soybean, whose iodine value is around 133.

The development of high oleic sunflowers was limited in the US during the 1980's and 1990's as a consequence of two patents (Fick, 1984; Fick, 1985), which ended in 2005. In Europe and South America, there was only a limited interest in some industries, which triggered a special production of this type of sunflower in Spain, France, Italy and Argentina. Thus, farmers received a premium price of between 10 and 30 percent depending on market demand with wave fluctuations, and high oleic sunflower oil had a from 20 to 40 percent higher price than conventional sunflower oil.

The NuSun or mid-range oleic sunflower, whose oleic acid levels vary from 50 to 70 percent, was introduced in 1999 into the U.S. as a response to U.S. food processors' desire for a vegetable oil low in saturated fatty acids and suitable for fast-food frying applications without hydrogenation. In 1999, the U.S Food and Drug Administration (FDA) proposed regulations that changed the Nutrition Facts label to allow consumers to identify the amount of *trans* fat in a food by listing the grams of *trans* fat. Later, the U.S.A. FDA published a final rule that amended its regulations on food labeling requiring *trans* fatty acids to be declared on the nutrition label of conventional foods and dietary supplements (68FR41434). This was effective January 1, 2006. The NuSun sunflower planting area grew very quickly in the US, and by 2004 it was already the dominant oil sunflower grown in the States, and in 2007 it reached about 90% of the oil type sunflower grown in this country. The high oleic type was grown in about 6%. Thus, high and mid oleic sunflowers constitute about 96% of US oilseed sunflowers (Kleingartner, 2004; Kleingartner, personal communication).

In the EU, the *trans* fatty acid concern is also causing an impact on the food industry. In 2002, the Health Council of the Netherlands recommended that *trans* fat should be limited to 1% of calories. In a report on dietary reference intakes, the Health Council of the Netherlands recommended, among other things, that *trans* fat should be limited to one percent of calories. In 2003, the Danish government announced that, as from January 2004, the amount of *trans* fat from partially hydrogenated oil would be limited to 2% of the total amount of fat or oil in the food. Despite the initiatives being taken for an EU regulation on *trans* fats, different industries have positioned their products in line with the concept of "free of *trans* fatty acid". Some industries have chosen the use of natural saturated oils, such as palm oil, or total hydrogenation of unsaturated vegetable oils, which does not cause *trans* fatty acids, aiming to

separate the concepts: "trans fatty acids" and "hydrogenation" (FEDIOL, 2006). In some cases, high oleic sunflowers has been chosen either pure or in blends with other vegetable oils. i.e., in 2003, SOS Cuetara, in Spain changed the formulation of fats in their cookies to reduce *trans* fatty acid content to below 0.5% of the oil fraction. They developed a special spread blend branded as Oleosan containing a large proportion of high oleic sunflower oil. This was achieved by blending high oleic sunflower oil with other vegetable oils. The Spanish fish canning industry also announces in TV the use of "high oleic" oils as premium quality.

As a consequence of the continuous demand increase of high oleic oils, the high oleic area planted in some European countries has grown rapidly during the last four years, while in others their presence is only testimonial. In 2007, the West European countries grew high oleic sunflowers in about 26% of the 1,349,400 sunflower-planted hectares. Two countries, France and Spain, were responsible for most of this (Fig. 3). In Eastern Europe, the high oleic area represents less than 3% of the sunflower area planted and only Hungary grew a significant 17% of its sunflower area with high oleic varieties.

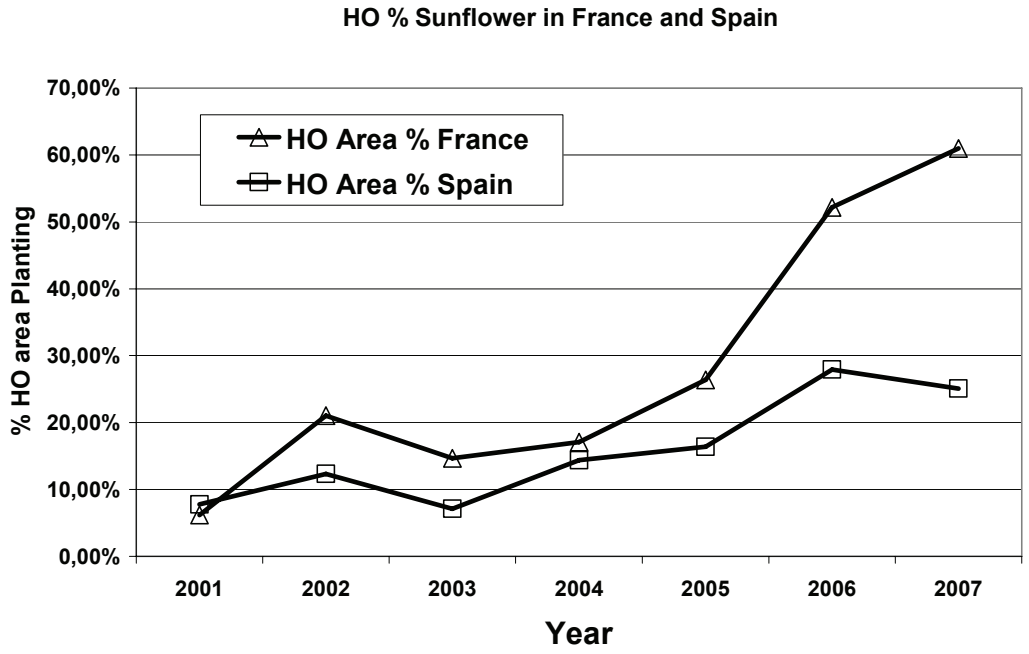

Fig. 3. High Oleic sunflower area planting growth in Spain and France in percentage of area planted.

The cosmetics and lubricant industries may also increase their use of high oleic sunflowers in the future. The EU uses more than 5 million tons per year of lubricants. This sector is dominated by mineral oils and only 2% is of vegetal origin. Besides its technical aspects as driving forces, the regulatory framework of the EU is also increasingly favouring the environment- friendly, readily biodegradable oil. Due to its high oxidative stability, high oleic sunflower oil is favourably compared to other natural oils as base oils or additives in lubricants. Compared to mineral oil-based lubricants, bio-lubricants still face disadvantages in product costs. Great perspectives are in store for very high oleic sunflower oil as it performs like synthetic esters (e.g. TMP-trioleates), but is somewhat lower in price (Vannozzi, 2006).

The continuous growth of high oleic sunflowers may be limited by two factors: the present sunflower oil prices and the lack of sunflower hybrids with the newest trait demands, such as herbicide tolerance, and/or different disease resistances.

Regarding the present very high sunflower oil prices, in order to make a contract with farmers and to preserve the identity of the seed in the whole factory supply chain, a premium has to be paid both to farmers and collectors. The amount of this premium can be either a fixed amount or a percentage of the commodity sunflower seed price. A fixed premium of 24 €/Tm of seed considered to be highly attractive by farmers two years ago, is not very encouraging in the present market situation.

The recent development in Europe, and worldwide, of herbicide-tolerant (HT) sunflowers, which allow the use of broad spectrum post-emergence herbicides is also segmenting the market very fast. The

development of performing HT and high oleic sunflower hybrids is also limiting the expansion of high oleic sunflowers.

In a similar way, the expansion of new races of well known diseases, such as Broomrape race F, caused by *Orobanche cumana*, also limits the high oleic sunflower potential area until performing high oleic and *Orobanche* resistant sunflower hybrids are developed.

Herbicide tolerant sunflowers: The new market development

Recently, two herbicide-tolerant sunflower types have been introduced into the market for weed control with post-emergence specific herbicide treatments. These sunflower hybrids provide growers with a post-emergent control option, without killing the crop. Broadleaf weeds, both annual and perennial, are the leading weed spectrums affecting sunflower yields but grasses and volunteer cereals may also be important. In the past, the lack of choice in weed control forced many sunflower farmers to rely on more expensive, less effective pre-emergent options that often did little to eliminate weed competition losses in their sunflower fields. The recent ban in the EU of the pre-sowing herbicide Trifluralin is making the use of herbicide-tolerant sunflowers and post-emergence herbicide weed control more attractive. Thus, both systems are spreading very quickly.

Clearfield Sunflower production system

The CLEARFIELD Production System for sunflowers is a unique production system comprised of non-GMO herbicide-tolerant sunflower hybrids and a herbicide of the Imidazolinone chemical family. At present, the herbicide used in sunflowers is imazamox, which is a member of the herbicide family of AHAS or ALS inhibitors. Some members of this family control susceptible weeds by inhibiting the acetohydroxyacid synthase (AHAS) enzyme also called acetolactate synthase (ALS). Several variant AHAS genes conferring imidazolinone tolerance were discovered in plants through mutagenesis and selection, and were used to create imidazolinone-tolerant maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), oilseed rape (*Brassica napus* L.), and sunflower (*Helianthus annuus* L.). These crops were developed using conventional breeding methods and commercialized as CLEARFIELD® crops from 1992 to the present. While the Clearfield system is available for several different field crops, including corn, wheat, rice, and canola, the "IMI" chemical formulations are not interchangeable. Clearfield sunflower is not cross-tolerant to the sulfonylurea (SU) family of herbicides.

In 1996, a wild sunflower population of *Helianthus annuus*, highly tolerant to the herbicide imazethapyr, was found by Al-Khatib in a soybean field in Kansas, U.S.A (Lilleboe, 1997). The Agricultural Research Service plant geneticist Jerry Miller learned of Al-Khatib's wild sunflower collection from John Nalawaja, a colleague at North Dakota State University-Fargo, and requested seed specimens. Their goal was to transfer resistance to cultivated sunflower. This prospect was very exciting because the list of broadleaf weeds and grasses controlled by imazethapyr herbicide and other herbicides of the imidazolinone chemical family (IMI) was extensive. Also, for sunflower to spread into no-till acreage, planting herbicide tolerant hybrids was the only alternative for postemergence weed control. In 2002, Miller and colleagues released two germplasm lines (USDA lines HA 425 and RHA 426) of imazamox (the new BASF IMI herbicide) -tolerant sunflower for commercial seed companies to use in developing their own hybrids (Miller and Al-Khatib, 2002). In 1997, I collected from the Kansas farm owned by Doug French the same wild sunflower population collected earlier by Al-Khatib and, back in Spain, Koipesol's technical team proved that this herbicide tolerance offered an excellent opportunity for the chemical control of the parasitic weed *Orobanche cumana* Wallr. (Alonso et al., 1998). *Orobanche* chemical control is not race specific and may serve both to prevent the parasitic plant to spread to new areas and to control it in already infected areas.

Clearfield sunflowers, which are registered for use only with imazamox herbicide (Beyond™ in U.S.A., Pulsar® 40 in Europe) manufactured by BASF Corporation represented the first real post-emergence option for controlling key problem weeds. Imazamox herbicide provides contact and residual activity on a number of grasses and broadleaf weeds as well, including nightshade, pigweed, foxtail species, wild oats, volunteer cereals, puncturevine, non-Clearfield wild or volunteer sunflower and broomrape.

The Clearfield Production System for sunflowers started in 2003, in the U.S., when the Environmental Protection Agency registered Beyond™ herbicide. In Europe it has been introduced into different countries since 2002 and Syngenta Seeds was the first seed company marketing Clearfield sunflower hybrids.

Express Sunflowers production system

Express Sunflowers was introduced by Pioneer Hi-Bred International, Inc., and DuPont Crop Protection in 2006 in some European countries, and in 2007 in the U.S. This system facilitates a broad spectrum weed control option for sunflower growers: Sunflower hybrids with the DuPont™ ExpressSun™ trait which provides tolerance to Express® herbicide. This herbicide is a sulfonylurea. Sulfonylureas are a powerful family of herbicides discovered by DuPont in 1975 and first commercialized for wheat and barley crops in 1982. Sulfonylureas comprise a family of compounds which kill broadleaf plants by blocking the plant enzyme acetolactate synthase (ALS), an enzyme important to the plant for the synthesis of some amino acids (leucine, isoleucine and valine)

The Express Sunflowers production system only offers broad leaf postemergence weed control while the Clearfield sunflowers production system offers postemergence control of many weeds such as; broad leaf, grasses and volunteer cereals as well as broomrape control.

The expansion of herbicide tolerant sunflower and future development

Herbicide-tolerant (HT) sunflowers gain a market share very quickly once the HT traits are incorporated into performing hybrids. Often their expansion has been limited by the lack of planting seed. In some cases the sunflower area planted with HT hybrids is above 25% in only three or four years after the introduction (Table 7). This tendency is going to continue as more performing hybrids will be available and it will not be long before most of the sunflower grown will be HT.

It is more difficult to forecast which of the two methods will dominate in the market and most probably both will be present. Currently, in some countries the Clearfield method represents nearly 100% of the HT planted area. This is the case of Turkey, Spain and Serbia. In other cases, Clearfield and Express methods are both spreading but keeping to about 50% each of the total HT market in the country. Finally, in some cases, the Express method represents 75% of the HT market, for instance in Bulgaria. These differences may be caused by the different dates of introduction of each method into the different countries. Thus, we shall still have to wait a few years before we see the real share of each in the market. However, it is reasonable to think that in the areas where broomrape (*Orobanche cumana*) is a problem, the Clearfield solution will probably dominate. Also, the Clearfield method could be a better option in those cases where sunflower is rotated with cereals in areas where cereals are repeated one to three years before planting sunflower, so that the grasses could create a weed problem.

Table 7. Percentage of sunflower area planted with herbicide-tolerant production systems (Clearfield and Express) in Argentina and Europe

Argentina	**Year of introduction**	**2002/3**	**2003/4**	**2004/5**	**2005/6**	**2006/7**	**2007/8**
Bs. As. (SO, SE y Oeste)	2002/3	2%	3%	9%	15%	27%	25%
S. Córdoba	2002/4	1%	2%	7%	21%	14%	21%
E. La Pampa	2002/5	1%	7%	12%	23%	22%	13%
Europe	**Year of introduction**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**
Turkey	2003	3%	10%	23%	25%	21%	24%
Bulgaria	2006	0%	0%	0%	9%	18%	33%
Romania	2005	0%	0%	1%	4%	9%	16%
Hungary	2006	0%	0%	6%	7%	14%	17%
Serbia	2006	0%	0%	4%	15%	5%	11%
Slovakia	2006	0%	0%	0%	0%	0%	6%
Russia	2006	0%	0%	0%	0%	1%	1%
Ukraine	2006	0%	0%	0%	1%	2%	3%
Spain	2003	0%	0,2%	0,2%	0,2%	1%	2%
France	2009	0%	0%	0%	0%	0%	0%
Croatia	2009	0%	0%	0%	0%	0%	0%
Czech	2009	0%	0%	0%	0%	0%	0%
TOTAL EUROPE		0%	0%	1%	2%	3%	6%

Source. Syngenta Seeds Marketing department

Both methods offer a tremendous potential for weed control and thus favour sunflower yield, particularly for the spreading of sunflower into no-till acreage as well as in very early planting in Mediterranean countries.

In several sunflower planting date studies made in Cordoba, Spain, during the 1980's, by the CIDA research group it was shown that sunflower planted during the winter (December and January) yielded about 30% more than when planted in March.

For some years, the Andalusian government sunflower trial network RAEA (Red Andaluza de Experimentación Agraria) planted, in the same location, sunflower hybrids at the end of January and in March in three years, 1994, 1998 and 2000. A number of common hybrids, (i.e., 32, 13 and 12 hybrids) were used both in winter and spring planting trials in 1994, 1998 and 2000, respectively (RAEA, 1994, 1998, 2000). The average yield for the three years in the nine trials (Fig. 4) was 2,069 kg/ha for the winter planting date and 1,694 kg/ha for the spring planting date. i.e., 375 kg/ha average difference. While in some locations the difference was minimal (near the coast and in very low yielding areas) the difference reached a peak of 911 kg/ha in the central part of Andalusia. Thus, the potential increase of yield due to early planting seems to be related to the flowering escape of these plantings from the extremely hot days that often occur in early June.

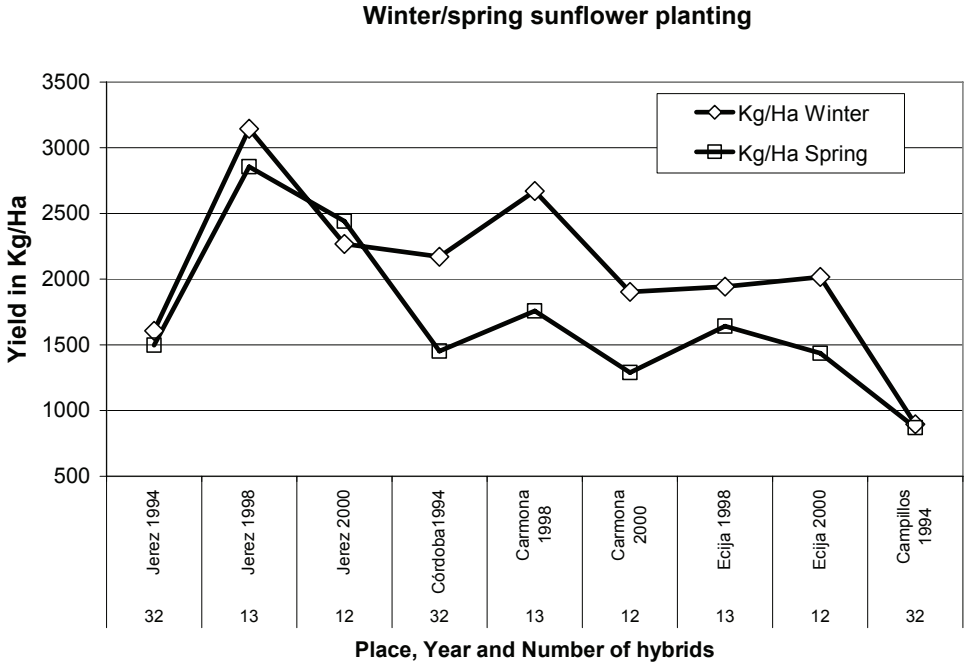

Fig. 4. Average sunflower yields of the same set (number) of sunflower hybrids planted in winter and spring in nine trials during three years. Source: RAEA (1994, 1998, 2000).

This potential yield increase was threatened by bird damage and herbicide damage from hormonal treatments in neighbouring cereal fields and weeds. The slow growth of sunflowers during January and February favoured the weed infection. There was no effective postemergence herbicide treatment for sunflowers at that time. The introduction of Clearfield sunflowers is allowing early planting in Andalusia with an effective control of *Orobanche* and weeds

CONCLUDING REMARKS

The sunflower planting area in Spain and other EU member states has been influenced in the past by political decisions and the CAP. This influence became less and less important through the successive CAP modifications. Yet, the recent political decisions at global levels on biodiesel production and use are again impacting on the prices of all oilseed oils.

The present high oilseed oil prices may be more related to production deficits than to biodiesel consumption. Sunflower oil use for biodiesel production is not significant. However, the present potential production capacity and the short term projected increase certainly may have an impact on preventing the

oilseed oil prices from going down. Even if sunflower oil remains mostly as food oil in the near future, the biodiesel demand may impact on all oilseed oil prices including sunflower oil.

A high oilseed oil price is an incentive to increasing production, but cereal prices are also very high causing a competition for land among crops. High transport costs as a consequence of high crude oil prices and high fertilizer prices will also play a role in the farmer's choice of what crops to plant. Sunflowers may be favoured in this complex equation, as it is an easy-to- grow crop which uses deep soil fertilizers which every year escape from cereals. Additionally, the low test weight of sunflower seed will encourage more and more local crushing. i.e., few seed exports/imports.

The niche high oleic sunflower market may become dominant in the next years at least in Western Europe as has happened in the U.S with the NuSun high oleic sunflower oil may also be of interest to the growing bio-lubricant industry and for some blending in bio-diesel production.

In the present oilseed oil price situation, and with high input costs, it is reasonable to think that sunflower area may grow again in Western Europe as well as in several ex-URSS countries. Weed and *Orobanche* control with HT-tolerant sunflowers offers an excellent opportunity to increase sunflower yield in different countries and lead this regrowth. This is particularly important for early planting dates in hot Mediterranean countries giving North African countries an excellent chance to increase their sunflower production.

REFERENCES

Alonso, L.C. 1997. El cultivo del girasol. Su historia e importancia mundial, actual y future. p. 307-348. In: Proc. 4º Symposium Nacional de Semillas. Sevilla 5-7 Noviembre 1997. Junta de Andalucia, Consejeria de Agricultura y Pesca. Colección: Congresos y Jornadas nº 43/97.

Alonso, L.C., M.I. Rodriguez-Ojeda, J. Fernandez-Escobar, and G. López-Ruiz-Calero. 1998. Chemical control of broomrape (*Orobanche cernua* Loefl.) in sunflower (*Helianthus annuus* L.) resistant to imazethapyr herbicide. Helia 21(29): 45-54.

Biodieselspain. 2007. http://www.biodieslspain.com

Committee for Agriculture. 2006. Working party on agricultural policies and markets 2006. Agricultural market impacts of future growth in the production of biofuels. Committee for Agriculture: Directorate for Food, Agriculture and Fisheries: Organisation for Economic Co-operation and Development. AGR/CA/APM(2005)24/FINAL.

EBB, 2007. European Biodiesel Board. Brussels, Belgium. http://www.ebb-eu.org

European Commission. 2007. The impact of a minimum 10% obligation for biofuel use in the EU-27 in 2020 on agricultural markets. European Commission; Directorate General for Agriculture and Rural Development, Brussels, 30 April 2007 AGRI G-2/WM D.

European Standard prEN 14214. 2002 Automotive fuels. Fatty acid methyl esters (FAME) for diesel engines. Requirements and test methods. European Committee for Standardization. Management Centre, 13 p.

FAOSTAT. 2007. Oilseeds, Oils and Meals. FAO Food Outlook; Global Market Analysis, November 2007. http://www.fao.org/giews/english/fo/index.htm

FEDIOL. 2006. Trans fatty acids and hydrogenation. The EU oil and protein meal Industry. Position paper 3.04.2006.

Fick, G.N., 1984. Sunflower products and methods for their production. U.S. patent number 4627192. Filed Nov.16th, 1984 and granted Dec, 9th 1986.

Fick, G.N., 1985. Novel Sunflower products and methods for their production. U.S. patent number 4743402. Filed Aug.26th, 1985 and granted May, 10th 1998.

Fischer Boel, M. 2008. Biofuels: not a magic wand, but a valuable policy tool. In: World Biofuels Markets Congress Brussels, 13 March 2008.

Hansen, S. 2006. Biodiesel Global Trends, EU Focus. Rabobank biofuels round table: Kuala Lumpur, August 24th, 2006.

Kleingartner, L.W. 2004. World outlook and future development of sunflower markets around the world. p. 69-77. In: Proc. 16th Int. Sunflower Conf., Fargo, ND, USA.

Lilleboe, D. 1997. Wild opportunity?. The Sunflowers April/May. p. 8-9.

MAPA. 2007. Ministerio de Agricultura, Pesca y Alimentación. http://www.mapa.es

Miller, J.F., and K. Al-Khatib. 2002. Registration of imidazolinone herbicide-resistant sunflower maintainer (HA 425) and fertility restorer (RHA 426 and RHA 427) germplasms. Crop Sci. 42:988-989.

Official Journal of the European Union. 2003a. Council Regulation (EC) No 1782/2003. (21-10-2003). Regulations (EEC) No 2019/93, (EC) No 1452/2001, (EC) No 1453/2001, (EC) No 1454/2001, (EC) 1868/94, (EC) No 1251/1999, (EC) No 1254/1999, (EC) No 1673/2000, (EEC) No 2358/71 and (EC) No 2529/2001: 21-10-2003, L270, 69 p.

Official Journal of the European Union. 2003b. Directive 2003/30/EC of the European Parliament and the Council: Promotion of the use of biofuels or other renewable fuels for transport. 17.5.2003, L123: 42-45.

Official Journal of the European Union. 2003c. Council Directive 2003/96/EC (27-10-2003) restructuring the Community framework for the taxation of energy products and electricity. L 283/51.

Oilworld. 2007. Oilword statistics. http://www.oilword.biz

Putt, E.D. 1978. History and present status: p. 1-29. In: Sunflower Science and Technology: Monography No. 19 Agronomy Series. American Society of Agronomy, Madison, WI, USA.

Soldatov, K.I. 1976. Chemical mutagenesis in sunflower breeding. p. 352-357. In: Proc. 7th Int. Sunflower Conf., Krasnodar, USSR.

Tan, C. 2007. Southeast Asia Biodiesel; Getting a Head Start. Rabobank International May 15th, 2007.

Vannozzi, G.P., 2006. The perspectives of use of high oleic sunflower for oleochemistry and energy raws. Helia 29(44):1-24.

RAEA. 1994. Red Andaluza de Experimentación Agraria: Girasol, campaña 1994. Instituto de Investigación y Formación Agraria y Pesquera. Conserjería de Innovación, Ciencia y Empresa y Consejería de Agricultura y Pesca. Junta de Andalucía, Seville, Spain.

RAEA. 1998. Red Andaluza de Experimentación Agraria: Girasol, campañas 1998. Instituto de Investigación y Formación Agraria y Pesquera. Conserjería de Innovación, Ciencia y Empresa y Consejería de Agricultura y Pesca. Junta de Andalucía, Seville, Spain.

RAEA. 2000. Red Andaluza de Experimentación Agraria: Girasol, campaña 2000. Instituto de Investigación y Formación Agraria y Pesquera. Conserjería de Innovación, Ciencia y Empresa y Consejería de Agricultura y Pesca. Junta de Andalucía, Seville, Spain.

USDA. 2008. United Status Department of Agricultura. http://www.usda.gov

Phomopsis control in sunflower using products of biogenic origin

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ABSTRACT

The regulation of growth and development in sunflower plants was studied. Resistance to Phomopsis and productiveness of sunflower plants were induced by treating seeds with a disease resistance inducer of elicitor nature based on chitosan together with plant growth regulators.

Key words: albite – chitosan – disease resistance inducer – growth regulator – Phomopsis – zircon.

INTRODUCTION

As the world literature indicates and also our data show, the fungus *Diaporte (Phomopsis) helianthi* (Munt.-Cvet et.al.), which affects both sunflower and wild plants, possesses a high infection potential that contributes to its expansion and injuriousness in Russia and other countries in which sunflower is cultivated. The available domestic and international sets of commercial and promising newly developed sunflower cultivars and hybrids are susceptible to this disease. Therefore, Phomopsis remains a problematic disease both for Russia and for other countries.

Within the current arsenal of pest control agents in crops, including those against Phomopsis in sunflower, chemical fungicides continue to be of priority. Research conducted in Yugoslavia, Romania, France, Russia and other countries demonstrated that the available range of chemical fungicides does not reduce the infection and, therefore, the effectiveness of protective measures remains low (Piven et al., 1997; Chaban, 1990).

In this connection, research on inducing the resistance based on strengthening the natural defense mechanisms of plants has very good prospects for developing environmentally safe and high-yielding crop production technologies. In fact, the concept of induced resistance appeared together with the purpose of using induced immunity in practice. The most important prerequisite to considering induced resistance as an actual phenomenon includes the fact that the defense reactions are triggered as a response to infection both in resistant and susceptible plants (Tyuterev, 1999).

Both synthetic and natural biologically active compounds may be used as disease resistance inducers. Among the latter, most attention is currently being paid to chitin and its derivative chitosan (Reunov, 2001; Reddy et al., 1999). For the first time, high activity of chitosan in protecting plants was demonstrated by L.A. Hadwiger in 1986. He determined that the seed treatment with chitosan protects the plants from fungal and bacterial diseases and increases their yields by an average of 20% (Hadwiger, 1989). Even in the recent past, the mechanism of the chitosan action on plants was practically unknown. Nowadays, convincing data have been obtained showing high effectiveness and revealing mechanisms of the chitosan action on biological objects. Some publications (Begunov et al., 2004). reported an effect of the induced biosynthesis of chitinases and chitisanases, that resulted from treating plant cells with chitosan; they showed very high resistance against plant-pathogenic fungi and bacteria. It was determined that not only the cells, but also the plants of rice, pea, carrot and other crops sprayed with chitosan synthesized pectins along with chitinolytic enzymes and their joint action led to the complex cell wall destruction of plant pathogens that prevented their invasion of plants. Long-term tests of chitosan under laboratory and field conditions conducted by Russian research institutes (All-Russian Research Institute of Biological Plant Protection, Krasnodar and All-Russian Research Institute of Plant Protection, St. Petersburg) showed positive results by using the models including root rot pathogens in cereals, vegetables, and rice and mildew in cucumber and other crops. The highest plant protection effect of using chitosan was produced due to seed treatments (Begunov et al., 2002).

Before our work started, no references to a complex application of disease resistance inducers and plant growth regulators against Phomopsis in sunflower were available in literature. Therefore, the work

proposed by us that includes studying growth and development regulation, Phomopsis resistance induction and increase in sunflower yields by using the sunflower seed treatment with a disease resistance inducer of an elicitor nature based on chitosan, is especially important.

MATERIALS AND METHODS

In 2006 research was conducted using two sunflower cultivars: a susceptible cultivar Rodnik - growing season duration: 80 days, potential yield: 3.2 t/ha, oil content: 55%; and a tolerant cultivar Master - growing season duration: 94 days, potential yield: 4.0 t/ha, oil content: 54%. The experiment layout, as well as phenological, phytopathological and biometric studies were designed according to conventional methods (Begunov et al., 2004). The plot area was 100 m^2, three replicates and randomized block design were used. The experiment plots were planted on May 15. The soil of the plots included deep low-humic leached chernozem. The humus level in the arable soil layer was 3.4-4.1 %, pH_{salt} was 6.5, pH_{water} was7.5. Winter wheat was a predecessor.

The experiment included the use of a biogenic resistance inducer based on chitosan – chitosanium glutaminium succinate - in the mixture with phytohormonal plant growth regulators based on hydroxycinnamic (zircon), polybetabutyric (albite), heteroauxinic (IAA) acids and trace elements of chelate form (hydromics). Hydromics, zircon, chitosanium glutaminium succinate are recommended in the Russian Federation to be used for sunflower seed treatments, while albite and IAA are new experimental plant growth regulators for sunflower plants.

The tested disease resistance inducer and plant growth regulators were applied as different combinations for the seed treatments of both sunflower cultivars a day before planting. The product Maxim (active ingredient: fludioxonyl) was used as a standard. A treatment without the application of the above products served as control. Experiment design, applied compositions and application rates are shown in Table 1.

The progress of Phomopsis in sunflower plants was evaluated using the VNIIMK scale.

Table 1. Experiment design

#	Experimental treatment	Rate of application, kg,l/t of seeds
1	Chitosanium-glutaminium succinate + zircon	0.2 + 0.2
2	Chitosanium-glutaminium succinate + phloroxan + IAA + zircon	0.2+0.0005 + 0.006+0.2
3	Chitosanium-glutaminium succinate + albite	0.2 + 0.1
4	Chitosanium-glutaminium succinate + phloroxan + IAA + albite	0.2 + 0.0005 + 0.006 + 0.1
5	Chitosanium-glutaminium succinate + hydromics	0.2 + 0.15
6	Chitosanium-glutaminium succinate + phloroxan + IAA + hydromics	0.2 + 0.0005 + 0.006 + 0.15
7	Maxim (standard)	5.0
8	Control	Untreated

Biological effectiveness was calculated with the formula:

$$B = \frac{Pc-Pt}{Pc} \times 100\ \%$$

where B – biological effectiveness, %;
Pc – Phomopsis progress in control;
Pt – Phomopsis progress in an experimental treatment.

The sunflower was harvested with a Sampo combine.

The agrometeorological conditions during the sunflower growing season were rather favorable both for sunflower growth and Phomopsis progress. The hydrothermic coefficient for the growing season was 1. The mathematical data processing was done using the variance analysis method.

RESULTS

Research on determining biological parameters of the chitosanium-glutaminium succinate application against Phomopsis showed that the highest disease resistance induction of 56-60 % was caused in sunflower plants as a result of treating their seed, *i.e.* when the defense mechanisms were launched at the

early plant ontogeny stages. The optimum rate of application was 0.2 kg/t of seeds (Begunov et al., 2004). In addition, it was shown that chitosan has good hydrophylic property, complex formation ability, film-forming capacity, absence of toxicity and broad-spectrum biological activity.

All these characteristics of chitosan provided a stimulus to search for the possibilities to enhance its biological activity, add new useful properties to this polymer and create its novel compositions with non-phytotoxic biologically active compounds.

The sunflower seed treatments using chitosanium-glutaminium succinate with zircon, IAA, albite and hydromics stimulated germination and activated initial growth and development of plants. For the Rodnik cultivar, the root growth stimulation reached 170% (Treatment 6) and the stem growth stimulation reached 140% (Treatment 1). For the cultivar Master, the maximum root and stem stimulation values were recorded in the Treatments 2 and 1 and they reached 150 and 119%, respectively. On the whole, the growth-stimulating process at the first ontogeny stages was more active in the early-ripening cultivar Rodnik than in the late-ripening cultivar Master (Table 2).

Table 2. Evaluation of the growth-stimulating action on sunflower plants produced by the tested formulations under field conditions

Treatment #	Cultivar Rodnik Germination on the 14th day				Cultivar Master Germination on the 14th day			
	Root (cm)	Percentage of control	Stem (cm)	Percentage of control	Root (cm)	Percentage of control	Stem (cm)	Percentage of control
1	9.3	137	22.0	140	9.4	130	17.5	119
2	8.7	128	19.7	125	10.7	150	16.4	111
3	9.6	140	19.8	125	9.0	120	17.0	116
4	8.5	125	19.3	120	10.5	140	15.4	105
5	9.4	138	19.3	120	10.5	140	16.3	110
6	11.6	170	18.2	115	10.5	140	16.3	110
7	8.5	125	17.7	110	10.5	140	16.0	110
8	6.8		15.8		7.3		14.7	
Control								
HCP_{05}	0.55		1.34		0.50		0.68	

Our further phenological observations showed that the sunflower plants of both cultivars formed the second pair of true leaves in the treatments where the tested compositions had been applied two or three days earlier than in the standard (Maxim) and control treatments. Also, the accelerated budding and flowering stages were recorded for the plants whose seeds had been treated with the tested compositions.

Effects of the compositions of the tested disease resistance inducer and plant growth regulators were tested under natural conditions at the field. It should be noted that the weather conditions were especially favorable for the progress of Phomopsis at all the developmental stages of the plants, from germination to flowering. For that period, 21 rainy days were recorded with 250 mm of total rainfall. The disease incidence and severity were evaluated at the budding, flowering and physiological ripeness stages.

The first symptoms of the Phomopsis leaf form were detected in the control plot at the budding stage on June 20 for the cultivar Rodnik and on June 29 for the cultivar Master. By July 29, 6% of the sunflower plants of Rodnik and 4% of Master had been affected by Phomopsis. The stem form of Phomopsis appeared in the plants of Rodnik on July 10 and in the plants of Master on July 24.

Table 3 shows the results of the evaluation of Phomopsis stem symptoms at the physiological ripeness stage. The cultivar Rodnik showed 42.3% of diseased plants, with 23.6% of disease severity; for the cultivar Master these values were 26.7 % and 12.2 %, respectively.

The data analysis showed that the combined application of chitosanium-glutaminium succinate togeteher with IAA, phloroxan, zircon, and albite produced the most effective protective action on plants. Induced biological effectiveness in these experimental treatments (2 and 4) was 71-73 % for Rodnik and 81-88 % for Master. Therefore, it was shown that plant growth regulators such as albite and zircon in combination with chitosanium-glutaminium succinate strengthened the defense mechanism of sunflower plants against Phomopsis.

Among the tested plant growth regulators, the seed treatments with the combination of disease resistance inducer and zircon (Treatments 1 and 2) and albite (Treatments 3 and 4) contributed to obtaining higher yields from both cultivars.

Table 3. Biological and economic effectiveness of the tested formulations

Treatment	Rodnik				Master			
	Incidence (%)	Severity (%)	Biological effectiveness (%)	Yield (t/ha)	Incidence (%)	Severity (%)	Biological effectiveness (%)	Yield (t/ha)
1	16.2	12.1	49	2.19	12.9	5.9	52	2.21
2	8.7	6.3	73	2.15	8.5	2.3	81	2.11
3	12.0	8.0	73	2.20	5.2	4.9	52	2.10
4	10.3	6.9	71	2.04	5.2	1.5	88	2.11
5	9.3	6.1	74	1.95	7.9	3.5	71	2.02
6	12.1	8.4	64	2.10	14.7	5.3	57	2.06
7	14.9	16.2	31	1.90	26.0	11.2	8	1.85
8 (Control)	42.3	23.6	-	1.89	26.7	12.2	-	1.76
HCP_{05}				0.083				0.085

DISCUSSION

The combined application of the tested biogenic disease resistance inducer and phytohormonal plant growth regulators albite and zircon enhances the Phomopsis resistance of sunflower plants and increases their yields.

Thus, this area of research on improving disease resistance in plants without changing their genome is a connecting link between fundamental immunology and practical crop protection. In this connection, using the products based on chitosan is very promising, and the induced resistance caused by them may be considered as one of the biological methods for disease control.

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REFERENCES

Begunov, I.I., K.V. Novozhilov, S.L. Tyuterev, et al. 2002. Composition including chitosan for enhancing resistance to plant diseases. U.S. Patent 6413910.

Begunov, I.I., V.D. Nadykta, V.I. Terekhov, and A.G. Begunova. 2004. Determining biological parameters for applying chitosan-based compositions against Phomopsis in sunflower. Biological plant protection as a basis for stabilizing agroecosystems. Krasnodar 2:318-321.

Chaban, V.S. 1990. Development and introduction of the systems for applying highly effective chemical and biological pest control agents safe for the environment and humans in sunflower. Ukrainian Research Institute of Plant Protection, Kiev. p.106.

Hadwiger, L.A. 1989. Method for treating cereal crop seed with chitosan to enhance yield, root growth and stem strength. U.S. Patent 4886541. Washington State University Research Foundation.

Piven, V.T., E.L. Slusar, and E.G. Dolzhenko. 1997. Effectiveness of fungicides against Phomopsis. Sci. and Techn. VNIIMK Bul., Krasnodar 118:75-77.

Reddy, M.V., I.J. Bhaskara, P. Angers, and L. Couture. 1999. Chitosan treatment of wheat seeds induces resistance to *Fusarium graminearum* and improves seed quality. J. Agric. Food Chem. 47:1208-1216.

Reunov, A.V. 2001. Immunization by biologically active compounds as a promising approach to crop protection. p. 170-172. In: Proc. of the Conf. Bioact. Comp. from Macro- and Microorg. in the Far East, Vladivostok.

Tyuterev, S.L. 1999. Scientific fundamentals for using chemical disease resistance activators within the crop protection from pathogens. Abstract of a thesis, St. Petersburg, Pushchino, p. 54.

Verticilosis en germoplasma de girasol

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RESUMEN

La verticilosis, causada por el agente patógeno *Verticillium dahliae* (Kleb), es una de las enfermedades más importantes que afecta al cultivo de girasol en Argentina. Se evaluó el comportamiento frente a *V. dahliae* de líneas de girasol del programa de mejoramiento de la EEA. Pergamino de INTA. La evaluación se realizó en 689 líneas aplicándose el método de inoculación artificial del patógeno en plántula. En la escala de severidad empleada se consideró: R: resistente, MR: moderadamente resistente, MS: moderadamente susceptible, S: susceptible y AS: altamente susceptible. De acuerdo al origen genético, se formaron 33 grupos, calculándose el porcentaje de líneas en cada una de las reacciones. La mayor amplitud de comportamiento se detectó en el compuesto P4. Se obtuvo mayor porcentaje de lecturas R en líneas provenientes de endocría de poblaciones mejoradas que en la descendencia de cruzas de líneas de diferentes orígenes. La combinación de las cruzas locales x exóticas, produjo mayor proporción de líneas de buen comportamiento que ambos grupos por separado. La resistencia a *Verticillium* puede ser obtenida a partir de diferentes fuentes de germoplasma.

Palabras clave: girasol - recursos genéticos - resistencia a enfermedades - *Verticillium* - Verticilosis.

ABSTRACT

Verticillium wilt , caused by pathogen *Verticillium dahliae* (Kleb), is one of the principal diseases of sunflower (*Helianthus annuus* L.) in Argentine. The objective of this research was to evaluate lines of the sunflower breeding program of E.E.A. Pergamino to *Verticillium* reaction. Seedling inoculation method was applied to evaluate the performance of 689 lines. The scale was R (resistant), MR (moderately resistant), MS (moderately susceptible), AS (very susceptible). According to their genetic background, 33 groups were formed and the reaction percentage in each group was calculated. The largest variability was detected in P4. A larger percentage of resistance lines was obtained from inbred lines derived from improved populations than from populations derived from crossing inbred lines of different origins. Lines derived from crosses of the type local x exotic performed better than both groups separately. Resistance to *Verticillium* can be obtained from different sources of germplasm

Key words: disease resistance – genetic resources – sunflower – *Verticillium* – Verticillium wilt.

INTRODUCCIÓN

La verticilosis, causada por el hongo *Verticillium dahliae* Kleb, se manifiesta inicialmente por un marchitamiento foliar ocasionado por el taponamiento de los tejidos de conducción que progresa desde la raíz. Se visualiza inicialmente en las hojas inferiores y luego en las superiores, posteriormente se puede observar áreas cloróticas internervales y necrosis (Zimmer and Hoes, 1978). Se afecta el rendimiento por reducción del peso de granos y el contenido de aceite (Bertero de Romano et al., 1994; Pereyra et al., 1999). Es una enfermedad de gran incidencia económica por lo cual la obtención de cultivares de buen comportamiento es una meta prioritaria en los programas de mejoramiento de la especie en Argentina (González et al., 2003).

El objetivo del presente trabajo fue evaluar el comportamiento frente a este patógeno de líneas del programa de mejoramiento de girasol de la EEA Pergamino INTA, provenientes de distinto origen genético.

MATERIALES Y MÉTODOS

En la E.E.A. Pergamino INTA se analizó la reacción de 689 líneas estabilizadas frente a *Verticillium dahliae* para evaluar la incidencia del patógeno. Se empleó el método de inoculación artificial en plántula en invernáculo (Bugbee y Presley, 1967) de alta correlación con la incidencia a campo. Se inocularon 20 plántulas de cada línea al estado de 3 hojas verdaderas (aproximadamente 20 días después de la siembra), Las lecturas se realizaron a las tres semanas de la inoculación con la siguiente escala: R (Resistente) sin

síntomas foliares, sana; MR (Moderadamente resistente) con áreas cloróticas; MS (Moderadamente susceptible) áreas cloróticas y necróticas; S (Susceptible) con predominio de manchas necróticas; AS (Altamente susceptible) con necrosis y deformaciones foliares. Los genotipos se agruparon por origen en 33 grupos en los cuales se calculó el porcentaje de líneas que tenia cada uno de los grupos en la escala de severidad descrita.

RESULTADOS Y DISCUSIÓN

En la Tabla 1 se presentan los resultados de la reacción frente al patógeno de las líneas derivadas de poblaciones y cruzamientos de líneas locales, exóticas, y locales por exóticas, en los 33 grupos (Anexo I: Descripción del germoplasma interviniente en la evaluación de *Verticillium*).

Tabla 1. Reacción frente al patógeno *V. dahliae* de las líneas derivadas de poblaciones y cruzamientos de líneas locales, exóticas y locales por exóticas

Origen	Reacción a *V. dahliae* (%)					
	Número de líneas	**R:** Resistente	**MR**: Moderada-mente resistente	**MS**: Moderada-mente susceptible	**S**: Susceptible	**AS**: Altamente susceptible
LINEAS LOCALES						
RK 489/AXB 3479	9	0	0	22	11	67
RK 456/BXC 3496	18	0	0	6	33	61
LXN 621/BXC3496	20	0	0	15	30	55
KLM 280/RK 489	17	0	6	24	11	59
KLM 280/GP 762	8	0	0	37	50	13
KLM 214/GP 762	3	0	0	0	67	33
GP 762/BXC 3496	39	0	0	10	18	72
GP 762/AXB 3479	15	0	6	47	27	20
DXT 3331/AXB 3479	5	0	0	0	40	60
BXC 97/01/KLM 214	10	0	10	20	50	20
BXC 97/01/DXT 3331	13	0	0	31	23	46
BXC 97/01/AXB 3479	28	0	7	11	46	36
RF 00/16	10	0	0	20	60	20
RF 00/01	10	0	0	30	70	0
RF 97/01	18	0	22	56	22	0
LINEAS EXÓTICAS						
ND 01	23	0	0	20	55	25
HA 89 x HAR 4	16	0	25	31	38	6
HA 301xCHERNY-66/	30	0	3	37	53	7
HA 337 / HA 335	21	0	0	48	47	5
HA 338/373 1x CHERNY-66	18	0	6	66	28	0
HA 343 x NOVINKA	10	0	0	10	70	20
LINEAS LOCALES POR EXÓTICAS						
LXN 621/HA 89	42	0	0	10	26	64
KLM 280/HA 822	28	0	0	22	39	39
HA 89/DXT 3330	4	0	0	100	0	0
AxB 3479-2-2-1/ DxT 3331-3-1-2/HA 300	11	9	36	32	23	0
LxN 621 / KLM 280/HA 300	48	0	8	23	67	2
RK 426-11 /KLM-280/HA 300	31	3	42	45	10	0
POBLACIONES						
Compuesto P2	12	0	0	17	58	25
Compuesto P3	9	0	0	44	44	12
Compuesto P4	15	7	13	20	33	27
Compuesto P6	5	0	0	20	20	60
VNIIMK 6540	66	0	18	58	24	0
VNIIMK 1646	77	22	51	19	8	0

Las mayores diferencias de comportamiento se dieron en las líneas derivadas del Compuesto P4 (de origen rumano, mezcla de Record, Sintética OS2 y Sintética Horizonte), y las menores en las líneas derivadas de la cruza de HA 89 / DXT 3330. Las líneas derivadas de las cruzas en que intervienen AXB 3479-2-2-1; DXT 3331-3-1-2 y HA 300 (derivada de Peredovik 301), tuvieron también alto porcentaje de

lecturas R y MR. Un comportamiento similar presentaron los genotipos originados en la cruza HA89 x HAR 4 (esta última línea originada a partir de Saenz Peña 74-1-2 de buena sanidad). Se obtuvieron también genotipos de buen comportamiento derivados de los cruzamientos entre RK 426-11, originada en el Compuesto RK y KLM 280, originada en el Compuesto KLM. Comparando las líneas obtenidas a partir de selección y endocría de poblaciones con los obtenidas a partir de cruzas entre líneas de distinto origen; se obtuvo mayor porcentaje de lecturas R explorando la variabilidad de las primeras (Tabla 1).

Comparando los grupos originados de líneas derivadas de locales con las de líneas exóticas y con la combinación de ambas; se obtuvo mayor porcentaje de líneas con resistencia en la combinación de ambas que en cada grupo por separado, destacándose AXB 3479/DXT 3331/HA 300 y RK 426-11/KLM 280/ HA 300. Se destaca la importancia de la variedad rusa VNIIMK 1646 como una fuente de resistencia a verticilosis, teniendo en cuenta el alto porcentaje de lecturas R y MR observado en líneas derivadas de la misma, siguiéndole en aptitud el Compuesto P4.Los resultados indicaron una amplia capacidad de respuesta en el fondo genético analizado. En consecuencia, sería posible obtener resistencia genética al patógeno a partir de fuentes de diverso origen.

Anexo I. Descripción del germoplasma interviniente en la evaluación de *Verticillium*

Designación	Origen genético / derivada de:
Locales (1)	
AxB 00/01	71/538, LC 206020
BxC 00/01	LC 206020, MP 555 (Rusa, silvestres)
BxC 97/01	LC 206020, MP 555 (Rusa, silvestres)
DxT 00/01	MP 557, Negro Bellocq
DxT 00/02	MP 557, Negro Bellocq
GP 762	Primeras líneas del programa de EEA Pergamino
KLM 214	Compuesto KLM (Klein, Local, Manfredi)
KLM 280	Compuesto KLM (Klein, Local, Manfredi)
LxN 621	Compuesto LxN (Local x Ruso)
Rf 00/16	A 871
Rf 00/01	M 731-243
Rf 97/01	S 3107
RK 426-11	Compuesto RK (Ruso, Klein)
RK 456	Compuesto RK (Ruso, Klein)
RK 489	Compuesto RK (Ruso, Klein)
Exóticas (2)	
CHERNY-66	Chernianka
HA 300	Peredovick 301, North Dakota, 1976
HA 301	North Dakota, 1976
HA 335	North Dakota, 1986
HA 337	North Dakota, 1986
HA 338	North Dakota, 1986
HA 343	North Dakota, 1986
HA 89	North Dakota, 1971
HA R 4	North Dakota, 1984 Saenz Peña 74-1-2
ND 01	North Dakota, 1984 Alto oleico
Novinka	Variedad rusa
Poblaciones	
Compuesto P2	6 B; Ienissei
Compuesto P3	Comangir (silvestre, cultivado)
Compuesto P4	Compuesto rumano: Record, Sintética OS2, Sintética Horizonte
Compuesto P6	Precoz, alto, aceite, Americano
VNIIMK 1646	Variedad rusa
VNIIMK 6540	Variedad rusa

(1) Locales: Obtenidas en INTA-EEA Pergamino; (2) Exóticas: No obtenidas en la EEA Pergamino

REFERENCIAS

Bertero de Romano, A., A. Vázquez, S. Piubello, and C. Sala. 1994. Quantifying the relationship between Verticillium wilt and yield loss of sunflower *(Helianthus annuus* L.), and feasibility of utilizing the hypodermic inoculation technique as a selection method. Helia 17(20):49-54.

Bugbee, W.M., and J.T. Presley. 1967. A Rapid inoculation technique to evaluate the resistance of cotton to *Verticillium albo-atrum*. Phytopatol. 57:1264.

González, J., N. Mancuso, P. Ludueña, y A. Ivancovich. 2003. Evaluación del comportamiento de líneas de girasol frente a *Verticillium dahliae*. Revista de Tecnología Agropecuaria 8(23):9-10.

Pereyra V., F. Quiroz, M.E. Agüero, and A. Escande. 1999. Relación del rendimiento del girasol con la intensidad de síntomas provocados por *Verticillium dahliae*. p. 35. In: X Jornadas Fitosanitarias Argentinas, Jujuy.

Zimmer, D.E., and J.A. Hoes. 1978. Diseases. In: In: J.F. Carter (ed.), Sunflower Science and Technology. Am. Soc. Agron., Madison, WI, USA.

Towards *Sclerotinia* resistance – *In vitro* screening of wild sunflower species

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ABSTRACT

This paper presents the work on testing the possibility of the use of *in vitro* screening for determination of wild *Helianthus* species resistance to *Sclerotinia*. For this purpose, micropropagated plants of different accessions of *H. maximiliani*, *H. mollis*, *H. rigidus* and *H. tuberosus* were grown on MS medium supplemented with 0, 0.5, 1 and 2 mM of oxalic acid. Fresh and dry weight of above-ground part, and dry weight of root could be considered as the potential parameters of wild species resistance/susceptibility to *Sclerotinia* in *in vitro* tests, as they were not affected by treatment in resistant (100%) accessions and were significantly decreased in susceptible (25%) ones in the presence of 2 mM of oxalic acid.

Key words: oxalic acid – *in vitro* screening – resistance – *Sclerotinia sclerotiorum* – wild sunflower.

INTRODUCTION

White rot caused by the fungus *Sclerotinia sclerotiorum* Lib. (de Bary) is the major disease of sunflower (*Helianthus annuus* L.) in countries with a humid climate, while in countries with moderate climate, it causes yield losses in rainy years (Škorić and Rajcan, 1992). Wild sunflowers (*Helianthus* spp.) constitute an important source of resistance against several major sunflower diseases including *Sclerotinia* (Georgieva-Todorova, 1993). Populations of several wild sunflower species were found to be tolerant to white rot (Škorić and Rajcan, 1992; Henn et al., 1997; Tavoljanski et al., 2002; Cerboncini et al., 2002; Vasic et al., 2004). Resistance screening was done either by observing naturally occurring infection (Tavoljanski et al., 2002) or by using different artificial inoculation methods (Henn et al., 1997; Cerboncini et al., 2002; Vasic et al., 2002; 2004).

De Bary was the first researcher to associate oxalic acid with *Sclerotinia* infection (Lumsden, 1979). Later, Noyes and Hankock (1981) demonstrated its importance as a factor in the pathogenicity of this fungus, while Hartman et al. (1988) found a correlation between oxalic acid production and virulence of different *Sclerotinia* isolates. There have been several attempts to create a bioassay in which resistance to oxalic acid would be used as an indicator of resistance to *Sclerotinia* (Hartman et al., 1988; Noyes and Hancock, 1981; Raducanu and Soare, 1992; Tu, 1985; Vasic et al., 1999; 2002). Whole plants or their parts were used, and correlation was found between field susceptibility/resistance of tested genotypes to *Sclerotinia* and reaction of the explants of the same genotypes when grown on a medium into which oxalic acid was added.

As maintenance of wild species collection and field screening are costly and labour-intensive, we have tested the possibility of the use of *in vitro* screening for determination of wild sunflower species resistance to *Sclerotinia*.

MATERIALS AND METHODS

Accessions of *H. maximiliani* Schrader (max), *Helianthus mollis* Lam. (mol), *H. rigidus* (Cass.) Desf. (rig) and *H. tuberosus* L. (tub) were obtained from wild *Helianthus* species collection of Institute of Fields and Vegetable Crops in Novi Sad, Serbia (Table 1). The accessions were pre-screened for *Sclerotinia* resistance by measuring sclerotia infection on stem (Vasic et al., 2004). Their resistance was determined as the percentage of healthy plants (Table 1).

The plants were propagated *in vitro* using culture of apical shoots (Vasic et al., 2001). Prior to transfer to a propagation medium, shoots were dipped into 0.1% indolebuteric acid (IBA) solution for 4 min. For the resistance screening, apical shoots of *in vitro* grown plants were placed in 250 ml Erlenmeyer flasks with 80 ml of MS medium (Murashige and Skoog, 1962), pH 5.7, supplemented with 5 g l^{-1} of sucrose, 6 g l^{-1} of agar, and different concentrations of oxalic acid (Table 1). Control plants were

grown on MS medium without oxalic acid. There were four Erlenmeyer flasks with four shoots per accession for each oxalic acid concentration. One Erlenmeyer flask was treated as one replication in the data analysis. The shoots were grown at 24ºC with a photoperiod of 16 h (light)/8 h (dark).

After six weeks of culture, the following parameters were measured: plant height, fresh and dry weight of above-ground part, root length, fresh and dry weight of root. The data were analysed using ANOVA and LSD test.

RESULTS AND DISCUSSION

Analysis of variance showed that both genotype and treatment had significant effect on the measured parameters.

Table 1. Reaction of tested wild sunflower accessions on treatment with different concentrations of oxalic acid[1,2].

Genotype	Resistance (%)	Concentration mM	h	rl	fm	dm	rfm	rdm
mol x	100	Control	10.875a	3.925a	0.476a	0.049a	0.478a	0.039a
		0.5	4.625b	5.225a	0.208b	0.0185b	0.132b	0.011c
		1	3.550b	3.650a	0.183b	0.019b	0.340ab	0.011c
		2	4.075b	5.075a	0.395a	0.040a	0.296ab	0.025b
		$LSD_{0.05}$	2.750	2.022	0.151	0.014	0.342	0.009
		$LSD_{0.01}$	3.855	2.835	0.212	0.020	0.479	0.014
mol 1298	100	control	15.200a	11.200a	0.293ab	0.030b	0.088ab	0.006b
		0.5	9.325c	2.750c	0.153c	0.022b	0.059b	0.004b
		1	13.675ab	7.525b	0.253bc	0.026b	0.117ab	0.007ab
		2	10.950bc	8.525ab	0.387a	0.042a	0.146a	0.010a
		$LSD_{0.05}$	3.328	2.732	0.113	0.011	0.062	0.003
		$LSD_{0.01}$	4.666	3.829	0.159	0.015	0.087	0.005
max 34	75	control	14.700a	11.875a	0.492a	0.048a	0.183a	0.013a
		0.5	12.425ab	3.550c	0.299b	0.025b	0.048b	0.004b
		1	11.775b	3.575c	0.277b	0.030b	0.074b	0.006b
		2	4.800c	6.550b	0.338b	0.048a	0.245a	0.017a
		$LSD_{0.05}$	2.862	2.812	0.139	0.016	0.067	0.004
		$LSD_{0.01}$	4.012	3.942	0.195	0.023	0.094	0.006
max 1631	50	control	15.400a	18.775ab	1.493a	0.105a	0.749a	0.048a
		0.5	16.225ab	14.650b	0.945a	0.066a	0.274b	0.016b
		1	14.100ab	22.325a	1.503a	0.104a	0.884a	0.051a
		2	11.825b	18.675ab	1.058a	0.077a	0.416ab	0.025ab
		$LSD_{0.05}$	3.836	4.436	0.737	0.047	0.473	0.028
		$LSD_{0.01}$	5.377	6.219	1.034	0.066	0.664	0.039
tub 675	50	control	12.600a	11.325a	0.690b	0.025c	0.257b	0.016a
		0.5	10.425a	11.175a	1.018a	0.054b	0.364a	0.021a
		1	8.600a	10.700a	0.512b	0.078a	0.201bc	0.029a
		2	2.600b	4.450b	0.134c	0.043bc	0.154c	0.048a
		$LSD_{0.05}$	4.535	4.291	0.259	0.021	0.101	0.048
		$LSD_{0.01}$	6.358	6.016	0.363	0.030	0.142	0.067
rig 1692	50	control	13.625a	12.850a	0.312b	0.030b	0.249b	0.020b
		0.5	14.750a	12.025a	0.486a	0.053a	0.380a	0.034a
		1	14.375a	10.700a	0.257b	0.025b	0.197b	0.014b
		2	11.500a	11.675a	0.259b	0.026b	0.173b	0.015b
		$LSD_{0.05}$	5.003	5.082	0.108	0.009	0.056	0.006
		$LSD_{0.01}$	6.697	5.880	0.159	0.015	0.085	0.008
mol 1530	25	control	12.175a	4.150a	0.678b	0.048b	0.215b	0.016b
		0.5	12.350a	3.475a	0.728b	0.070ab	0.497ab	0.042ab
		1	13.525a	4.050a	1.359a	0.115a	0.926a	0.067a
		2	5.350b	3.925a	0.626b	0.055b	0.314b	0.031ab
		$LSD_{0.05}$	2.239	1.290	0.623	0.050	0.483	0.038
		$LSD_{0.01}$	3.140	1.809	0.873	0.070	0.677	0.053
rig 1843	25	control	11.125a	6.575ab	0.529b	0.062b	0.473b	0.047b
		0.5	8.975a	6.350ab	0.448b	0.059b	0.372b	0.041b
		1	7.950ab	7.500a	0.781a	0.089a	0.835a	0.083a
		2	4.800b	4.825b	0.530b	0.063b	0.346b	0.038b
		$LSD_{0.05}$	3.466	$LSD_{0.05}$	0.180	0.022	0.258	0.025
		$LSD_{0.01}$	4.860	$LSD_{0.01}$	0.252	0.031	0.362	0.035

[1]Within each column, genotype means followed by different letter differ significantly at the level p=0.05.
[2]Legends for traits: h - plant height, rl - root length, fm - fresh weight of above-ground part, dm – dry weight of above-ground part, rfm - fresh weight of root, rdm - dry weight of root.

The choice of oxalic acid concentrations was made based on the research done on cultivated sunflower protoplasts (Vasic et al., 1999) and intact plants grown *in vitro* (Vasic et al., 2002). Results obtained with 0.5 and 1 mM concentrations of oxalic acid were not conclusive as there was neither any difference between resistant and susceptible accessions nor a regular pattern in measured parameter variation (Table 1). This is in accordance with the results obtained on the sunflower plants grown in the presence of oxalic acid (Vasic et al., 2002). The same applies in the reaction of tolerant accessions (*H. maximiliani* 1631, *H. tuberosus* 675 and *H. rigidus* 1692) to oxalic acid treatment, and is probably the consequence of differences in morphology and biochemistry between wild sunflower species (Heiser et al., 1969).

Concentration of 1 mM of oxalic acid had a stimulant effect on the most susceptible accessions – *H. mollis* 1530 and H. r*igidus* 1843 (Table 1). Stimulant effect of non-selective concentrations of stress agents in *in vitro* culture was also observed in the experiments with herbicides, and is thought to be a consequence of the phenomenon that stress agents when present in small concentrations act as nutrients (Olofsdotter et al., 1994).

Similarly to the work of Vasic et al. (2002), concentration of 2 mM of oxalic acid discriminated between resistant and susceptible genotypes. This oxalic acid concentration only affected plant height in the resistant accessions (*H. mollis* x and 1298) and almost all the traits in susceptible ones, except for the root length in *H. mollis* 1530 (Table 1). This is in disagreement with the results of Mouly (1989) who found that concentrations of oxalic acid lower than 4.44 mM were not selective in a bioassay with sunflower leaves. The same author recommended a concentration of 8.88 mM as optimal.

Fresh and dry weight of above-ground parts and dry weight of root could be considered the potential parameters of wild sunflower resistance/susceptibility to *Sclerotinia* in *in vitro* tests, as they were not affected by treatment in resistant accessions and they were significantly decreased in susceptible ones in the presence of 2 mM of oxalic acid (Table 1). In contrast to the results obtained in cultivated sunflower (Vasic et al., 2002), plant height and root length were not good indicators of wild sunflower resistance/susceptibility to *Sclerotinia*. This may be due to structural differences between cultivated and wild sunflowers and their biochemical reaction to *Sclerotinia*, as previously observed in *H. resinosus* Small (Mondolot-Cosson and Andary, 1994).

The results obtained in our study showed that there is potential for the use of oxalic acid bioassays for screening wild sunflower species for resistance to *Sclerotinia*. Fresh and dry weight of above-ground parts and dry weight of root were found to be good morphological parameters for discrimination between resistant and susceptible accessions, in combination with an oxalic acid concentration of 2 mM. However, more work should be done in determining the optimal oxalic acid concentration. Also, the morphological and biochemical differences between different sunflower species should be taken into account in further studies.

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REFERENCES

Cerboncini, C., G. Beine, P.C. Binsfeld, B. Dresen, H. Peisker, A. Zerwas, and H. Schnabl. 2002. Sources of resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary in a natural *Helianthus* gene pool. Helia 25:167-176.

Georgieva-Todorova, J. 1993. Interspecific hybridization and its application in sunflower breeding. Biotechnol. Biotechnol. Eq. 7:153-157.

Hartman, C.L., P.A. Donald, G.A. Secor, and J.F. Miller. 1988. Sunflower tissue culture and use in selection for resistance to *Phoma macdonaldii* and white mold (*Sclerotinia sclerotiorum*). p. 347-351. In: Proc. 12th Int. Sunflower Conf., Novi Sad, Yugoslavia.

Heiser, C.B., D. Smith, S. Clevenger, and W. Martin. 1969. The North American sunflower (*Helianthus*). Mem. Torrey Bot. Club 22:1-218.

Henn, H.J., U. Steiner, R. Wingender, and H. Schnabl. 1997. Wildtype sunflower clones: Source of resistance against *Sclerotinia sclerotiorum* (Lib.) de Bary stem infection. Angew. Bot. 71:5-9.

Lumsden, R.D. 1979. Histology and physiology of pathogenesis in plant diseases caused by *Sclerotinia* species. Phytopathology 69:890-896.

Mondolot-Cosson, L., and C. Andary. 1994. Resistance factors of a wild species of sunflower, *Helianthus resinosus*, to *Sclerotinia sclerotiorum*. Acta Hortic. 381:642-645.

Mouly, A. 1989. Recherche de marqueurs moleculaires de la tolerance du tournesol a *Sclerotinia sclerotiorum*. PhD Thesis, UPS Toulouse, France.

Murashige, T., and F. Skoog. 1962. A revised medium for growth and bioassays with tobecco tissue cultures. Physiol. Plant. 15:473-497.

Noyes R.D., and J.G. Hancock. 1981. Role of oxalic acid in the *Sclerotinia* wilt of sunflower. Physiol. Plant Pathol. 18:123-132.

Olofsdotter, M., A. Olesen, S.B. Andersen, and J.C. Streibig. 1994. A comparison of herbicide bioassays in cell cultures and whole plants. Weed Res. 34:387-394.

Raducanu, F., and G. Soare. 1994. An approach of *in vitro* testing sunflower for resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary. Rom. Agric. Res. 2:35-39.

Škorić, D., and I. Rajcan. 1992. Breeding for *Sclerotinia* resistance in sunflower. p. 1257-1262. In: Proc. 13th Int. Sunflower Conf., Pisa, Italy.

Tavoljanski, N., A. Yesaev, V. Yakutkin, E. Akhtulova, and V. Tikhomirov. 2002. Using the collection of wild species in sunflower breeding. Helia 25:65-78.

Tu, J.C. 1985. Tolerance of white bean (*Phaseolus vulgaris*) to white mold (*Sclerotinia sclerotiorum*) associated with tolerance to oxalic acid. Physiol. Plant Pathol. 26:111-117.

Vasic, D., G. Alibert, and D. Škorić. 1999. *In vitro* screening of sunflower for resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary. Helia 22:95-104.

Vasic, D., D. Škorić, G. Alibert, and V. Miklič. 2001. Micropropagation of *Helianthus maximiliani* (Schrader) by shoot apex culture. Helia 24:63-68.

Vasic, D., K. Taski, S. Terzic, S. Kevresan, and D. Škorić. 2002. Transferring of *Sclerotinia* resistance from wild into cultivated sunflower - combining of conventional and laboratory techniques. Proc. Nat. Sci., Matica Srpska 102:29-33.

Vasic, D., N. Dusanic, V. Miklič, and D. Škorić. 2004. Transferring of *Sclerotinia* resistance from wild into cultivated sunflower – Evaluation of wild sunflower species. Acta Agronom. Serb. 65:5-10.

Correlation between macronutrient content and sunflower resistance to *Sclerotinia sclerotiorum* measured by sclerotia infection of stem

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ABSTRACT

Nutrition of plants has a substantial impact on the predisposition of plants to be attacked or affected by pests and diseases. Since availability and nutrient quantity in the host plants is a limiting factor for *Sclerotinia sclerotiorum* infection development in sunflower, the aim of this work was to determine macronutrient content in photosynthetic tissue (leaves) of sunflower plants before and after development of fungus infection, and to find the correlation between macronutrient content and resistance to sclerotium infection. The study was carried out on eight sunflower inbred lines. Macronutrient (N, P, K, Ca and Mg) content was determined in dry plant material of control and sclerotium infected plants. There was a high positive correlation between N content in infected plants and resistance, which points to the important role of this nutrient in sunflower defence from *Sclerotinia* attack. Moderate negative correlation between K and Ca content in control plants showed that the content of these nutrients in healthy plants could be used as an indicator of cultivated sunflower resistance/susceptibility to *Sclerotinia* infection.

Key words: macronutrients – resistance – *Sclerotinia sclerotiorum* – sunflower.

INTRODUCTION

Nutrition of plants has a substantial impact on the predisposition of plants to be attacked or affected by pests and diseases. By affecting the growth pattern, the anatomy and morphology and particularly the chemical composition, the nutrition of plants may contribute either to an increase or decrease of the resistance and/or tolerance to pests and diseases (Krauss, 2001).

White rot, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary and *S. minor* Jager, is the most important sunflower (*Helianthus annuus* L.) disease in the regions with moderate climate (Masirevic and Gulya, 1992; Vasic et al., 2004). The nutrition of *Sclerotinia* during all stages of disease development is probably the most important factor in determining the extent of infection development (Lumsden, 1979). Smirnova (1967) determined that, for normal development of *Sclerotinia*, all nutrients are needed (N, S, K, Mg, and Fe), while Purdy and Grogan (1954) observed that growth and sclerotium formation only occur when the inorganic macronutrients P, K, Mg and S are present in the medium. Availability and nutrient quantity in the host plants is a limiting factor for *Sclerotinia* infection development in sunflower (Acimovic, 1998).

Because of the importance of nutrients for *S. sclerotiorum* infection development, the aim of this work was to determine the nutrient content in photosynthetic tissue (leaves) of sunflower plants before and after development of fungus infection, and to find the correlation between nutrient content and resistance to sclerotium infection.

MATERIALS AND METHODS

Six cms inbred lines (PR-ST-3A, CMS_3-8A, PH-BC_1-40A, Ha-48A, Ha-74A, and CMS_1-50A) and two restorer lines (RUS-RF-OL-168 and RHA-583), all selected in Institute of Field and Vegetable Crops, Novi Sad, Serbia, were used in the study.

The experiment was set in two variants - SC_1 – non-infected control, and SC_2 – stem infection with sclerotium. In each variant, four rows with 12 plants per genotype were sown. Plants in SC_2 plot were inoculated by incorporation of sclerotia into the middle part of the stem, at the stage of bud appearance (E4). Wounds with sclerotia were covered with wet cotton and aluminium foil as described by Vasic et al. (2002). After the infection, plants in both variants were irrigated three times a week, for three hours.

Screening was done at the stage of physiological maturity (M0), and resistance was determined as percentage of healthy plants.

Leaf samples for physiological analyses were taken 28 days after the inoculation in both SC_1 and SC_2 variants. In both cases, three healthy leaves from the upper part of the plant were taken from 10 plants from the inner rows. In SC_2 variant, leaves were taken only from the plants showing symptoms of the white rot. Macronutrient (N, P, K, Ca and Mg) content was determined in dry plant material.

Nitrogen content was determined by the Kjehdal method (Nelson and Sommers, 1973) and phosphorus content spectrophotometrically by the ammonium molybdate-vanadate method (Gericke and Kurmies, 1952). Leaf samples were dry-ashed and dissolved in 25% HCl and analyzed for potassium content by flame photometry and for calcium and magnesium content by atomic absorption spectroscopy.

Resistance of genotypes to sclerotia infection was correlated with macronutrient content in infected and non-infected plants in order to estimate their relationship.

RESULTS AND DISCUSSION

Results obtained showed that there was a difference between tested genotypes in resistance to *Sclerotinia* sclerotium infection. The most resistant genotype was Ha-48A (>90%), and the most susceptible ones were RUS-RF-OL-168 (60.0%) and Ha-74A (60.0%). However, all tested genotypes could be considered tolerant since they all had resistance over 50% (Table 1).

Table 1. Macronutrient content in tested sunflower inbred lines.

Genotype	Variant	Resistance (%)	N	P	K	Ca	Mg
			mg/100g				
PH-BC_1-40A	Control		3820	246.7	3223	3069	1250.0
	Infection	75.0	3582	313.0	3210	3299	1157.0
Ha-48A	Control		4520	295.7	3478	2873	758.7
	Infection	93.9	4425	254.3	3112	2883	1043.0
CMS_3-8A	Control		3854	288.0	2916	2921	1299.0
	Infection	77.8	3310	293.7	2969	2572	1265.0
PR-ST-3A	Control		3691	307.0	3576	2893	866.7
	Infection	73.7	3834	249.0	2914	2745	1075.0
CMS_1-50A	Control		4065	266.7	3646	2796	856.7
	Infection	60.6	3888	273.7	3516	2623	950.0
RUS-RF-OL-168	Control		4133	284.0	4552	2991	887.7
	Infection	60.0	3385	253.0	3181	3694	1397.0
Ha-74A	Control		3093	323.7	3462	3103	1095.0
	Infection	60.0	3290	309.0	3620	3646	1271.0
RHA-583	Control		3412	290.3	4096	2359	619.3
	Infection	71.9	3643	268.7	3645	2132	580.3

Nutrient content in sunflower leaf tissue depended both on genotype and the presence or the absence of infection (Table 1). Genotype dependence of nutrient content in sunflower tissue was also observed by other authors (Saric et al., 1991; Vasic et al., 2001).

Nitrogen concentration in leaves of tested genotypes ranged from 4472 mg/100g (average for both variants – infection, control) in genotype Ha-48A, which was at the same time the one most resistant to sclerotium infection (93.9%), to 3192 mg/100g in genotype Ha-74A (Table 1). There was a low positive correlation between resistance and N content in leaves of control plants, and a high positive correlation between resistance and N content in leaves of infected plants (Table 2). This is not in accordance with the conclusions presented in the work of HuiLian (2004). This author connected increased susceptibility of field crop plants to pathogen attack with the increased nitrogen compound content in the plant. However, facultative parasites, such as *Sclerotinia*, require weak plants to infest and kill in order to survive (Marchner, 1995). Vigorous plant growth stimulated by ample N would suppress infestation by this group of pathogens. This may explain the differences in expression of plant diseases in relation to the nutrition of the host and N content.

Phosphorus content in leaves of control plants was in positive correlation with resistance (Table 2). This is in accordance with the results of Sindhan and Parashar (1996), who found that leaves of groundnut (*Apios americana* Medic.) cultivars resistant to *Cercospora arachidicola* contained more phosphorus than leaves of susceptible cultivars.

Potassium concentration in leaves of control plants ranged from 4552 mg/100g in genotype RUS-RF-OL-168 to 2916 mg/100g in genotype CMS_3-8A (Table 1). Although the results varied, a negative correlation between resistance and K content in leaves of the control, as well as in leaves of infected plants was found (Table 2). Correlation coefficients were moderate and low, respectively, but results clearly showed that a higher K content in leaves has as a consequently lower resistance to sclerotium infection. This is in contrast with the data obtained by Perrenoud (1990) concerning the relationship of K and plant health. This author reviewed some 2450 references and showed that in 70% of all quoted cases K induced a significant reduction in fungal disease incidences. However, in a more recent publication, Mondal et al. (2001) found a negative correlation between K content and disease incidence in soybean (*Glycine max* (L.) Merrill) and sesame (*Sesamum indicum* L.).

Table 2. Correlation of resistance of inbred lines to sclerotia infection with macronutrient content in infected and non-infected sunflower plants.

Macronutrient	Variant	Resistance (%)	Correlation coefficients
N	Control		0.1137
	Infection	75.0	0.5064
P	Control		0.2140
	Infection	93.9	-0.0554
K	Control		-0.3582
	Infection	77.8	-0.1485
Ca	Control		-0.3161
	Infection	73.7	-0.4121
Mg	Control		-0.1917
	Infection	60.6	-0.4040

There was a moderate negative correlation between Ca content in leaves of control and infected plants and resistance to sclerotium infection, meaning that plants with a higher Ca content were more susceptible to the infection (Table 2). This is in contrast with the results obtained by other authors regarding the role of Ca in sunflower resistance and reaction to *Sclerotinia* infection. Antonova et al. (1984) tested chemical composition of leaves and flowers of sunflower plants resistant and susceptible to *Sclerotinia sclerotiorum* and found that flowers of resistant plants contain more Ca.

Infection led to increase in Mg content in leaves of five genotypes (Table 1). Increases in Mg content in infected plants could be a consequence of chlorophyll degradation and disturbed processes of re-translocation and reutilization of Mg ions, since a pathogen attack causes chlorotic to necrotic changes on all plant parts, which are a consequence of chlorophyll degradation (Singh et al., 1998).

Similarly to the results obtained by other authors, nutrient content in leaves of tested sunflower inbred lines was genotype specific. It has also depended on the development of *Sclerotinia* infection, i.e. genotype resistance. There was a high positive correlation between N content in infected plants and resistance, which points to important role of this nutrient in sunflower defence from *Sclerotinia* attack. Moderate negative correlations between K and Ca content in control plants showed that the content of these nutrients in healthy plants could be used as an indicator of cultivated sunflower resistance/susceptibility to *Sclerotinia* infection. Further studies are in progress in order to find out more on the role of nutrients in sunflower response to *Sclerotinia* attack and to test the value of the results of this study on a larger number of sunflower genotypes.

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REFERENCES

Acimovic, M. 1998. Sunflower diseases, Institute of Field and Vegetable Crops, Novi Sad, Serbia. (in Serbian).

Antonova, T.S., V.F. Zaichuk, and T.V. Kalinchenko. 1984. Role of calcium in the resistance of sunflower anthodium to grey rot. Selskohozyajstvennaya Biologiya 11:67-68.

Gericke, P., and S. Kurmies. 1952. Die kolormetriche Phosphorsaürebestimmung mit Ammonium-Vanadate-Molybdat und ihre Anwendung in der Pflanzenanalyse. Z. Pflanzenernähr. Düng. Bodenkd. 59:32-35.

HuiLian, X. 2004. Accumulation of nitrogen metabolites in relation with incidences of diseases and pest insects in crop plants. p. 1-15. In: S.G Pandalai (ed.), Recent Research Developments in Plant Pathology. International Nature Farming Research Center, Nagano, Japan.

Krauss, A. 2001. Potassium and biotic stress. p. 281-293. In: Proc 1st FAUBA-FERTILIZAR-IPI Workshop, Buenos Aires, Argentina.

Lumsden, R.D. 1979. Histology and physiology of pathogenesis in plant diseases caused by *Sclerotinia* species. Phytopathology 69:890-896.

Marchner, H. 1995. Mineral nutrition of higher plants, 2nd edn. Academic Press, New York, USA.

Masirevic, S., and T.J. Gulya. 1992. *Sclerotinia* and *Phomopsis* - two devastating sunflower pathogens. Field Crops Res. 30:271-300.

Mondal, S.S., Pramanik C.K., and J. Das. 2001. Effect of nitrogen and potassium on oil yield, nutrient uptake and soil fertility in soybean (*Glycine max*) - sesame (*Sesamum indicum*) intercropping system. Indian J. Agric. Sci. 71:44-46.

Nelson, D.W., and L.E. Sommers. 1973. Determination of total nitrogen in plant material. Agron. J. 65:109-112.

Perrenoud, S. 1990. Potassium and plant health. IPI Research Topics No. 3, 2nd rev. Edn. Basel, Switzerland.

Purdy, L.H., and R.G. Grogan. 1954. Physiological studies of *Sclerotinia sclerotiorum* in liquid agar culture. Phytopathology 48:36-38.

Saric, M., B. Krstic, and D. Škorić. 1991. Element diversity in sunflower inbred lines. Helia 14:41-48.

Sindhan, G.S., and R.D. Parashar. 1996. Biochemical changes in groundnut leaves due to infection by early and late leaf spot pathogens. Indian J. Mycol. Plant Pathol. 26:210-212.

Singh, M.J., J. Singh, and S.S. Cheema. 1998. Effect of cucumber mosaic virus on chlorophyll content and mineral elements in chilli. Plant Dis. Res. 13:125-128.

Smirnova, A.D. 1967. Vlijanije uslovij pitanija na razvitije vozbuditelja sklerotiniji podsolnecnika. Bjulleten Nauc-tehnic informaciji po maslicinim kulturam, p. 10-13.

Vasic, D., S. Pajevic, M. Saric, Lj. Vasiljevic, and D. Škorić. 2001. Concentration of mineral elements in callus tissue culture of some sunflower inbred lines. J. Plant Nutr. 24:1987-1994.

Vasic, D., K. Taski, S. Terzic, S. Kevresan, and D. Škorić. 2002. Transferring of *Sclerotinia* resistance from wild into cultivated sunflower - combining of conventional and laboratory techniques. Proc. Nat. Sci., Matica Srpska 102:29-33.

Vasic, D., R. Marinković, F. Miladinovic, S. Jocić, and D. Škorić. 2004. Gene actions affecting sunflower resistance to *Sclerotinia sclerotiorum* measured by sclerotia infections of roots, stems, and capitula. p. 603-608. In: Proc. 16th Int. Sunflower Conf., Fargo, ND, USA.

Races of *Plasmopara halstedii* on sunflower in separate agrocenosises of Adigeya Republic, Krasnodar and Rostov regions in Russia

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ABSTRACT

During the past four years, the occurrence of *Plasmopara halstedii* and the regional distribution of its pathotypes in some districts of Northern Caucasus and Rostov region were studied. More than 1000 isolates of the pathogen were collected in separate agrocenoses of Adigeya Republic, Krasnodar and Rostov regions in 2004-2007 period. A large-scale identification of these isolates was undertaken. Pathotype characterization, based on sunflower differential lines and evaluated according to a triplet code system, indicated the existence of at least seven pathotypes. Among them, race 330 was predominant. Races 710 and 730 dominated in some fields of Adigeya Republic and Krasnodar region. Races 100, 300, and 310 were only sporadically present. Race 700 was discovered in some fields of Krasnodar region, representing up to 11% of all isolates from these places. At present only races 330, 710 and 730 are economically important in the studied regions of Northern Caucasus.

Key words: downy mildew – *Plasmopara halstedii* – pathotypes ratio – races – sunflower.

INTRODUCTION

Downy mildew of sunflower caused by the pathogenic fungus *Plasmopara halstedii* (Farl.) Berl. et de Tony is a worldwide major disease of this crop. In a recent survey of pathogen race spreading, it was shown that more than 30 pathotypes of this Oomycete on sunflower were discovered in the World (Gulya, 2007). The quantity and composition of pathogen races vary in different countries and are the objective of study by leading phytopathologists (Masirevic, 1998; Molinero-Ruiz et al., 1998; Kormany and Viranyi, 1997; Penaud, 1998; Tourvieille de Labrouhe et al., 2000b; Shindrova, 2000, 2005; Rozynek and Spring, 2000; Shirshikar, 2005). Sunflower is the main oil crop in Russia. Adigeya Republic, Krasnodar and Rostov regions are the territories of intensive sunflower cultivation in Russia. Downy Mildew is one of the most potentially important sunflower diseases here, but for a long period of time a structure of *P. halstedii* population on sunflower in all of Northern Caucasus was a white spot on the world map of pathogen races distribution. This disease was first observed in Russia in the mid 1950s (Novotelnova, 1966). Successful development in this country of a hybrid *H. tuberosus* x *H. annuus* resistant to the disease and breeding based on its new open-pollinated varieties permitted to control the pathogen during three decades. The first information about the appearance of a new virulent pathotype of pathogen in Krasnodar region goes back to the beginning of the 1980s (Tihonov and, Zaichuk, 1981). At that time, the sunflower differential line HA-274 was resistant, but at that time both this differential line as well as the resistant varieties began to be infected. The investigations of Antonova et al. (2000) revealed the presence of races 100, 310 and 330 in Krasnodar region. The favourable weather conditions for disease appearance in 2004-2007 permitted to collect an ample collection of pathogen isolates (about 1000) in different districts of Northern Caucasus, which was preserved at a temperature of -80^{o}C, The aim of this study was to identify these isolates and to determine the race ratio in separate agrocenosises by the use of an international method proposed by a group of scientists (Gulya et al., 1998; Tourvieille de Labrouhe et al., 2000a).

MATERIALS AND METHODS

In order to determine the race variability of sunflower downy mildew in the Northern Caucasus and the ratio of the individual races in separate agrocenosises, expeditions for collecting isolates of *Plasmopara halstedii* from infected plants of different hybrids and varieties were organized in the period 2004-2007. About 1000 isolates of the Oomycete were collected from 14 regions of the Northern Caucasus (Adigeya republic, Krasnodar and Rostov areas) (Table 1, Fig. 1). Leaves from systemically infected plants in the field were harvested and kept in darkness at 6^{o}C in 100% humidity for 12 hour for induction of fungus sporulation. The leaves with sporulation were kept in polyethylene bags at -80^{o}C. At this temperature,

zoosporangia do not lose their viability for some years. For differentiation of pathogen races according to the new nomenclature system, nine sunflower differential lines were used: set 1 – HA 304 (D-1), Rha-265 (D-2), Rha-274 (D-3); set 2: PM-13(D-4), PM-17 (D-5), 803-1(D-6); set 3: HAR-4 (D-7), HAR-5 (D-8), HA-335 (D-9). Races were determined on the basis of the response of the lines from each group (sporulation on the first true leaves) (Tourvieille de Labrouhe et al., 2000a). Seeds of differentials were placed for germination in rolled up filter paper at 25°C. At the radicle length of 1.0-2.0 cm the seedlings were laid in rows in growth plates with wet sterilized sand covered by filter paper (10 seedlings of each line in one plate). The roots of seedlings were covered by strips of filter paper and wet cotton wool. A suspension of zoospores was prepared from frozen spores (the concentration was 10^6 spores/ml) at 16°C; 150 ml of suspension was added to each growth plate and they were kept for 3-5 hours at the same temperature. Plants were grown 7-9 days at 25°C in the daytime and 18°C at night (16 h photoperiod). The growth plates were placed in a wet chamber at 16°C for 12-24 hours for sporulation. Plants with sporulation on true leaves were classified as susceptible. If any of the differential lines displayed partial infection, these lines were re-inoculated a second time, using spores from the universal susceptible or the line in question.

RESULTS

During the period 2004-2007, the climate conditions were favorable for downy mildew on sunflower in more regions of Northern Caucasus. Therefore, a numerous collection of *P. halstedii* isolates (more than 1000) was collected from infected sunflower plants of different varieties, hybrids and lines in 14 areas of Northern Caucasus (Fig. 1).

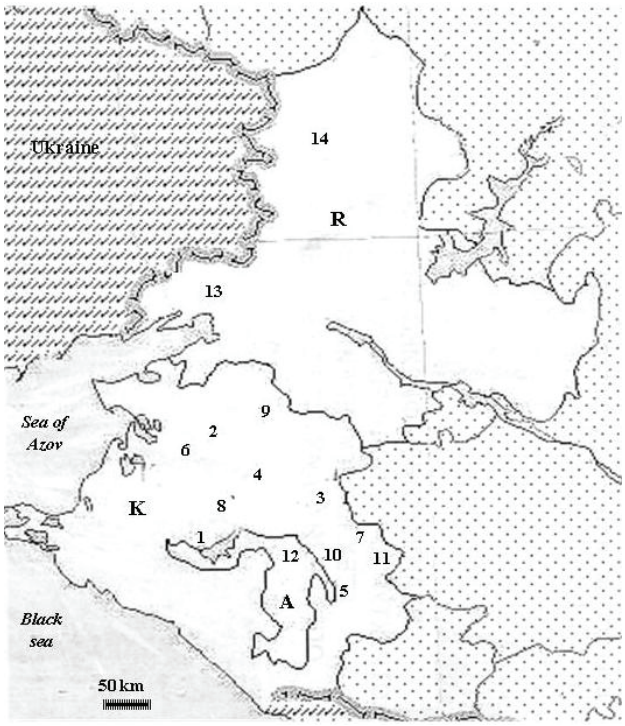

Fig. 1. Location of districts of gathering *Plasmopara halstedii* isolates from infected sunflower plants: ***K****-Krasnodar region: 1- Krasnodarskiy, 2 - Leningradskiy, 3- Caucazskiy, 4 - Viselkovskiy, 5 - Labinskiy, 6-Kanevskoy, 7 - Novokubanskiy, 8 - Korenovskiy, 9 - Krilovskoy, 10 - Kurganinskiy , 11 - Uspenskiy ;* ***A*** *- Adigeya Republic: 12- Shovgenovskiy;* ***R*** *- Rostov region: 13 -Matveev-Kurganskiy., 14 - Millerovskiy.*

Our identification of collected isolates in accordance with new nomenclature system, proposed by Tourvieille de Labrouhe et al. (2000a), was the first circumstantial investigation of race composition of *P. halstedii* population on sunflower in this part of the world. It revealed 7 races (Table 1). Pathotypes 100, 300, and 310 were only sporadically present in some areas of the above regions. Race 330 was revealed in

all investigated fields everywhere (Table 1). Races 710 and 730 were discovered in 9 and 7 areas, respectively. Race 700 was revealed in Adigeya Republic and in 3 areas of Krasnodar region. All seven races were identified in fields of VNIIMK.

Table 1. *Plasmopara halstedii* races infecting sunflower in some regions of Northern Caucasus during 2004-2007.

District	Year of collection of isolates	Host of pathogen	Races						
			100	300	310	330	700	710	730
		Adigeya Republic							
Schovgenovskiy	2004	Master*	-	-	-	+	+	+	+
		Rostov region							
Matveev Kurgan	2004	R- 453 (Rodnik)*	-	-	+	+	-	-	-
Millerovskiy	2004	Signal**, Donskoy krupnoplodniy*	-	-	-	+	-	+	+
		Krasnodar region							
Leningradskiy	2004	R- 453 (Rodnik)*	-	-	+	+	-	-	-
Caucazskiy	2004	Master*	-	-	-	+	-	-	-
Viselkovskiy	2004	PR64A83,**	-	-	+	+	+	+	+
	2005	R- 453 (Rodnik)*, Signal**							
Labinskiy	2005	R- 453 (Rodnik)*	-	+	-	+	+	+	+
Kanevskoy	2005	Master*, Flagman*, R- 453 (Rodnik)*, Signal**	+	-	-	+	-	+	+
Novokubanskiy	2005	VK 276 B***	-	-	-	+	-	-	-
Korenovskiy	2005	VK-653***	-	+	-	+	-	-	-
Krilovskoy	2006	Konditerskiy (SPK)*, Donskoy krupnoplodniy*	-	-	-	+	+	+	+
Krasnodarskiy	2004-2007	Different varieties, hybrids and lines on the fields of VNIIMK	+	+	+	+	+	+	+
Kurganinskiy	2007	Konditerskiy (SPK)*	-	-	-	+	-	+	-
Uspenskiy	2007	R- 453 (Rodnik)*	-	-	-	+	-	+	-

* open pollinated variety; **hybrid; *** inbred line.

The analysis of the race ratio was carried out for isolates of 24 separate agrocenoses. In Schovgenovskiy district of Adigeya Republic from 23 isolates of fungus, collected on one field from infected plants of open pollinated variety Master, 69.6 % belonged to race 710 and 13.0% were classified as race 330. Races 700 and 730 represented 8.7% each (Table 2). In Millerovskiy district of Rostov region (18 isolates) the highest number corresponded to races 330 (77.7%), 730 (16.7%) and 710 (5,6%). In Matveev Kurgan district, race 330 represented 80% and race 310 was 20%, but the number of isolates was small (6 isolates).

Table 2. The ratio (%) of *Plasmopara halstedii* races of infected sunflower in separate agrocenoses of Adigeya Republic and Rostov region

Location of the field	Year of collection of isolates	Host of pathogen	N. of isolates	Races				
				310	330	700	710	730
Schovgenovskiy district of Adigeya Republic	2004	Master*	23	0	13.0	8.7	69.6	8.7
Millerovskiy district of Rostov region	2004	Donskoy krupnoplodniy*	18	0	77.7	0	5.6	16.7
Matveev-Kurganskiy district of Rostov region	2004	R- 453 (Rodnik)*	6	20	80	0	0	0

*open pollinated variety

Table 3. The ratio (%) of *Plasmopara halstedii* races of infected sunflower in separate agrocenoses in different districts of Krasnodar region

N. of the field	Year of collection of isolates	Host of pathogen	N. of isolates	Races						
				100	300	310	330	700	710	730
Viselkovskiy district										
1	2004	PR64A83**	93	0	0	1.2	23.4	11.2	60.5	3.7
2	2005	R- 453 (Rodnik)*	79	0	0	0	12.7	0	29.1	58.2
3[1]	2005	Signal**	6	0	0	0	50	0	50	0
Kanevskoy district										
4	2005	Flagman*	60	0	0	1.7	91.7	0	3.3	3.3
5	2005	R- 453 (Rodnik)*	63	0	0	1.6	98.4	0	0	0
6	2005	R- 453 (Rodnik)*	12	0	0	0	100	0	0	0
7	2005	Master*	14	7.1	0	0	92.9	0	0	0
8[1]	2005	Signal**	12	0	0	0	100	0	0	0
Krilovskoy district										
9	2006	Konditerskiy (SPK)*	82	0	0	0	90.2	1.2	3.7	4.9
10	2006	Donskoy krupnoplodniy*	15	0	0	0	53.3	0	26.7	20
11[1]	2006	Konditerskiy (SPK)*	11	0	0	0	100	0	0	0
Kurganinskiy district										
12	2007	Konditerskiy (SPK)*	43	0	0	0	97.7	0	2.3	0
Krasnodarskiy district (fields of VNIIMK)										
13	2005	VK-678*** (first planting date)	28	0	0	3.6	89.3	0	7.1	0
14	2005	VK -678*** (second planting date)	45	0	0	2.2	55.6	0	11.1	31.1
15	2005	Different genotypes	30	3.3	3.3	6.7	73.4	3.3	3.3	6.7
16	2005	R- 453 (Rodnik)*	12	0	0	8.3	66.7	0	0	25
17	2005	Different genotypes	10	0	0	0	40	0	30	20
18	2005	Different genotypes	29	0	3.5	10.3	58.6	3.5	17.2	6.9
19	2006	Different genotypes	7	0	0	0	100	0	0	0
20	2006	Different genotypes	15	0	0	0	100	0	0	0
21	2006	Volunteer plants	14	0	0	0	100	0	0	0

[1]Treated fields with metalaxyl-protected seeds; *open pollinated variety; **hybrid; *** inbred line.

Race 330 dominated in 18 out of the 21 studied agrocenoses in Krasnodar region and constituted 40-100% (Table 3). In Viselkovskiy district, two adjacent fields were analyzed. In the first field 93 isolates of the pathogen were collected from infected plants of hybrid PR64A83 in 2004. In the second field (adjacent to the first), 79 isolates were collected from open pollinated variety R- 453 (Rodnik) in 2005. In the first field, race 710 dominated (60.5%), whereas in the second dominated race 730 (58.2%) (Table 3).

Race 730 in the first field only accounted for 3.7%, but in the second field it represented 58.2%. Race 330 was 23.4 % in the first field and 12.7% in the second. Races 310 and 700 were only identified in the first field (1.2% and 11.2%, respectively). In the third field, treated with metalaxyl-protected seeds of hybrid Signal, only 6 isolates were collected and identified, with races 330 and 710 representing 50% each.

Another race composition was found on five fields of Kanevskoy district. Race 330 here was up to 100%. Races 710 and 730 were discovered only in one field, each of them made up to 3.3%. Race 100 made up to 7.1% on another field and race 310 represented 1.6 and 1.7 % in two fields. Races 300 and 700 were not found in these five fields (Table 3).

In Kurganinskiy district, the percentage of race 330 was 97.7% and race 710 constituted 2.3% (Table 3). In all the three studied fields in Krilovskoy district, race 330 also dominated (53.3-100%). From the 82 isolates collected from infected plants of variety Konditerskiy (SPK) races 700, 710 and 730 were also identified, representing only 1.2, 3.7, and 4.9%, respectively. The percentage of race 710 was 26.7% and of race 730 20% in 15 collected isolates from infected sunflower of variety Donskoy krupnoplodniy (Table 3).

All isolates from Krasnodarskiy district were collected from infected plants of different genotypes of sunflower (Russian and foreign open pollinated varieties, hybrids, inbred lines) in VNIIMK`s fields. In all 8 presented agrocenoses, race 330 predominated over the other races. The line VK-678 was planted in one field at two different planting dates. For both dates, apart from race 330, also were present races 310 and 710. Race 730 was not observed among the samples on the first date of planting but it was identified in the samples on the second date of planting (31.1%) . In one of the fields, all the 7 races were observed: races 100, 300, 700 and 710, represented each 3.3% of the samples from this field, races 310 and 730 made up to 6.7% each. In one of the remaining fields, 6 races were discovered; races 300 and 700 constituted 3.5 % each, race 310 was 10.3%, race 710 was 17.2%, and race 730 was 6,9% (Table 3).

DISCUSSION

According to this observation, seven races *of Plasmopara halstedii* were identified in regions of Northern Caucasus during the last four years. The distribution area and the ratio of pathotypes in separate agrocenoses were different. We assumed that at present races 100, 300, and 310 are disappearing here because of the following reasons: firstly, they have been discovered only sporadically; secondly, all their isolates form an extremely poor sporulation even on susceptible differential lines. In addition, races 100 and 300 are the oldest races, at least in Krasnodar territories. Prolonged sunflower breeding for resistance to these pathotypes over the span of some decades must result in their maximal ousting from the populations. At present, the main race which is widely distributed everywhere in the studied territories is 330. This race dominates up to 100% in 18 out of 21 studied agrocenoses in Krasnodar region and in the two studied agrocenosises in Rostov region. But in separate agrocenoses of Adigeya Republic and Krasnodar region, races 710 and 730 are dominant. Race 700 was found in 5 areas and it represented up to 11.2%. Its condition in pathogen population here is not clear now. Maybe it will spread more widely in the future. At present, only races 330, 710 and 730 are economically important in these territories of Northern Caucasus. Strictly speaking, the quantity of races of pathogen depends on the diversity of cultivated sunflower genotypes. The rapid change over the last decades of sunflower crop variety structures in the studied territories, together with a wide distribution of foreign hybrids everywhere in Russia, will change the race structure of *P. halstedii* populations in this country. Data from our investigation showed the necessity to concentrate efforts on the development of native sunflower varieties and hybrids resistant to races 330, 710 and 730. In addition, our investigation will serve as a starting point for controlling the population structure of *Plasmopara halstedii* in Northern Caucasus territories.

ACKNOWLEDGEMENTS

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REFERENCES

Antonova, T.S., N.M. Araslanova, A.V. Golovin, T.A. Tchelustnikova, and S.L. Saukova. 2000. To the question about race belonging of sunflower Downy Mildew casual agent in Northern Caucasus. Sci.-tecn. Bulletin of VNIIMK 123:21-23 (In Russian).

Gulya, T.J. 2007. Distribution *Plasmopara halstedii* races from sunflower around the world. In: Proceedings of the 2nd International Downy Mildews Symposium. Advances in downy mildew research. Palacky University in Olomouc and JOLA. v.o.s., Kostelec na HanI (Czech Republic) 3:121-134.

Gulya, T., D. Tourvieille de Labrouhe, S. Masirevic, A. Penaud, K. Rashid, and F. Viranyi. 1998. Proposal for standardized nomenclature and identification of races of *Plasmopara halstedii*. p. 130-136. In: Symposium III: Sunflower Downy Mildew, Fargo, ND, 13-14 January. Int. Sunflower Assoc, Paris.

Kormany, A., and F. Viranyi. 1997. Studies on the virulence and aggressiveness of *Plasmopara halstedii* (sunflower downy mildew) in Hungary. Med. Fac. Landbouw. Univ. Gent., 62(3b):911-918.

Masirevic, S. 1998. Races of sunflower downy mildew pathogen (*Plasmopara halstedii*) in Yugoslavia. p. 21-23. In: Symposium III: Sunflower Downy Mildew, Fargo, ND, 13-14 January. Int. Sunflower Assoc, Paris.

Molinero-Ruiz, L., J.M. Melero-Vara, and T.J. Gulya. 1998. Pathogenic characterization of *Plasmopara halstedii* isolates from Spain. p. 26-29. In: Symposium III: Sunflower Downy Mildew, Fargo, ND, 13-14 January. Int. Sunflower Assoc, Paris.

Novotelnova, N.S. 1966. Downy Mildew of sunflower. M.;L. Science. 150 p.(In Russian).

Penaud, A. 1998. Survey of downy mildew on sunflower in France. p. 8-9. In: Symposium III: Sunflower Downy Mildew, Fargo, ND, 13-14 January. Int. Sunflower Assoc, Paris.

Rozynek, B., and O. Spring. 2000. Pathotypes of sunflower downy mildew in Southern parts of Germany. Helia 23(32):27-34.

Shindrova, P.S. 2000. Distribution and race composition of downy mildew (*Plasmopara halstedii* Farl. Berl. and de Toni) in Bulgaria. Helia 23(33):25-32.

Shindrova, P.S. 2005. New nomenclature of downy mildew races in sunflower (*Plasmopara halstedii* (Farl.) Berl. and de Toni) in Bulgaria (race composition during 2000-2003). Helia 28(42):57-64.

Shirshikar, S.P. 2005. Present status of sunflower downy mildew disease in India. Helia. 28 (43):153-158.

Tihonov, O.I., and V.F. Zaichuk. 1981. New race of downy mildew. Oil Crops 6:20-21 (in Russian).

Tourvieille de Labrouhe, D., T.J. Gulya, Y.K. Rashid, and F. Viranyi. 2000a. New nomenclature of races of *Plasmopara halstedii* (sunflower downy mildew). p. 161-166 (vol. 2). In: Proc. 15th Int. Sunflower Conf., Toulouse, France.

Tourvieille de Labrouhe D., E. Pilorge, P. Nicolas, and F. Vear. 2000b. Le mildiou du tournesol. Published by INRA and CETIOM, Paris. France, 176 p.

Differences in some DNA RAPD-loci of *Plasmopara halstedii* races affecting sunflower in Krasnodar region of Russia

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ABSTRACT

Forty-three isolates of *P. halstedii* races 300, 310, 330, 700, 710, 730 affecting sunflower in Krasnodar region were studied by means of 22 RAPD-loci. Primer P38 did not produce amplification in the studied isolates. The other primers produced a total of 92 fragments with an average frequency of 4.4 fragments per primer. Eight primers (L14, Y11, P28, P46, P5, OPM08, OPM20, OPJ15) were not polymorphic among the studied isolates. Thirteen primers (OPB07, OPB17,OPC08, OPC15, OPD 11, OPD 18, OPE 03, OPD 20, OPF09 OPG02 OPG05, OPG06, and OPJ13) allowed the identification of race 300 from the other races, based on the presence of amplified DNA fractions lacking in other races, or the absence of fractions typical for races 310, 330, 700, 710, 730. The resemblance measured between races 330, 700, 710 and 730, counted by means of Jacquard coefficient, was near 1.00. The resemblance between race 300 and the others was 0.29. Accordingly, the genetic distance (D_{xy}) between race 300 and the others was 0.71. These data suggested that local races 310, 330, 700, 710 and 730 in Krasnodar region could not originate from race 300. One primer (OPG06) showed intraracial polymorphism on the presence-absence of a fragment with a length of 1125 bp in all races, except 700 and 710. Monomorphic condition (invariable presence of the 1125 bp fragment) of locus OPG06 in race 710 isolates from five remote districts of Krasnodar region pointed to its stability. The monomorphic condition of this locus (invariable absence of the 1125 bp fragment) in race 330 isolates from Kanevskoy district and polymorphic condition in one isolate from Viselkovskiy district are discussed.

Key words: downy mildew – molecular markers – *Plasmopara halstedii* – races – sunflower – RAPD markers.

INTRODUCTION

The fungus *Plasmopara halstedii* (Farl.) Berl. et de Toni is an obligate parasite of sunflower, causing downy mildew, a worldwide major disease of this crop. The pathogen exists as many physiological races, which over the last few decades has grown into a complex, with at least 36 pathotypes being identified in different countries (Gulya, 2007). Physiological races of obligate parasites are always difficult to differentiate. Different physiological races of this pathogen have been described according to their reactions on various sunflower lines. An international nomenclature based on a series of well defined host plants is starting to be used, which should make it possible to establish the presence of the same races in different continents and to define the specific races in each country (Gulya et al., 1998; Tourvieille de Labrouhe et al., 2000). This international method of *P. halstedii* races differentiation and their new nomenclature application was successfully used by many pathologists from different countries (Rozynek, Spring, 2000; Molinero-Ruiz et al., 2002; Shindrova, 2000, 2005; Shirshikar, 2005; Antonova et. al, 2006; Iwebor et. al., 2005, 2007). But sometimes this cannot guarantee the clear differentiation of some races, especially if they are from different countries. The molecular methods for genomic analysis of this fungus are especially applied at present (Roeckel-Drevet et al., 1997, 2003; Giresse at al., 2007). The relationships between all known races of *P. halstedii* from different countries have been investigated by means of 21 RAPD primers (Tourvieille, 2000; Roeckel-Drevet et al., 2003), but races of fungus from Russia were not used in that investigation. The downy mildew pathogen on sunflower in Russia until recently has been scarcely studied either on racial structure or on the molecular structure of the genome. At this time, a successful control of the disease demands a regular survey of pathogen populations and incorporation of resistance to as many races as possible in sunflower breeding programs.

The objective of our investigation was the analysis by means of RAPD-PCR markers of molecular-genetic polymorphism of *P. halstedii* races present in Krasnodar region of Northern Caucasus.

MATERIALS AND METHODS

The research included 43 field isolates of *P. halstedii* belonging to six races of the pathogen with code numbers: 300, 310, 330, 700, 710, 730 (accordingly a quantity of isolates: 2, 1, 12, 4, 13, 11). The isolates were collected from the affected sunflower plants in different areas of Krasnodar region in 2005-2007. The leaves with sporulation were kept in polyethylene bags at -80°C. The seedling inoculations were implemented by using the method described in these Proceedings (Antonova et al., 2008). The physiological races were determined according to the international nomenclature, which has been proposed by Tourvieille de Labrouhe et al. (2000). All isolates were maintained on seedlings of sunflower open-pollinated variety VNIIMK 8883, which has never been used in breeding for resistance to downy mildew. DNA was extracted from conidial sporulation of the Oomycete on cotyledons of sunflower seedlings; these were artificially infected by zoospores of every isolate separately. Spores were collected and kept at -20°C until DNA extraction, which was performed within 1 month. DNA was extracted by a modified method based on Zolan and Pukkila (1986).

For RAPD-analysis, 22 decamer primers (L14, Y11, P 28, P 53, M 08, M 20, J 15, P38, B 07, B 17, C 08, C 15, D 11, D 18, E 03, D 20, F 09, G 02, G 05, J 13, G 06) were used. The first six primers were used by us early on sunflower (Guchetl et al. 2004). The others have been used for differentiation of 5 races of *P. halstedii* collected from different districts of France (Roeckel-Drevet et al., 1997; Tourvieille et al., 2000). These primers were kindly given to us by those authors. Each 25 μL of reaction volume contained 67 mM tris-HCl, ph 8.8; 16.6 mM $(NH_4)_2SO_4$; 1.5-3.0 mM $MgCl_2$; 0.001 % Tween 20; 0.2 mM deoxynucleoside triphosphates, 10 μM primer; 10 ng template DNA and 1.0 unit *Tag* DNA polymerase (Gosniigenetic, Russia). Amplification was performed in thermocycler (AO DNA-technology, Russia). PCR was conducted at regime standard for RAPD-primers: 1 cycle at 94°C for 2 min (initial denaturation) and 30 cycles – in consecutive temperature change: 1 min. at 94°C (denaturation), 1 min. at 36°C (annealing), 2 min. at 72°C (elongation), 4 min.at 72°C (final elongation).

Electrophoresis of PCR products was carried out in agarose gel (1.5 % agarose, 1x TAE-buffer in horizontal camera during 1.5-2.0 h at I=50 mA, U= 70-90 V; 10 μL of reactionary mixture were introduced in gel together with dye-stuff bromphenol blue. GeneRuler 1 kb DNA Ladder (MBI "Fermentas") was used as marker for DNA fragments lengths. Ethydium Bromide was used for subsequent staining of DNA fragments. Data were documented by means of trans-illuminator and video system (AO DNA-technology, Russia) with computer program "Gel-Imager 2". Experiments were carried out in triplicate.

The differences between isolates were expressed by the presence or the absence of bands on gel corresponding to DNA fragments of the definite length. The resemblance measure between races was calculated by means of Jacquard coefficient (Sneath and Sokal, 1973) using the formula: $\mathbf{J_{xy} = n_{xy} / n_x + n_y}$, where $\mathbf{J_{xy}}$ is the Jacquard's coefficient; $\mathbf{n_{xy}}$, is the number of DNA fragments of patterns **x** and **y** coinciding by their electrophoretical mobility; $\mathbf{n_x}$ and $\mathbf{n_y}$ are the number of amplified DNA fragments of patterns **x** and **y.**

RESULTS AND DISCUSSION

In the first stages of DNA experiments, both the parasite and its host variety VNIIMK 8883 were amplified. The major DNA fragments of the Oomycete and sunflower reproduced always differed by the quantity of nucleotides pairs (Fig. 1). This suggested the correctness of the pathogen sporulation picking up. From 22 RAPD-primers used, one (P38) did not give any amplified DNA spectra. The others produced a total of 92 fragments with average frequency of 4.4 fragments per primer. Eight primers: L14, Y11, P28, P46, P5, OPM08, OPM20, and OPJ15 were not polymorphic in the studied isolates.

Thirteen primers: OPB07 OPB17OPC08, OPC15, OPD 11, OPD 18, OPE 03, OPD 20, OPF09 OPG02 OPG05, OPG06, and OPJ13 allowed the identification of race 300 isolates from other races, based on the presence of amplified DNA fragments lacking in other races, or the absence of fractions typical for races 310, 330, 700, 710, 730 (Fig. 2). These 13 primers produced a total of 76 fragments (from 1 to 12 polymorphic fragments per primer). From them, we identified 62 polymorphic loci. Our results showed that these 62 polymorphic loci only allowed a clear identification of race 300 from the six races studied.

The resemblance measure between race 300 and the others was accounted for by means of Jacquard coefficient (J_{xy}). This method is more suitable for accounting RAPD-data (Link et al., 1995). The resemblance measure between races 330, 700, 710 and 730 was near to 1.00. The resemblance measure between race 300 and the others was defined by a fairly small value of 0.29. Accordingly, the genetic

distance (D_{xy}) between race 300 and the others (which is calculated by the formula $D_{xy}=1- J_{xy}$) was 0.71. The resemblance measure between races 300 and 710 in France was 0.88 (Roeckel-Drevet et al., 1997).

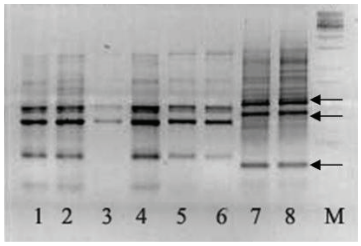

Fig. 1. Amplified DNA electrophoretical spectra of *P. halstedii* and sunflower obtained with primer L 14. Lanes: 1-6 - isolates of *P. halstedii*; 7, 8 – sunflower variety VNIIMK 8883. M – molecular mass marker (GeneRuler 1 kb DNA Ladder, MBI "Fermentas") The arrows show the reproduced DNA fractions having the length: 550 bp, 460 bp and 160 bp (respectively from top to bottom).

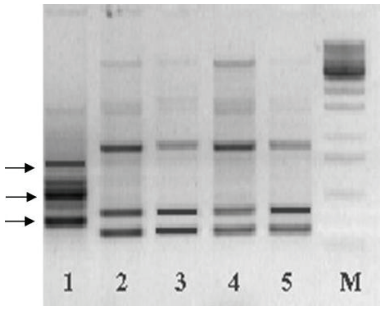

Fig. 2. Amplified DNA electrophoretical spectra of *P. halstedii* obtained with primer OPJ13. Lanes (races): 1 - 300, 2 - 330, 3 – 700, 4 -710, 5 – 730. M – molecular mass marker (GeneRuler 1 kb DNA Ladder, MBI "Fermentas" . The arrows show race 300 DNA fragments with length (from top to bottom) 710bp., 480 bp and 330 bp that distinguished it from the others.

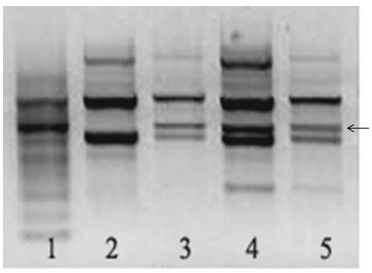

Fig. 3. Amplified DNA electrophoretical spectra of *P. halstedii* obtained with primer OPG06. Lanes (races): 1 - 300, 2 - 330, 3 – 700, 4 -710, 5 – 730. The arrow shows DNA fraction 1125 bp.

Although the race composition of *P. halstedii* population in Russia was not controlled for a long time, since first investigations date back to the beginning of the 1980s, it may be assumed that race 300 appeared in Krasnodar region later than race 100 (Antonova et al., 2000). Apparently, it was introduced into the former USSR with sunflower seeds. Its origin from another continent could explain the the distinctions of race 300 from the others on RAPD-loci studied in our experiments. Our data agreed with investigations of Tourvieille et al. (2000) grouping in separate clusters European and American isolates of this Oomycete.

Only one primer OPG06 produced intraracial polymorphism on the presence-absence of one fraction whose length is 1125 bp (shown as $OPG06_{1125}$) (Fig. 3, Table 1). Two isolates of race 300 which were

Table 1. The characteristic of DNA RAPD-locus $OPG06_{1125}$ of *P. halstedii* races, collected on sunflower from different districts of Krasnodar region, Russia, 2005

Isolate number	Race	District of isolate collection	$OPG06_{1125}$*
1	300	Fields of VNIIMK	1
2	300	Labinskiy	0
3	310	Viselkovskiy	0
4	330	Viselkovskiy	0
5	330	Viselkovskiy	0
6	330	Viselkovskiy	1
7	330	Fields of VNIIMK	1
8	330	Fields of VNIIMK	1
9	330	Fields of VNIIMK	1
10	330	Kanevskoy	0
11	330	Kanevskoy	0
12	330	Kanevskoy	0
13	330	Kanevskoy	0
14	330	Kanevskoy	0
15	330	Kanevskoy	0
16	700	Labinskiy	1
17	700	Labinskiy	1
18	700	Viselkovskiy	1
19	700	Krilovskoy	1
20	710	Fields of VNIIMK	1
21	710	Fields of VNIIMK	1
22	710	Viselkovskiy	1
23	710	Viselkovskiy	1
24	710	Viselkovskiy	1
25	710	Viselkovskiy	1
25	710	Viselkovskiy	1
27	710	Viselkovskiy	1
28	710	Viselkovskiy	1
29	710	Labinskiy	1
30	710	Labinskiy	1
31	710	Kanevskoy	1
32	710	Krilovskoy	1
33	730	Fields of VNIIMK	0
34	730	Fields of VNIIMK	0
35	730	Fields of VNIIMK	0
36	730	Fields of VNIIMK	1
37	730	Viselkovskiy	0
38	730	Viselkovskiy	0
39	730	Viselkovskiy	1
40	730	Viselkovskiy	1
41	730	Viselkovskiy	1
42	730	Viselkovskiy	1
43	730	Viselkovskiy	1

* 0- absence, 1- presence of amplified DNA fragment

collected in different districts of Krasnodar region had different genotypes for this character. The only isolate of race 310 studied showed the absence of fraction. Races 300 and 310, which were found only sporadically in the districts of Krasnodar region, gave an extremely poor sporulation and we failed to collect enough material for analysis. Twelve isolates of race 330 from three districts have shown interesting results. All the six isolates from Kanevskoy district lacked this fraction. All three isolates from fields of VNIIMK have shown its presence and, although two out of three isolates from the Viselkovskiy district did not have it, the third one did. All four isolates of race 700 have shown the presence of fraction 1125 bp in locus OPG06 and they were collected in three districts which are situated quite far from each other (Table 1). The distances between Viselkovskiy district and two others: Krilovskoy and Labinskiy are about 130 and 250 km, respectively. Therefore, despite the small quantity of studied isolates, we presume that for race 700 the presence of this fraction is uniform.

Thirteen isolates of race 710 were collected in five different districts and all of them have shown the presence of this fraction (Table 1). Eleven isolates of race 730 collected in two districts have shown the presence or absence of this fraction. Data of Table 1 show some stability of race 710 in locus OPG06 because the isolates were collected in five different districts and all of them had the fraction 1125 bp. As shown in another manuscript of these proceedings (Antonova et al., 2008), race 330 was predominant in Kanevskoy district, up to 100% in the majority of studied fields, and its isolates from there have shown the condition of locus OPG06 as the absence of the fragment. In some fields of the Viselkovskiiy district, races 710 and 730 predominated, whereas in the others there was race 330. The presence-absence of fraction varied in isolates of races 330 and 730, but it was always present on the isolates of race 710. This gives an idea about the hybridization of races in that district. Apparently, locus OPG06 is not coupled with genes of virulence. In this case, the coexistence in one population of individuals with different allelic conditions is possible.

In conclusion, our study has confirmed the considerable molecular homogeneity previously observed among the French races (Roeckel-Drevet et al., 1997; Tourvieille et al., 2000). Our data suggests that the origin of local races 330, 700, 710 and 730 in Krasnodar region could not be race 300. However, this should be further confirmed, because of the scanty quantity of race 300 isolates available for this study.

Monomorphic condition of locus OPG06 of race 710 isolates from five remote districts of Krasnodar region suggested its stability, i.e. the invariable presence of fraction 1125 bp. Monomorphic condition of locus OPG06 (the absence of fraction 1125 bp.) of race 330 isolates from one district (Kanevskoy) may be connected with this pathotype's prevailing domination there of up to 100% in the majority of studied agrocenoses. And we suppose that for "pure" race 330 the absence of fraction 1125 bp in OPG06 locus is a constant character. The polymorphic condition of this locus revealed in isolates of race 330 from Viselkovskiy district may possibly be explained as the result of hybridization between races 330 and 710 or 730 in that place.

ACKNOWLEDGEMENTS

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REFERENCES

Antonova, T.S., N.M. Araslanova, A.V. Golovin, T.A. Tchelustnikova, and S.L. Saukova. 2000. To the question about the race belonging of sunflower Downy Mildew casual agent in Northern Caucasus. Sci. Techn. Bulletin of VNIIMK, 123:21-23 (In Russian).

Antonova, T.S., N.M. Araslanova, and M.V. Iwebor. 2006. New races of sunflower downy mildew causal agent. Proceedings (Vestnik) of Agricultural Academy of Russia 3:37-38 (In Russian).

Antonova, T., M. Iwebor, and N. Araslanova. 2008. Races of *Plasmopara halstedii* on sunflower in separate agrocenosises of Adigeya Republic, Krasnodar and Rostov regions in Russia (these Proceedings).

Giresse, X., D. Tourvieille de Labrouche, S. Richard-Cervera, and F. Delmotte. 2007. Twelve polymorphic expressed sequence tags-derived marker for *Plasmopara halstedii*, the causal agent of sunflower downy mildew. Mol. Ecol. Notes 7:1363-1365.

Gulya, T.J. 2007. Distribution *Plasmopara halstedii* races from sunflower around the world. In: Proceedings of the 2nd International Downy Mildews Symposium. Advances in downy mildew

research. Palacky University in Olomouc and JOLA. v.o.s., Kostelec na HanI (Czech Republic) 3:121-134.

Gulya, T., D. Tourvieille de Labrouhe, S. Masirevic, A. Penaud, K. Rashid, and F. Viranyi. 1998. Proposal for standardized nomenclature and identification of races of *Plasmopara halstedii*. p. 130-136. In: Symposium III: Sunflower Downy Mildew, Fargo, ND, 13-14 January. Int. Sunflower Assoc, Paris.

Guchetl, S.Z., T.A. Tchelustnikova, S.A. Ramazanova, and T.S. Antonova. 2004. Molecular-genetic characteristic of sunflower inbred lines on isozymes markers and DNA-profiles. Scientific-Technical Bulletin of VNIIMK, 2(131):42-46 (in Russian).

Iwebor, M.V., T.S. Antonova, and N.M. Araslanova. 2005. New races of *Plasmopara halstedii* – the causal agent of sunflower downy mildew in Northern Caucasus. Science of Kuban 2:162-164 (in Russian).

Iwebor, M.V., T.S. Antonova, and N.M. Araslanova, 2007. The identification of race composition of *Plasmopara halstedii* population in regions of Northern Caucasus. Science of Kuban 2:47-51 (in Russian).

Link, W., C. Dixkens, M. Singh, M. Schwall, and A.E. Melchinger 1995. Genetic diversity in European and Mediterranean faba bean plasma reveal by RAPD markers. Theor. Appl. Genet. 90:27-32.

Molinero-Ruiz, M.L., J. Dominguez, and J.M. Melero-Vara. 2002. Races of isolates of *Plasmopara halstedii* isolates from Spain and studies on their virulence. Plant Disease 86:736-740.

Novotelnova, N.S. 1966. Downy Mildew of sunflower. M.;L. Science. 150 p. (in Russian).

Roeckel-Drevet, P., V. Coelno, J. Tourvieille, P. Nicolas, and D. Tourvieille de Labrouhe. 1997. Lack of genetic variability in French identified races of *Plasmopara halstedii*, the cause of downy mildew in sunflower *Helianthus annuus*. Can. J. Microbiol. 43:260-263.

Roeckel-Drevet, P., J. Tourvieille, T.J. Gulya, G. Charmet, P. Nicolas, and D. Tourvieille de Labrouhe. 2003. Molecular variability of sunflower downy mildew, *Plasmopara halstedii* from different continents. Can. J. Microbiol. 49:492-502.

Rozynek, B., and O. Spring. 2000. Pathotypes of sunflower downy mildew in Southern parts of Germany. Helia 23(32):27-34.

Shindrova, P.S. 2000. Distribution and race composition of downy mildew (*Plasmopara halstedii* Farl. Berl. and de Toni) in Bulgaria. Helia 23(33): 25-32.

Shindrova, P.S. 2005. New nomenclature of downy mildew races in sunflower (*Plasmopara halstedii* (Farl.) Berl. and de Toni) in Bulgaria (race composition during 2000-2003). Helia 28(42):57-64.

Shirshikar, S.P. 2005. Present status of sunflower downy mildew disease in India. Helia 28(43):153-158.

Sneath, P.H., and R.R. Sokal. 1973. Numerical taxonomy: the principles and practice of numerical classification. W.H. Freeman (ed.). San Francisco.

Tihonov, O.I., and V.F. Zaichuk. 1981. New race of Downy Mildew. Oil Crops 6:20-21 (in Russian).

Tourvieille, J., J. Millon, P. Roeckel-Drevet, P. Nicolas, D. Tourvieille de Labrouhe, and T.J. Gulya. 2000. Molecular variability of *Plasmopara halstedii*. p. 67-72 (vol. 1). In: Proc. 15th Int. Sunflower Conf., Toulouse, France.

Tourvieille de Labrouhe, D., T.J. Gulya, Y.K. Rashid, and F. Viranyi. 2000a. New nomenclature of races of *Plasmopara halstedii* (sunflower downy mildew). p. 161-166 (vol. 2). In: Proc. 15th Int. Sunflower Conf., Toulouse, France.

Zolan, M.E., and P.J. Pukkila. 1986. Inheritance of DNA methylation in *Corpinus cinereus*. Mol. Cell Biol. 6:195-200.

Relations between spring rainfall and infection of sunflower by *Plasmopara halstedii* (downy mildew)

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ABSTRACT

The incidence of spring rainfall on the severity of primary downy mildew attack of sunflower was studied in field trials with staggered sowing dates. Infection potential of soil depended partly on rainfall probably because, in damp conditions, oospores germinate and must infect sunflower rapidly. In contrast, under dry conditions, they remain dormant and thus maintain their infection potential. Disease risk appears greatest if there is heavy rainfall when sunflower seedlings are at their most susceptible stage, between germination and emergence. Heavy rainfall before sowing had no effect on percentage of diseased plants and heavy rainfall after seedling emergence did not increase primary downy mildew attack.

Key words: disease risk epidemiology – downy mildew – *Helianthus annuus* – infection potential – soil test.

RESUME

La mise en place de semis décalés sur deux années présentant des conditions climatiques différentes a permis de préciser l'influence des précipitations printanières sur l'expression de la forme la plus dommageable du mildiou qui est l'infection primaire tellurique. D'une part, le potentiel infectieux du sol évolue au grès des pluviométries. Les pluies continues épuisent le stock d'inoculum alors qu'une période sèche fait grimper le pouvoir infectieux de sol au niveau de l'horizon correspondant au lit de semence. D'autre part, le risque mildiou est étroitement lié à la présence de pluies abondantes au moment où la plantule présente une forte sensibilité aux infections telluriques. C'est-à-dire entre le début de la germination et l'émergence des cotylédons. Les fortes pluies qui interviennent avant la germination n'ont que peu d'impact sur le taux de plantes malades. Les fortes pluies qui arrivent après la levée n'augmentent pas le nombre d'infections telluriques. L'utilisation de ces informations épidémiologiques devrait être intégrées à la construction d'un modèle d'analyse de risques.

Mots clés: analyse de risque –épidémiologie – *Helianthus annuus* – mildiou – potentiel infectieux – test sur terre.

INTRODUCTION

Plasmopara halstedii is a soil-borne parasite, which remains in the soil in the form of oospores. These spores are produced by sexual reproduction and may remain dormant but viable for up to 10 years. Under favourable conditions, oospores germinate to give zoosporangia which, in the presence of free water, liberate mobile zoospores. These cause primary infections of sunflower radicles, leading to systemic attacks that cause most loss to the crop. Rainfall is a major climatic factor determining disease risk. Delos et al. (2000) considered that rainfall just before or after sowing was the most favourable for downy mildew infections of sunflower. To determine in more detail the effect of rainfall on percentage attack, in 2006 and 2007, sunflowers were sown at weekly intervals and detailed records were made of rainfall, soil temperature and numbers of plants showing systemic downy mildew symptoms. The infection potential of the soil in the fields concerned was measured by a growth chamber test developed by Tourvieille de Labrouhe and Walser (2005). Correlations between the different factors were determined in order to define those that should be included in models predicting disease risk.

MATERIALS AND METHODS

Sunflower genotypes

The inbred sunflower line GB (INRA, Clermont-Ferrand) was used both for soil tests in the laboratory and to measure downy mildew attack in the field. This line has no known downy mildew resistance gene.

Field trials

Trials were carried out in fields near Clermont-Ferrand (Auvergne) naturally infected with race 710 of *P.halstedii.* Each year, 10 zones were defined in the field and, in each, one 3m row was sown with the inbred line GB every week from 21st of March to 6th of May in 2006 (8 sowing dates) and from 13th of March to 15th of May in 2007 (10 sowing dates).

Observations of weather conditions

Rainfall was obtained from Météo-France at Clermont-Ferrand airport (at 1km). Soil temperature was measured with a recorder (xvacq de TMI Orion) measuring the temperature at a depth of 3cm every hour.

Field observations

The trials were observed each week from seedling emergence to 2 pairs of leaves. The numbers of plants showing systemic downy mildew symptoms resulting from primary infections through the roots were counted.

Soil test

The day before soil sampling, sunflower seeds were germinated at 100% RH after soaking in water for 2 hours. Soil was sampled at a depth corresponding to that at which sunflower seeds are normally sown. The samples were placed in trays and the germinating seed sown at a depth of 1cm. After 48h at 18°C, the trays were immersed in water for 8h. They were then incubated for 12 days at 18°C and 12000 lux light 16h/24. To determine presence of downy mildew, the trays were covered with a plastic bag to obtain 100%RH for 48h and then observations were made of sporulation on cotyledons and true leaves (Tourvieille de Labrouhe and Walser, 2005).

RESULTS

Weather conditions

Rainfall, mean daily temperatures and sowing dates are presented in Fig. 1 and 2.

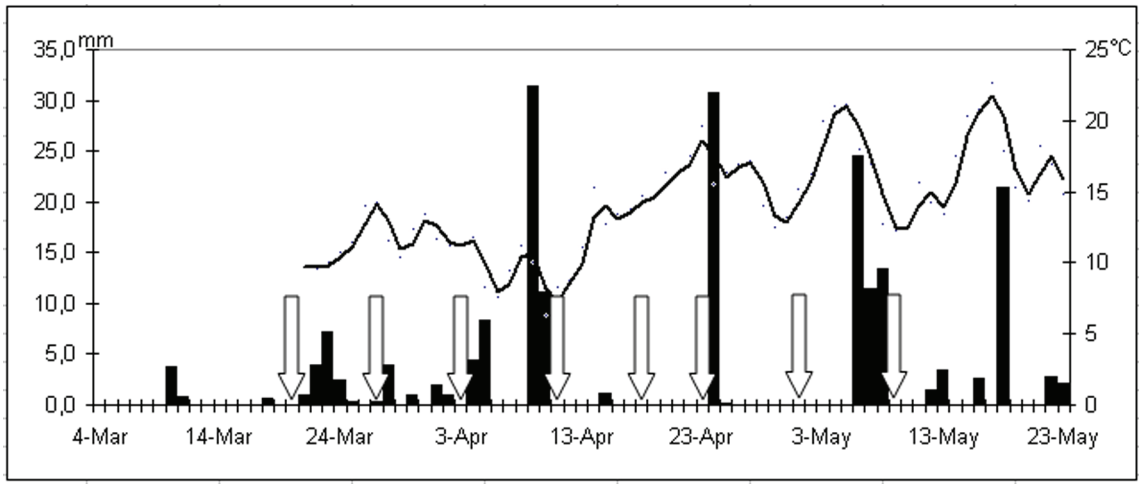

Fig. 1. Soil temperature and rainfall in 2006. Arrows indicate sowing and soil sampling dates

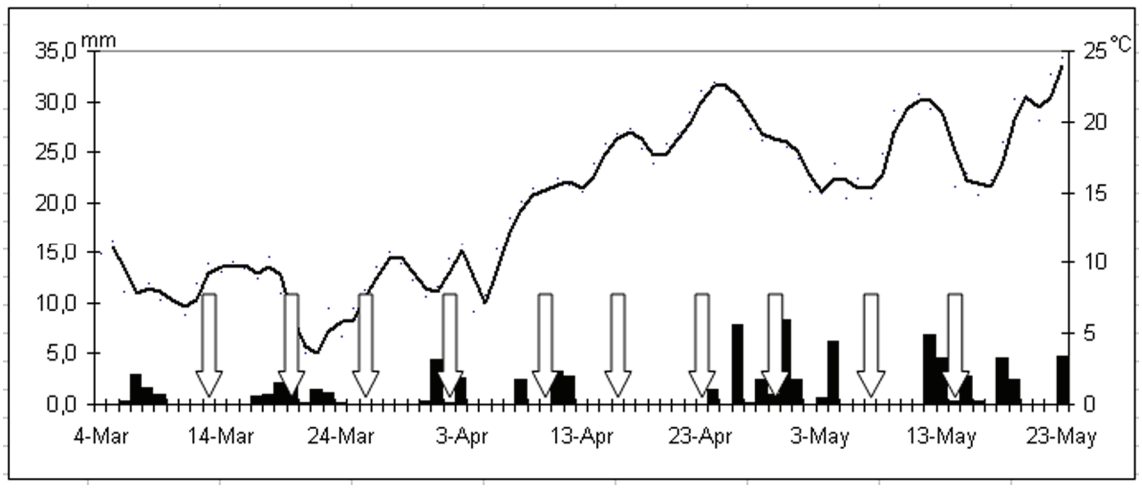

Fig. 2. Soil temperature and rainfall in 2007. Arrows indicate sowing and soil sampling dates

Weather conditions were very different in 2006 and 2007. In 2006, the period studied was cool (mean temperature 15°C) with some heavy rain (178mm, including 42mm 9-10/4, 31mm 24/4, 49mm 6-8/5 et 21mm 18/5). 2007 was much warmer (mean temperature 18°C) and drier (less than 100mm, with 12mm 29/4-1/5 and 14mm 12-15/5).

Variation in downy mildew attack according to sowing date
Table 1 presents percentage primary attacks in 2006 and 2007.

Table 1. Mean percentage downy mildew attack for each sowing date (10 plots for each date)

2006		2007	
Sowing date	% attack	Sowing date	% attack
		March 13rd	0.0
March 20th	16.3	March 20th	1.1
March 27th	44.0	March 26th	0.4
April 3rd	25.1	April 2nd	0.0
April 11th	32.5	April 10th	2.2
April 18th	23.3	April 16th	13.2
April 23rd	34.9	April 23rd	12.9
May 1st	16.0	April 30th	2.0
May 9th	11.6	May 7th	0.2
		May 14th	0.8

In 2007, only 2 dates (16/4 and 23/4) showed more than 10% attack (with a maximum of 39% for one plot), in contrast with 2006 when mean attack was 11 to 44%, with a plot maximum of 83%. Both years showed a considerable variation between sowing dates.

Soil infection potential according to sampling date
Fig. 3 and 4 show the variations in infection potential measured by the laboratory soil test. Potential infection varied considerably according to the zones in the field where soil was sampled but also according to sampling date. The lowest levels were found in March, and in April the greatest potentials were observed for 2 dates: 3/4 and 23/4 in 2006 and 2/4 and 23/4 in 2007.

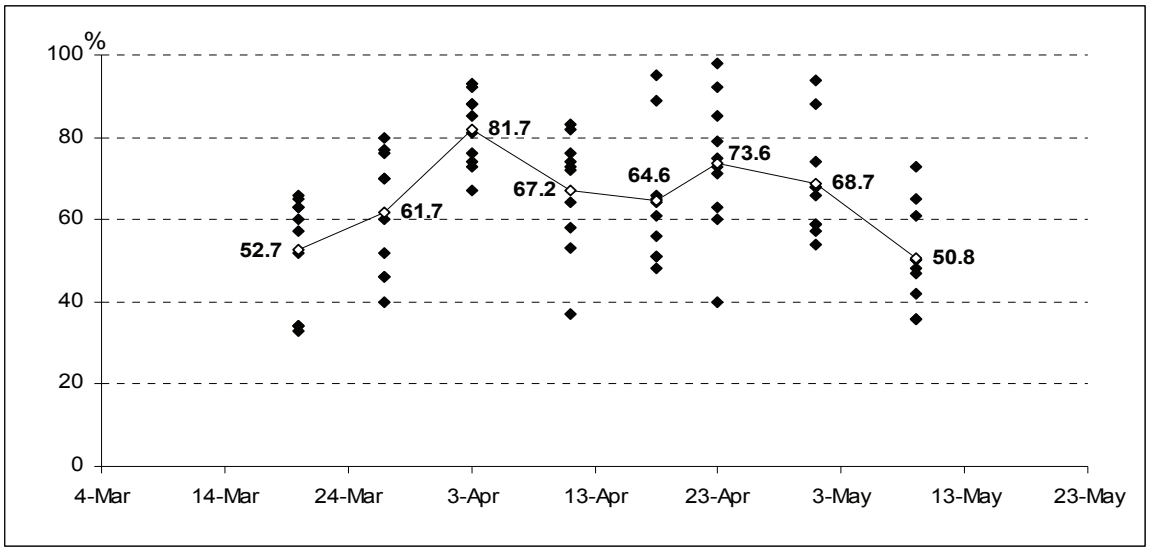

Fig. 3. Variation in percentage of seedlings showing downy mildew symptoms according to date of soil test (10 samples per date) for 2006

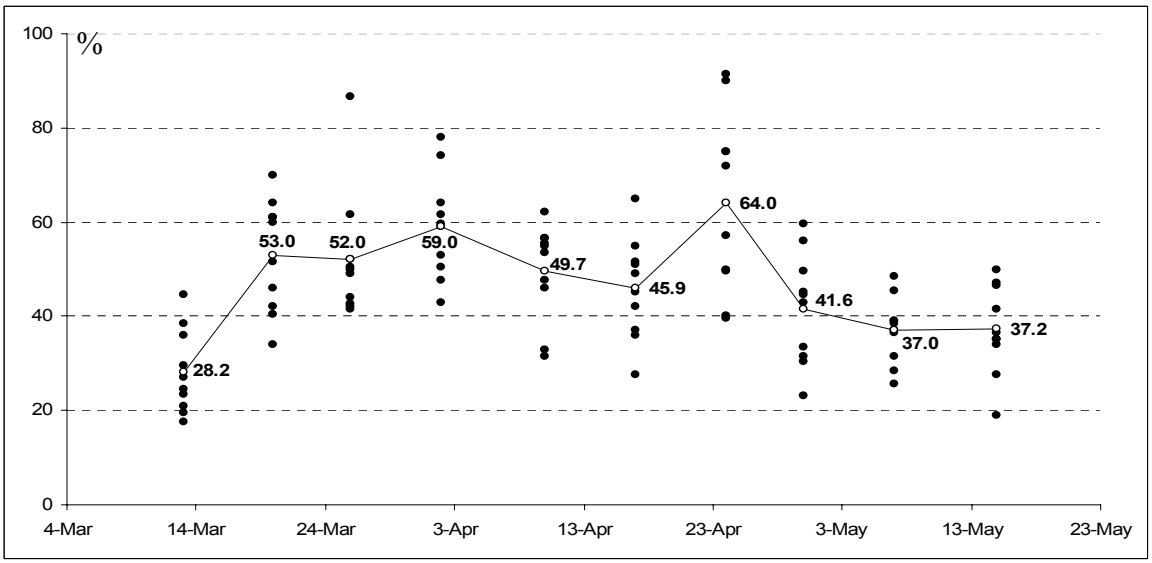

Fig. 4. Variation in percentage of seedlings showing downy mildew symptoms according to date of soil test (10 samples per date) for 2007.

Relations between rainfall and soil infection potential

There was a negative relation between rainfall and soil infection potential. This was confirmed by comparison between total rainfall 10 days before sampling and the percentage of diseased plants in soil tests (Table 2). The negative correlation coefficient was highly significant in 2007, an unfavourable year for downy mildew, especially if only April is considered.

Table 2. Correlation coefficients (Pearson) between rainfall and soil infection potential

	2006		2007	
	March-May	April	March-May	April
Number of replicates (10 plots by date of sowing)	80	40	100	50
Correlation coefficient				
- Infection potential / rainfall 10 days before sampling	r= - 0.166	r= -0.337*	r= - 0.416**	r= -0,513**
- Rate of attack / rainfall between "sowing + 166 h. with > 7°C" and "sowing + 360 h. with > 7°C"	r= 0.383**	r= 0.403**	r= 0.513**	r= 0.522**

* significant at p = 0.05, ** significant at p = 0.01

Relation between rainfall and downy mildew attack in the field

Correlations were calculated between percentage of primary attack and rainfall in the pre-emergence period taking into account soil temperature, which is important for rapid emergence The closest correlation was with rainfall in the period from sowing +166h at above 7°C and sowing + 360h at 7°C (Table 2). For both years, the correlation coefficients were highly significant.

DISCUSSION

The effect of rainfall on the level of downy mildew attack was analysed for 2 very contrasting years. In neither year was there any water deficit, but whereas 2006 had some days of heavy rain interspersed with dry periods, in 2007 there were no heavy rains but regular damp periods. These differences are useful to make general conclusions.

The fields used in 2006 and 2007 had shown comparable levels of downy mildew attack in preceding years, suggesting that they should have similar inoculum potentials (Tourvieille de Labrouhe et al., 2008). The present results indicate quite clearly that the risk of downy mildew attack depends closely on pattern and intensity of rainfall. Délos et al. (2000) reported that rainfall ought to be sufficient to give the free water in the soil necessary for zoospore movement. The low levels of attack in March 2006 and 2007 may be explained not only by lack of rainfall but also low infection potentials, as measured by the soil test. The limiting factor could be the level of inoculum maturation, which depends on soil temperature.

The low levels of attack on the last sowing dates, in May, when there was considerable rainfall, especially in 2006, also suggest that the limiting factor was also a low level of inoculum. This was confirmed by soil tests. It appears likely that the inoculum present around seeds had been used up by the end of April. Infection potential varied between weeks, and soil samples taken after a dry period (when zoosporangia may not have been produced) always showed an increase in infection potential. This observation was confirmed by the negative correlation between rainfall in the 10 days preceding soil sampling and the infection potential of this sample. As suggested by Délos et al. (2000), rainfall may be necessary to obtain free water in the soil for zoospore movement, but it could also be suggested that rainfall leads to production of inoculum which can only cause infection over quite a short period (Goossen and Sackston, 1968), so that none remains in later weeks. Thus, in addition to a variable level of infection potential over the whole period, with a maximum in mid-April, (Tourvieille de Labrouhe et al., 2008), daily variation may occur according to soil humidity. The trials have not been made under very dry conditions so no data is available as to the length of time necessary for oospores to produce zoosporangia when conditions become favourable for the parasite. It would be useful to carry out laboratory tests on soil samples subjected to variable periods of drying.

Délos et al. (2000) reported that attack levels were closely correlated with rainfall from 5 days before sowing to 5 days after sowing. The present results did not show this correlation. Taking into account minimum growth temperature (6°C; Merrien, 1992) and sunflower germination optimum (8°C; Anonymous, 2003), a close relation appeared between rainfall at sowing date + 166h above 7°C (beginning of radicle growth) and sowing date + 360h at above 7°C (emergence). This is generally equivalent to 5 to 15 days after sowing, later than that suggested by the earlier results.

These epidemiological results should help in the construction of a model concerning disease risk. The probability of the appearance of plants showing downy mildew symptoms depends on many factors, of which the following appear to be the most important:

- The level of infestation of the field , which will depend on the number of diseased plants in the preceding sunflower crop (Tourvieille de Labrouhe et al., 2008).
- The use of resistant sunflower varieties and the development of downy mildew races virulent on these varieties (Vear et al., 2007).
- Seed dressing with fungicide and resistance of downy mildew isolates to this fungicide (Gulya, 2000).
- crop management practices which could affect disease development (Covarelli et Tosi, 2007; Escande et al., 2007).
- Rainfall between germination and emergence.
- Soil infection potential on level of seed bed.

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REFERENCES

Anonymous. 2003. Le tournesol. INA P-G – Département AGER. http://138.102.82.2/agronomie/phytotechnie/pdf/tournesol.pdf

Délos, M., N. Eychenne, I. Birba, and C. Fabry. 2000. Etude des facteurs expliquant les fluctuations des attaques de *Plasmopara halstedii* en France. p. I43-I48. In: Proc. 15th Int. Sunfl. Conf., Toulouse, France.

Escande, A., F. Quiroz, J.P. Fernandez, S. Lenardon, J. Gonzalez Montaner, D. Alvarez, N. Huguet, V. Pereyra, P. Calvino, O. Marcellan, M.E. Bazzalo, A. Romano, and A. Vazquez. 2007. Las enfermedades y el manejo del cultivo. Qué hemos aprendido? p. 133-144. In Proc. 4to Congreso Argentino de Girasol, B.A., Argentina.

Goossen, P.G., and W.E. Sackston. 1968. Transmission and biology of sunflower downy mildew. Can. J. Bot. 46:5-10.

Gulya, T.J. 2000. Metalaxyl resistance in sunflower downy mildex and control through genetics and alternative fungicides. p. I79-I84. In: Proc. 15th Int. Sunfl. Conf., Toulouse, France.

Merrien A., 1992. Les points techniques du CETIOM: Physiologie du tournesol.CETIOM, 66 p.

Tourvieille de Labrouhe, D., and P. Walser. 2005. Un test simple pour déceler la présence du mildiou sur une parcelle. Oleoscope 83:12.

Tourvieille de Labrouhe, D., E. Pilorgé, P. Nicolas, and F. Vear. 2000. Le Mildiou du Tournesol. Versailles, France: CETIOM-INRA, 70p.

Tourvieille de Labrouhe, D., A. Penaud, P. Walser, E. Mestries, J. Moinard, F. Serre, C. Thiery, B. Garric, and X. Pinochet. 2008. Determine the sunflower downy mildew risk by soil analysis. In: Proc. 17th Int. Sunfl. Conf., Cordoba, Spain.

Vear, F., F. Serre, S. Roche, P. Walser, and D. Tourvieille de Labrouhe. 2007. Recent research on downy mildew resistance useful for breeding industrial-use sunflowers. Helia 30:45-54.

Structural aspects regarding formation and emission of *Diaporthe* (*Phomopsis*) *helianthi* ascospores

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ABSTRACT

Ascospores are the main infection source of Phomopsis in sunflower. However, the concept of their structure is still being contradicted and is not complete enough. The objective of this work was to study the structural organization of the ascospore formation and emission for the sunflower *Phomopsis* pathogen. Perithecia of the fungus both in their intact and crushed condition were studied using light microscopes, digital photography and computer-aided analysis. It was determined that 16 ascospores were produced within an ascus and that was twice as many as those considered earlier. The ascospores are pressed out of the water-swollen perithecium wall in the form of a colorless sphere, whose membrane has a granular structure. The infection source contained within the sphere is transmitted by wind.

Key words: ascospore – fungus – perithecium – Phomopsis – structure – sunflower.

INTRODUCTION

Severe infection of sunflower stems caused by Phomopsis (*Diaporthe helianthi* Munt.-Cvet. et al.) was first observed in Yugoslavia (Mihalicevic et al., 1980) and quickly spread in other countries. The severe damage caused by this disease prompted general interest in the Phomopsis pathogen biology. It was determined that the fungus penetrated into the plant through the leaves, spread along the vessels and then entered the stem (Petrov et al., 1981; Bertrand and Tourvieille, 1987). Pathohistological bases of the fungus penetration and resulting stem tissue changes (Muntanola-Cvetkovic et al., 1989) were studied. Nowadays, it is recognized everywhere that ascospores developing in perithecia are a main infection source. Perithecia, of an irregular round shape and 150-430 x 180-850 μm in size, are produced on overwintering plant residues and contain elongated clavate asci (44-67.5 x 4.5-12.0 μm in size), each of which includes 8 ascospores (Assemat and Fayret, 1988; Yakutkin, 1988). The ascospores, which are colorless, with one partition, of an elongated elliptic shape, 15-17 x 5-7.5 μm in size, slightly pressed in the middle beside the partition, have two equal cells containing generally two fat drops per cell and, as some researchers consider, have constricted ends (Maric et al., 1982). According to other scientists' observations they have rounded ends (Assemat and Fayret, 1988) and may reach 7.5 μm in length (Yakutkin, 1988). It is accepted that the ascospores are thrown out from the perithecium to the height of about 3 mm above the plant residue surface. Sometimes, a mucoid drop or a white mass containing ascospores form on the rostrum apex (Maric et al., 1982; Yakutkin, 1988).

The structure of fruit bodies, conidia and ascospores serves as a basis for fungus identification. However, until now their understanding has been incomplete and rather contradictory.

The objective of this work was to study the structural organization of the ascospore formation and emission for the sunflower Phomopsis pathogen.

MATERIALS AND METHODS

For two years, the fungal fruit body formation was observed in the Phomopsis-affected seeds and stems of the sunflower cultivars Rodnik and Berezansky, the hybrids Fly (Monsanto) and Melody (Syngenta), as well as the stems of *Sonchus oleraceus* (L.) Scop. by keeping them under natural or storage or stationary (refrigerator) conditions.

The stem fragments of the above plants and sunflower seeds with the Phomopsis infection symptoms were washed with water, disinfected with ethyl alcohol; afterwards, the stems were flamed with a gas-stove burner and the seeds were washed with sterile water and placed on sterile wet filter paper inside Petri dishes, which were incubated in the environmental chamber (Sanyo), at 25°C, 16-hour photoperiod

(3000 lux) and 80% of humidity. A part of the experiment was conducted under the same conditions but at the night temperature of 12-15^{o}C.

Intact fruit bodies of Phomopsis forming on the stem or on seeds were examined with a stereozoom trinocular microscope (MLS) and perithecia isolated from the plant substrate and crushed – with a laboratory (ML2300) microscope and trinocular microscopes (Meiji, Japan). Their typical structures were photographed with the digital cameras Canon Digital (Canon) and Cyber-short (Sony) and CCD Digital Microscope camera with software. The pictures were analyzed and processed on a computer.

RESULTS

It was determined that the content of the clavate ascus transformed from an undifferentiated condition to the ascospore formation. The ascospore formation happened stepwise. First, 8 colorless structures with an elongated elliptic shape and slightly deppressed beside the partition were produced within the asci. Each of these structures had two equal cells containing generally two fat drops or, although seldom, one fat drop, and constricted ends (Fig. 1a). Their size exceeded 10 µm, but did not reach 20 µm. They did not leave the asci, which were still so firm that they did not collapse while the preparation was produced by crushing the perithecium on the microscope slide. Being in the ascus, the structures started to change: their membranes becoming thinner and their ends rounded (Fig. 1b). Further, each cell of these structures transformed to a bicellular colorless structure of an elongated elliptic shape, depressed in the middle near the partition, with two rounded fat drops – one at each of the opposite ends (Fig. 1a). These structures were identified by us as ascospores, and the initial ones were called biascospores. Two bicellular ascospores, whose length did not exceed 10 µm, were generated by each biascospore. The biascospore cell that had one fat drop turned into a unicellular ascospore with one fat drop in its center (Fig. 1c).

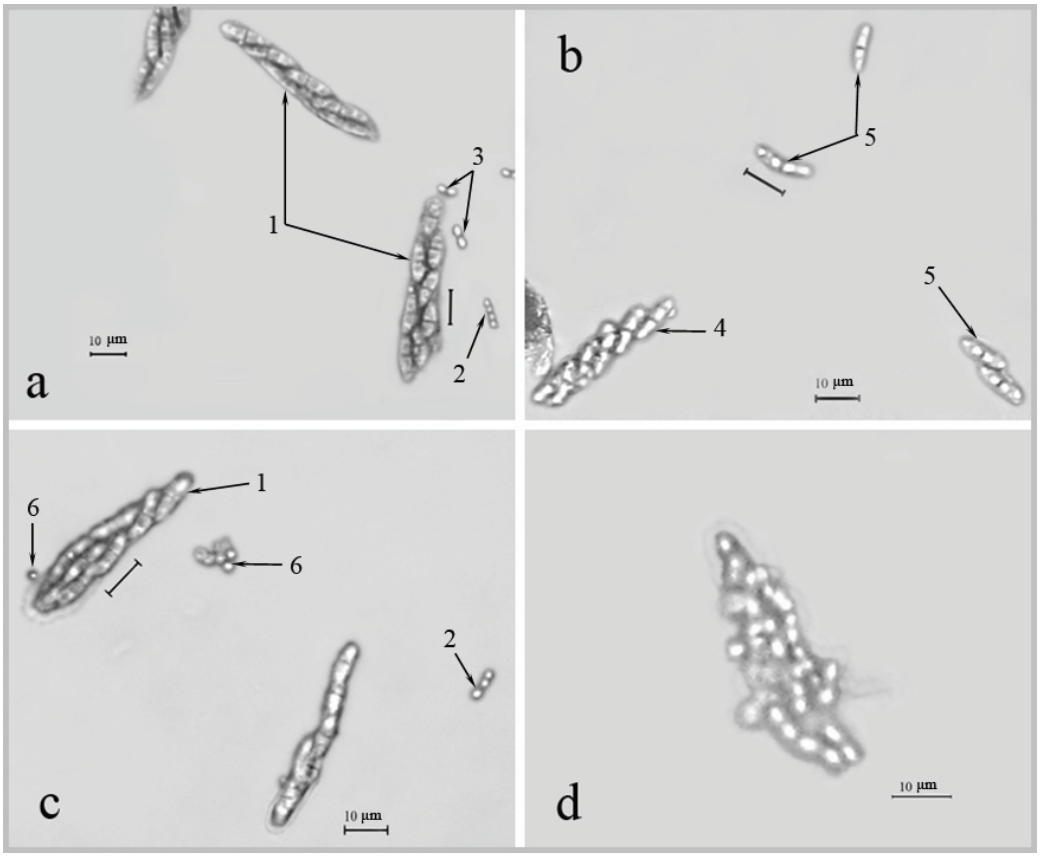

Fig. 1. Structures emerging from a crushed perithecium at their different developmental stages: 1- intact asci with biascospores; 2- ascospores still forming a pair and connected by a thin layer of a common membrane; 3 – bicellular ascospores; 4- a collapsed ascus with biascospores that already have rounded ends; 5- biascospores with rounded ends; 6- unicellular ascospores; d- a collapsing ascus with ascospores (ML2300) x 400.

The transformation of biascospores to ascospores did not happen within an ascus simultaneously, but sequentially: it started at the narrow end and finished at the broad part (from bottom to top). At that period the asci, which developed irregularly within the same perithecium, became fragile because of the thinning of their membranes especially at the sites where the ascospores had already been produced. Thus, the following structures emerged from the crushed perithecium at that period: intact asci with biascospores (Fig. 1a); collapsed asci with biascospores already having rounded ends; ascospores keeping to the arrangement in the form of the ascus already collapsed (Fig. 1d); ascospores still united into a pair by a thin layer of a common membrane (Fig. 1a; Fig. 1c); separate ascospores, generally bicellular and rarely unicellular. 16 ascospores developed in each ascus. No paraphyses were identified. As the last biascospore turned into two ascospores, the ascus membrane collapsed (Fig. 1d). From the preparation generated from a crushed ripe perithecium only free ascospores emerged (Fig. 2). Most of them were bicellular. The quantity of unicellular ascospores is, as a rule, not high and depends on the perithecium formation conditions. For example, during a drought, the number of unicellular ascospores increases.

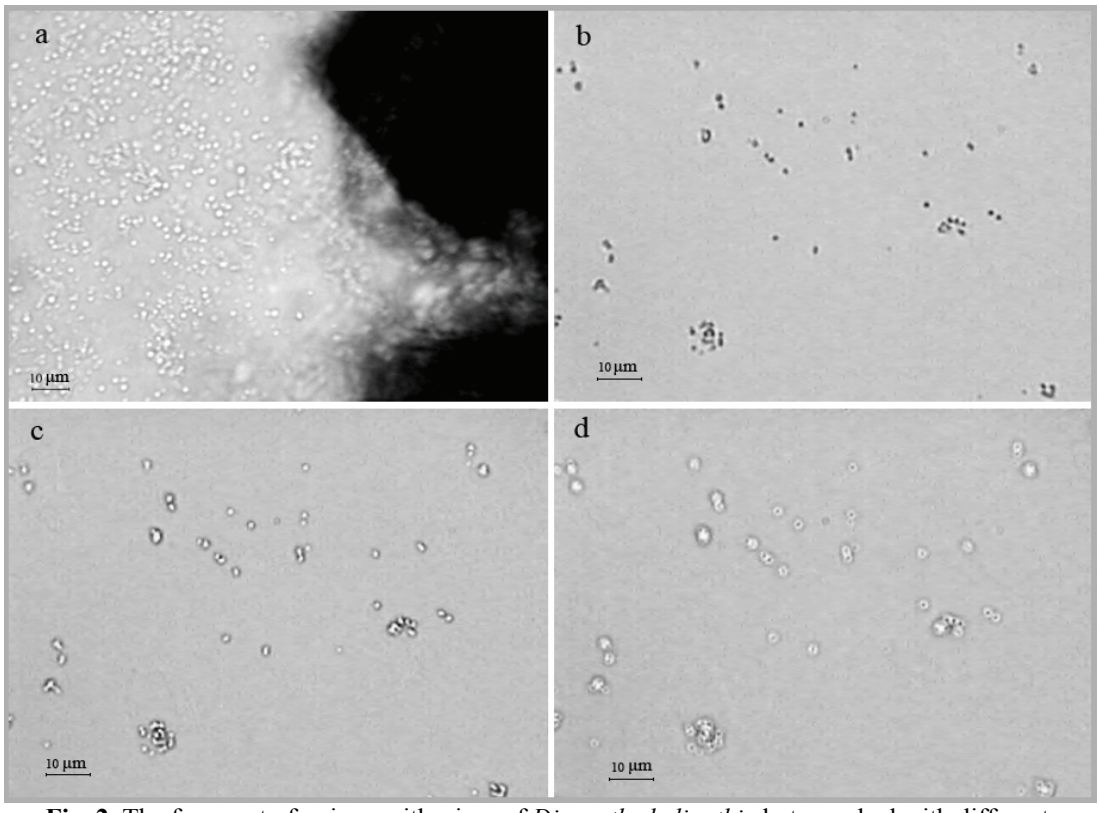

Fig. 2. The fragment of a ripe perithecium of *Diaporthe helianthi* photographed with different sharpnesses of the microscope: a- ascospores emerging from the crushed perithecium, (ML2300) x 400; b- on its surface; c- inside a cell; d- with optical border effect on the membrane and partition, CCD camera x 400.

Before the ascospore emission, a colorless transient sphere (Fig. 3a) with a granular structure of its membrane and smooth surface (Fig. 3b) appeared at the rostrum apex under the pressure of the perithecium walls swollen with water. The ascospores (Fig. 3c) started to be pressed into this sphere. The size of the sphere was from 150 to 200 µm. The color of the filled sphere changed from beige to bright yellow and depended on color and size of fat drops in ascospores, the larger the drops the yellower the sphere (Fig. 3d). The filled spheres became detached from the rostrums and fell into the humid chamber (Fig. 4). Under natural conditions they are caught by ascending air currents and borne by the wind.

Thus, it was determined that 16 ascospores developed in the perithecium ascus of *D. helianthi* which was twice as many as had been considered earlier. Bicellular, or more rarely unicellular, ascospores developed as pairs, one pair per each cell of a bicellular biascospore, and each ascus contained 8 biascospores. The ascospores were pressed by the water-swollen perithecium walls into a colorless sphere, whose membrane had a granular structure and whose surface was not smooth. The infection was transmitted by wind together with the sphere.

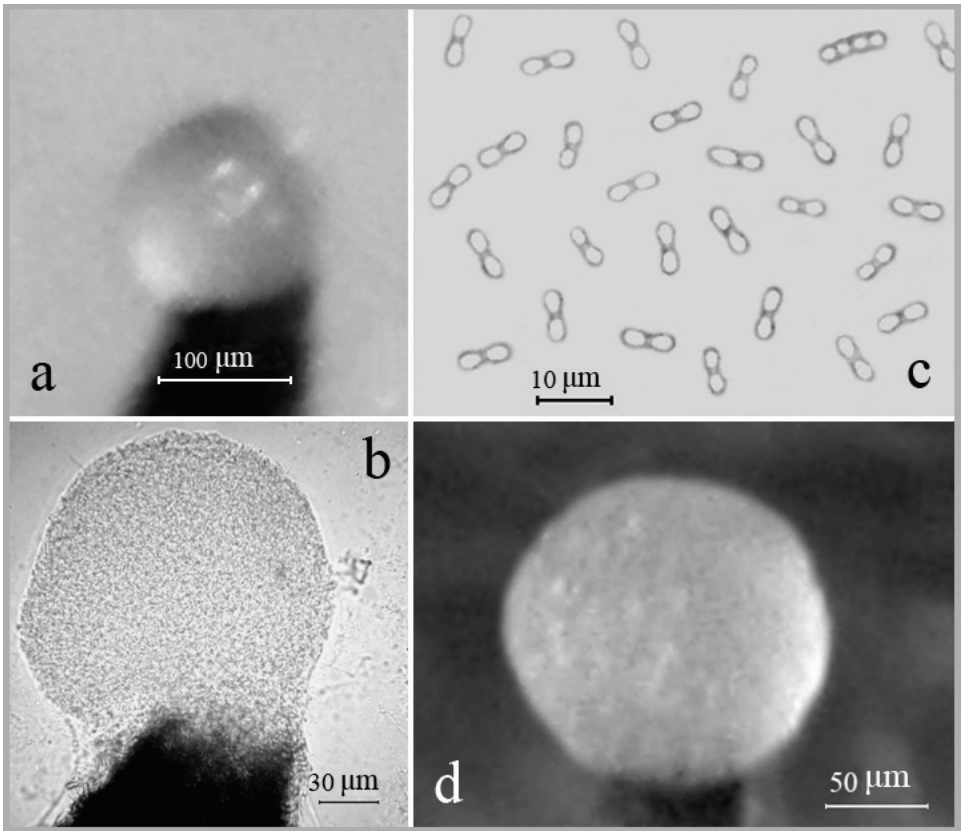

Fig. 3. Apexes of rostrums with spheres: a- an empty sphere under the stereozoom microscope. MLS x 12; b- membrane of an empty sphere. (ML2300) x 400; c- ascospores. (ML2300) x 400; d- a sphere filled with ascospores. MLS x 42.

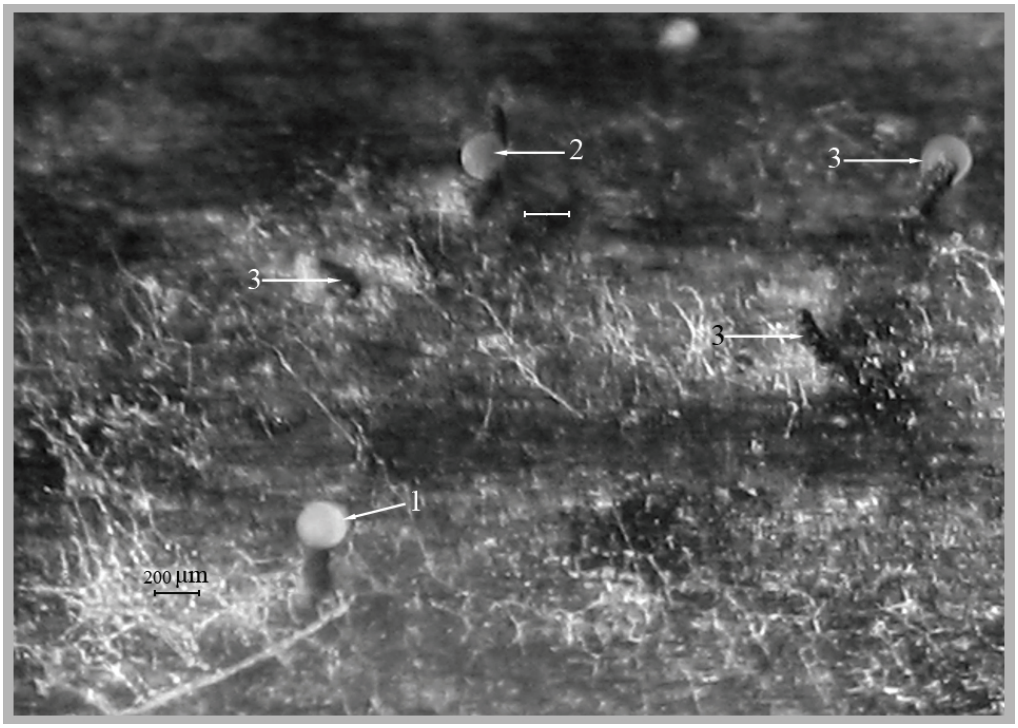

Fig. 4. Appearance of the *Diaporthe helianthi* infection source from perithecia. MLS x 12: 1- a sphere filled with ascospores; 2 – a sphere detached from the rostrum; 3- rostrum.

DISCUSSION

Earlier unknown phenomena have been demonstrated: spreading the infection source by spheres and a stepwise formation of ascopspores including their development within the larger structures (biascospores) formed earlier in an ascus. According to their structure the biascospores correspond to spores, and depending on their function – to additional asci or capsules as they are not spread from the perithecium and do not affect plants.

The results presented differ from the already known concepts regarding the structure and formation of the *D. helianthi* ascospores. However, they solve the contradictions described in the introduction to this paper. Now there are no doubts that bicellular ascospores have rounded ends, which contain one fat drop and reach about 10 μm in length. It should be noted that the formation of ascospores happens stepwise. This fact was unknown earlier and conventional approaches were used here: some researchers took larger biascospores for ascospores, others – bicellular ascospores with rounded ends. These contradictions resulted in indicating significantly different sizes and forms of the ascospore ends in the world literature.

It is not clear what the reason for this stepwise formation of ascospores within the *D. helianthi* asci is. Why isn't the fungus spread by biascospores that have a thicker membrane (i.e., are better protected)? Evidently, the answer to this question is concealed in the perfect stage characteristics of the fungus.

The infection distribution by spheres does not exclude the emission of ascospores from the perithecium as described above. It is possible that they coincide. The spheres could be easily taken for mucoid drops mentioned in earlier publications. Moreover, the filled spheres subjected to internal pressure easily collapse at the slightest pressure from outside and it is impossible to detect their membranes. However, some empty and full drop-shaped structures were noticed on the rostrum apexes. But a drop cannot be empty – then, it is nothing but a membrane. This phenomenon has been shown by us. The formation of spheres on the rostrum apexes often occurs under nightly temperature drop conditions. It should be noted that the spheres are a very convenient means of transporting ascospores because the infection source is well protected inside them. Due to the spheres there may be a high concentration of spores on the leaves of infected plants. Though the sphere is large and contains a great number of ascospores it cannot land for a long time, as its surface is not even.

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REFERENCES

Assemat, P., and J. Fayret. 1988. Biologie culturale et differenciation des peritheces in vitro chez *Diaporthe (Phomopsis) helianthi* Munt.-Cvet. p. 84-89 (vol. 2). In: Proc. 12th Int. Sunflower Conf., Novi Sad. Yugoslavia.

Bertrand, F., and D. Tourvieille. 1987. Phomopsis de selection. p. 12-13. In: Informations Techniques CETIOM 98. France.

Maric, A., S. Masirevic, and Su Li. 1982. Prilog proucavanju *Phomopsis* spp. (*Diaporthe* sp.) prowsrokovace sive pecevosti stabea suncokreta. Zastita Bilja 33, 4:403-419.

Mihalicevic, M., M. Muntanola-Cvetkovic, and M., Petrov. 1980. *Phomopsis* sp. a new parasite of sunflower in Yugoslavia. p. 531-539. In: Savrem. Poljopr. 28. Yugoslavia.

Muntanola-Cvetkovic, M., J. Vukojevic, and M. Mihalicevic. 1989. Pathohistology of sunflower stems attacked by *Diaporthe helianthi*. Can. J. Bot. 67:1119-1125.

Petrov, M., M. Muntanola-Cvetkovic, and M. Mihalicevic. 1981. New findings on the disease of sunflower plants caused by *Phomopsis helianthi Munt.-Cvet. et al.* (*Diaporthe helianthi* Munt.-Cvet. et al.). Arh. Biol. Nauka 33:13-19.

Yakutkin, V.I. 1988. Methods for identification and evaluation of sunflower *Phomopsis*. p. 191-207. In: Collection of Methodological Recommendations in Crop Protection. St.- Petersburg, Russia.

Research on a growth chamber test to measure quantitative resistance to sunflower downy mildew

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ABSTRACT

A test on sunflower seedlings in a growth chamber would facilitate measurement of factors determining quantitative resistance to downy mildew compared with the complex field trials necessary at present. Tests based on the usual method to determine major gene resistance were made on genotypes with no efficient major gene. Observations were made of the percentage of plants showing either damping off or sporulation on cotyledons and true leaves, with the aim of representing the percentage of systemically diseased plants observed in field trials. It was found that radicle length needs to be between 3 and 10mm at infection to obtain the closest correlation between field and growth chamber results for inbred lines. For hybrids, there was no significant correlation with field results and hybrids made between one tester line and varied inbreds showed reduced variability compared with per se values. Use of this test to understand quantitative resistance and to improve durability of resistance is discussed.

Key words: germination – *Helianthus annuus* – *Plasmopara halstedii* – quantitative resistance – sporulation

RESUME

Afin de phénotyper des génotypes de tournesol pour leur caractère de résistance partielle non-race spécifique, nous avons recherché un test en laboratoire. L'intérêt du protocole de notation a été validé par comparaison avec le comportement des génotypes observé en infections naturelles en absence de toutes méthodes de lutte culturale ou chimique. Les tests sont réalisés en chambre de culture dans les conditions identiques à celles qui sont utilisées pour la caractérisation des résistances monogéniques dominantes**.** Nous utilisons un pathotype virulent vis-à-vis des génotypes en évaluation. Les observations portent sur l'importance des symptômes: la fonte de semis et l'étendue des sporulations sur les organes aériens. Le taux de plantules présentant des symptômes forts et caractéristiques de mildiou lors du test en laboratoire est bien corrélé avec le taux de plantes montrant une infection primaire (infection tellurique) lors des observations réalisées en plein champ. L'intérêt de ce test en laboratoire pour sélectionner des variétés de tournesol présentant un bon niveau de résistance non race spécifique au mildiou est discuté.

INTRODUCTION

Downy mildew resistance in sunflowers has mainly been based on use of major resistance genes, denoted *Pl*. However, selection pressure on the parasite, *Plasmopara halstedii* has led to the appearance of new races (Tourvieille de Labrouhe et al., 2005) which could cause reduced crop yields in areas where weather conditions at sowing are favourable to the disease (Délos et al., 2000). To obtain more durable resistance, research was made for quantitative resistance and field trials showed high levels of partial resistance in cultivated sunflower which would be useful in breeding (Tourvieille de Labrouhe et al., 2008). However, field trials with downy mildew are complex and limited to the naturally occurring race. To make possible studies of reaction to different races, or large scale early breeding tests, a laboratory test on sunflower seedlings, measuring frequency or extent of downy mildew symptoms, is required. This paper reports experiments measuring the frequency of sporulation on the first true leaves, and the effects of radicle length when infected, for both inbred lines and hybrids. The results were compared with those obtained in field trials.

MATERIALS AND METHODS

Plasmopara halstedii race. Race 710 was shown to be naturally present in the field at Clermont-Ferrand, by observation of differential lines (Gulya et al., 1998). In the growth chamber, the same race was used.

Sunflower genotypes. Two series of genotypes were used (Table 1):

- 44 inbred lines and 45 hybrids obtained from crosses between these lines, considered as representing the variability present in modern cultivated sunflower.
- 40 recombinant inbred lines (RIL) chosen, among a population obtained from a cross between 2 INRA lines, XRQ and PSC8, for their diversity of reaction in downy mildew field trials. They carried either no *Pl* gene or *Pl*2, which is not effective against race 710. Hybrids between these lines and a very susceptible line GB (Vear et al., 2006) were also studied.

Table 1. Numbers of inbred lines and hybrids used in each experiment

Germination stage	Radicle length	Inbred - hybrid comparison	Heredity
44 inbreds	40 RIL	40 RIL	11 inbreds
45 hybrids	40 GB x RIL	40 GBxRIL	28 hybrids

Field trials. Methodology used was that of Tourvieille de Labrouhe et al. (2008). The level of attack of each genotype was defined according to the percentage showing damping off, yellowed leaves or dwarfing, characteristic of primary downy mildew attacks (Tourvieille de Labrouhe et al., 2000). Three to 4 weeks after sowing (cotyledon stage), the number of plants emerged in each plot was counted (including those showing symptoms of damping off). Two to 3 weeks later (2-3 pairs of leaves), the number of healthy plants per plot were counted (rather than the number of diseased plants since some of these had already withered). Percentage infection was then calculated (from 100-% healthy plants).

Growth chamber experiments. Growth chambers, in accordance with quarantine regulations, had 16h light (12000 lux) with a temperature of 18±1°C and 65-90% RH. Methodology was based on that of Roche et al. (2005) for testing major gene resistance. Sunflower seeds with radicle lengths between 1 and 30mm were soaked for 3h in fresh zoosporangia suspensions (100,000/ml), obtained from infected seedlings covered with polythene bags for 48h. For the germination stage trial, germinating seeds were divided into 2 groups: those with radicles of <5mm and those with radicles of >5mm. For the radicle length trial: germinated seeds were photographed before infection to permit measurement of radicle lengths. Seedlings were then pricked out in trays with Klasmann Seedlingsubstrat NF U 44-551 compost.

After 12 days, seedlings were maintained at 100%RH for 48h. Since all genotypes were susceptible to race 710, they all showed some sporulation on the shoot. However, symptom intensity varied and plants were placed in 1 of 3 classes: 1= Damped off (rotting before or after emergence); 2= Sporulation on cotyledons and at least 1 true leaf; 3= More or less sporulation on cotyledons but no sporulation on true leaves. From these observations, the percentage of "completely susceptible" (%CS) was calculated from the sum of classes 1 and 2 compared with the total.

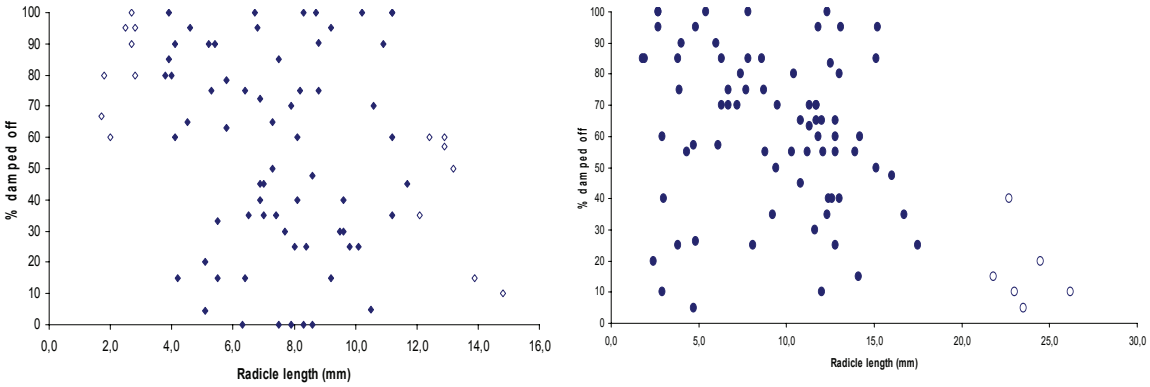
RESULTS

Effect of germination stage. The %CS was significantly higher for seedlings with radicles <5mm (Table 2) but the results of the 2 series were significantly correlated (Pearson correlation): inbred lines r= 0.774** , hybrids: r= 0.572**. However, 7 inbred lines and 15 hybrids showed more symptoms with radicles >5mm.

Table 2. Percent of seedlings showing complete susceptibility (%CS) according to germination stage when infected

		Radicle length	
		< 5 mm	≥ 5 mm
44 inbred lines			
	Mean	73.1%	58.2%
Extremes			
	inbred F340	100.0	35.7
	inbred MO502	44.0	65.0
45 hybrids			
	Mean	65.2%	52.8%
Extremes			
	hybrid CD x 90R18	100.0	0.0
	hybrid SL x PAZ2	47.6	75.0

Effect of radicle length on proportion of damped off seedlings. Fig. 1a and 1b present the relations between radicle length measured from photographs before infection and proportion of damped off seedlings of RIL and their hybrids, respectively. For the RIL, the 8 genotypes showing the shortest radicles (<3mm) were all damped off at >60% whereas the 8 with radicles of >12mm always showed less than 60% damping off.



a.) b.)

Fig. 1. Proportion of damping off according to radicle length: a.) 40 RIL; b.) 40 hybrids (2 replications)

The hybrids showed a more rapid germination than the inbred lines and there was no clear effect of short radicles, but the hybrids with long radicles (>16mm) again had a low level of damping off (<50%).
Comparison RIL - hybrids: Since germination rate appeared to be important in measurement of quantitative resistance, the vigour provided by hybrid seed could help to provide uniformity between genotypes. The reactions of 40RIL were thus compared with those of their hybrids with GB (Fig. 2). The hybrids showed a greater proportion of CS (68.2%) than the RIL (56.2%), probably related to the high susceptibility of GB. As could be expected from crosses with a single tester line, there was less variation among the hybrids than among RIL, but the results were significantly correlated (r= 0.392*).

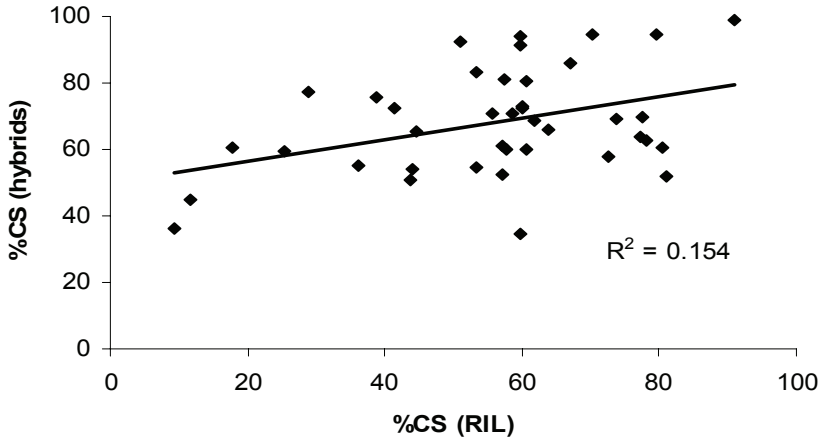


Fig. 2. Relations between 40 RIL and 40 (GB x RIL) hybrids: mean % completely susceptible in 4 tests.

Choice of test conditions according to relations between growth chamber tests and field trials. Fig. 3a and 3b show that when the germinated seed was divided into 2 groups, radicles <5mm or >5mm, the closest correlation between %CS in growth chamber and % infection in the field was obtained with radicles <5mm. For the RIL and their hybrids, where radicle length was measured at infection and long radicles showed little damping off whereas short radicles of inbred lines showed a high level of damping

off, correlations were made with field attack, including and excluding the genotypes concerned. Exclusion of seedlings which had probably not been infected correctly did not exclude extreme reactions and improved the correlation for inbred lines but made no difference for hybrids (Table 3). In addition, when reactions of inbred lines in the field were compared with those of their hybrids under test, there were no significant correlations. It may thus be concluded that to judge inbred lines it is better to test the lines than hybrids made with a single tester genotype.

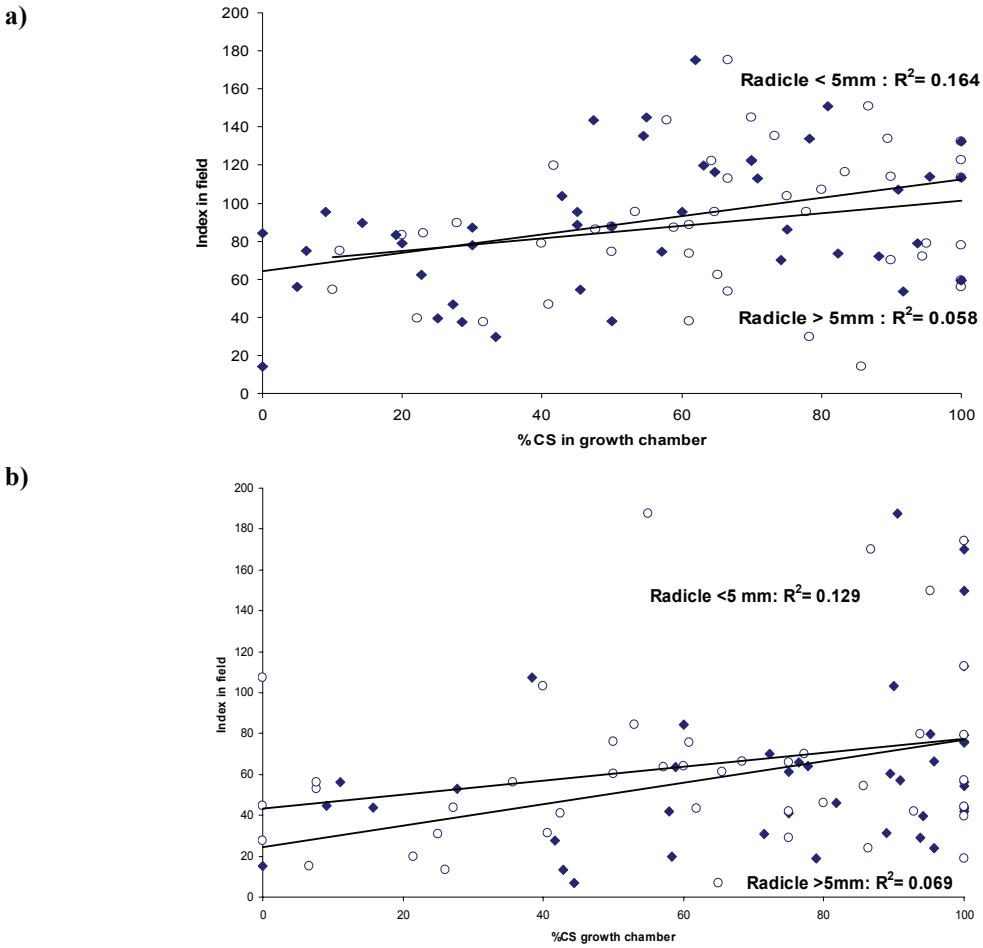


Fig. 3. Comparison of downy mildew attack on 45 hybrids (a) and 44 inbred lines (b) in the field and in the growth chamber test according to radicle length in the test.

Table 3. Correlations between percent downy mildew attack in field trials and in growth chamber tests, including and excluding genotypes with very short or very long radicles when infected in the growth chamber

	Complete series		Excluding genotypes with very short or very long radicles.	
	Nb genotypes	Pearson correlation	Nb genotypes	Pearson correlation
RIL in growth Chamber[(2)] / RIL in field trials[(1)]	40	r= **0.484****	35	r= **0.548****
Hybrids in growth Chamber[(2)] / Hybrids in field trials[(1)]	40	r= 0.188 ns	34	r= 0.274 ns
Hybrids in growth Chamber[(2)] / RIL in field trials[(1)]	40	r= 0.214 ns	35	r= 0.248 ns

[(1)] % attack compared with mean of 4 check lines (4 replications of 30 plants)
[(2)] % completely susceptible plants (%CS) (2 replications of at least 10 plants)

Inheritance of percent completely susceptible plants in growth chamber tests. The 28 hybrids from a factorial cross of 7 female lines and 4 restorers, and the parental lines were tested in the growth chamber. Results are presented in Table 4.

Table 4. Percent completely susceptible plants in growth chamber tests on hybrids of a factorial cross and their parental lines.

Males / Females	83HR4	PR56	PAZ2	90R18	Mean hybrid value	Inbred line
FU	61.1	4.2	11.1	22.2	24.7	15.8
FRIGA	80.0	42.9	10.0	100.0	58.2	38.5
IR	66.7	78.3	40.9	85.7	67.9	100.0
GX	58.8	31.6	90.0	65.2	61.4	89.5
SL72	100.0	83.3	47.6	100.0	82.7	100.0
GU	86.7	75.0	89.5	95.0	86.5	90.5
HA89	40.0	23.1	20.0	100.0	45.8	100.0
Mean hybrid value	70.5	48.3	44.2	81.2		
Inbred line	100.0	57.9	11.1	95.7		

The female line FU appears best with hybrids showing low percent of completely susceptible plants, and this is also true for the restorers PR56 and PAZ2. The mean parent - hybrid correlation was highly significant (r= 0.617**) (Fig. 4).

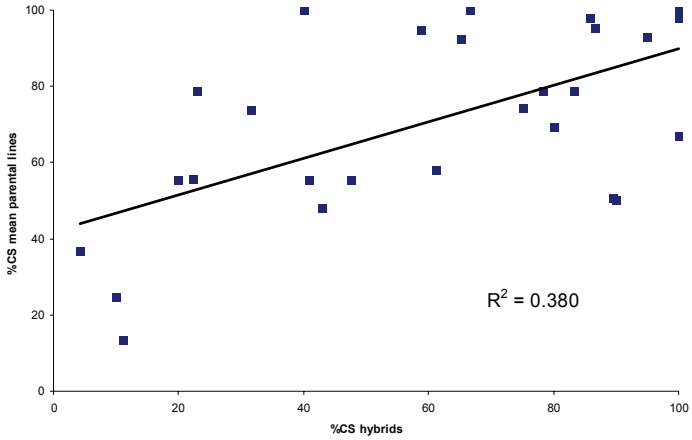


Fig. 4. Relations between percent completely susceptible plants in growth chamber test of hybrids compared with parental inbred lines

DISCUSSION

Horizontal reactions (according to Robinson, 1973) of sunflower genotypes to parasites depend on many environmental factors. It is often difficult to design a laboratory test which gives a good indication of the mean response from multi-location trials over several years in the field (Ladsous et al., 1991; Viguié et al., 2000; Eva, 2002; Serre et al., 2004). However, in controlled conditions, it may be possible to obtain more detailed knowledge of the processes involved in resistance even if the results of a single test do not represent the overall field reaction. The results reported here concern the probability that a systemic downy mildew attack will develop after infection of sunflower seedlings at their most susceptible stage under environmental conditions favourable for the disease.

The percentage of completely susceptible plants for each genotype depends clearly on radicle length at infection. This is probably because the pathogen infects plant tissues through the extremities of root hairs (Allard, 1978) and to provoke symptoms characteristic of systemic infection, it must reach the apical meristem very quickly. The test described here measures this possibility and the results show that for the germination stage to be the most uniform possible, infection should be made of seedlings with radicles

measuring between 3 and 10mm. This measurement of the proportion of completely susceptible seedlings in the growth chamber is indicative of reaction in field trials for many inbred lines, but it does not represent all possible resistance factors. Genotypes such as "IR" and "90R18", which have high levels of resistance in the field, appear very susceptible in the growth chamber. Factors other than the germination rate are certainly involved; some examples could be resistance to infection or tissue receptivity (Mazeyrat et al., 1999). These cannot be studied in the field and will require additional growth chamber or laboratory tests.

In 2000, changes in downy mildew races in France suggested that sole use of monogenic resistances was a strategic error (Tourvieille de Labrouhe, 2000) and more recently it has been confirmed that major gene resistance alone does not provide durable control of downy mildew (Tourvieille de Labrouhe et al., 2005). Quantitative resistance is most often non-race specific and the levels observed in cultivated sunflowers suggest that this type of resistance should be used in breeding, at least to complement major genes. The test proposed here is a first step in understanding this resistance and making routine breeding programmes for this character possible.

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REFERENCES

Allard, C. 1978. Invasion et colonisation systémique de la plantule de tournesol (*Helianthus annuus* L.) par le *Plasmopara halstedii* (Farl.) Berl. Et de Toni. Ann. Phytopathol. 10:197-217.

Délos, M., N. Eychenne, I. Birba, and C. Fabry. 2000. Etude des facteurs expliquant les fluctuations des attaques de *Plasmopara halstedii* en France. p. I43-I48. In: Proc. 15th Int. Sunflower Conf., Toulouse, France.

Eva, B. 2002. The evolution of pathogenic agents attack in some sunflower (*Helianthus annuus* L.) hybrids, under conditions of the agricultural research and development station of Podu-Iloaiei, Iasi District. Cercetari Agronomice in Moldova 35:3-4.

Gulya, T.J., D. Tourvieille de Labrouhe, S. Masirevic, A. Penaud, K.Y. Rashid, and F. Virányi. 1998. Proposal for Standardized Nomenclature and Identification of Races of *Plasmopara halstedii* (Sunflower Downy Mildew). p. 130-136. In: Proceeding of ISA, Symposium III, Sunflower Downy Mildew. Fargo, ND, USA.

Ladsous, O., F. Vear, and D. Tourvieille de Labrouhe. 1991. Heredity of resistance to *Botrytis cinerea* in sunflower (*Helianthus annuus*) measured by artificial infections. Plant Breed. 107:235-241.

Mazeyrat, F., S. Mouzeyar, I. Courbou, S. Badaoui, P. Roeckel-Drevet, D. Tourvieille de Labrouhe, and G. Ledoigt. 1999. Accumulation of defence related transcripts in sunflower hypocotyls (*Helianthus annuus* L.) infected with *Plasmopara halstedii*. Eur. J. Plant Pathol. 105:333-340.

Robinson, R.A. 1973. Horizontal resistance. Revue of Plant Pathology 52:483-501.

Roche, S., P. Walser, and F. Serre. 2005. Test de résistance du tournesol au mildiou (*Plasmopara halstedii*) en conditions contrôlées. Cahier des techniques INRA N° spécial, p. 101-105.

Serre, F., P. Walser, D. Tourvieille de Labrouhe, and F. Vear. 2004. *Sclerotinia sclerotiorum* capitulum resistance tests using ascospores: Results over the period 1991-2003. p. 129-134. In: Proc. 16th Int. Sunflower Conf., Fargo, ND, USA.

Tourvieille de Labrouhe, D. 2000. Disease control concerns everybody: The example of sunflower downy mildew. p. Pl.C-1 – Pl.C-8. In: Proc. 15th Int. Sunflower Conf., Toulouse, France.

Tourvieille de Labrouhe, D., E. Pilorgé, P. Nicolas, and F. Vear. 2000. Le Mildiou du Tournesol. Versailles, France: CETIOM-INRA, 70p.

Tourvieille de Labrouhe, D., E. Mestries, and P. Walser. 2005. Quelles perspectives pour la lutte génétique vis-à-vis du mildiou du tournesol? Oléagineux Corps Gras Lipides 12:85-93.

Vear, F., F. Serre, S. Roche, P. Walser, and D. Tourvieille de Labrouhe. 2006. Recent research on downy mildew resistance useful for breeding industrial – use sunflowers. I.S.A. Symposium on Sunflower Industrial Uses , 10-13 September 2006, Udine, Italy.

Viguié, A., F. Serre, P. Walser, F. Vear, and D. Tourvieille de Labrouhe. 2000. The use of natural infections under controlled conditions and of artificial infections to estimate *Phomopsis* resistance of sunflower hybrids: conclusion after ten years of trials. p. K78 – K83. In: Proc. 15th Int. Sunflower Conf., Toulouse, France.

Can management of *Pl* genes influence aggressiveness in *Plasmopara halstedii* (sunflower downy mildew)?

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ABSTRACT

Evolution of aggressiveness in populations of race 710 of *Plasmopara halstedii* was measured under different strategies of *Pl* gene management: mixture, alternation and monoculture of major resistance genes in comparison with a population under no selection pressure. Two sunflower lines showing different levels of non-race-specific resistance were used to measure four aggressiveness criteria: length of latent period, sporulation density, percentage infection, and hypocotyl length. The sunflower inbred line BT, rather susceptible in the field, presented a higher percentage infection, a higher sporulation density, a lower latent period and less reduced hypocotyl length than inbred line FU, which has greater resistance in the field. Differences were observed between *P. halstedii* populations under different strategies of *Pl* gene management. Strains multiplied under varietal mixtures gave the greatest sporulation densities and shortest hypocotyl lengths, those multiplied under alternation gave a reduced latent period and shorter hypocotyl lengths compared with those not influenced by selection pressure. There were no significant differences between populations multiplied under monoculture of resistance genes and those under no selection pressure. These differences appear to be linked to the number of diseased plants present. The results suggested that the method of *Pl* gene management affects aggressiveness because it determines the number of susceptible plants harbouring the parasite. Applications of these strategies of *Pl* gene management are discussed.

Key words: alternation – mixture– monoculture – pathogenicity – *Pl* gene

RESUME

L'évolution de l'agressivité des populations du profil 710 de *Plasmopara halstedii* a été mesurée sous différentes stratégies de gestion de gènes *Pl*: l'alternance, l'assemblage et la monoculture de source de la résistance en comparaison avec une population n'ayant subit aucune pression. Deux génotypes du tournesol présentant des niveaux différents de résistance non-race spécifique ont été utilisés pour mesurer quatre facteurs de l'agressivité: le taux de réussite de l'infection, la durée de latence, la densité de sporulation et la longueur de l'hypocotyle. Les souches récoltées sous les systèmes de l'alternance et de l'assemblage présentent des durées de latence les plus courtes significativement sur le génotype résistant et des longueurs de l'hypocotyle les moins grandes sur le génotype le plus sensible par rapport aux souches multipliées sous la monoculture de source résistant. De plus, les souches récoltées sous le système de l'alternance présentent des densités de sporulation plus élevées sur les deux génotypes. Cette évolution semble directement liée à la présence de nombreuses plantes malades dans ces dispositifs. Nos résultats suggèrent l'existence d'un impact du mode de gestion des gènes *Pl* sur l'évolution de l'agressivité. Seules les stratégies qui maintiennent des effectifs de la population parasitaire assez élevés permettent une évolution de l'agressivité de *P.halstedii*. Les résultats sont discutés aux regards à la mise en œuvre de ces méthodes de gestion.

Mots-clés: alternance – assemblage – monoculture – pathogenicité – *Pl* gene

INTRODUCTION

Selective effects on pathogenicity due to host resistance are an important aspect of plant-pathogen interactions, which can be divided into two parts: virulence (specific disease-causing abilities) and aggressiveness (non-specific disease-causing abilities) according to Van der Plank (1968). There have been many reports concerning increase of virulence in relation to host resistance in pathogens of economically important crops (McDonald and Linde, 2002). Similarly, Gandon and Michalakis (2000) predicted that increased levels of quantitative host resistance may select for increased aggressiveness of parasites, leading to increased crop losses. Cowger and Mundt (2002) showed that wheat cultivars with good partial resistance selected more aggressive isolates of *Mycospharella graminicola*. However, this is not always true, Sullivan et al. (2005) reported that tobacco cultivars with high levels of quantitative resistance did not select for more aggressive isolates of *Phytophthora parasitica* var. *nicotiana*e. Also, Flier et al. (2007) showed that, following large-scale introduction of more resistant potato varieties in organic production systems in Europe, there was no shift towards increased levels of aggressiveness of *Phytophthora infestans* populations.

Plasmopara halstedii (downy mildew) is a pathogen specific to sunflower, present in most areas of the world where this crop is grown. It shows physiological races (pathotypes) capable of infecting a variable range of sunflower genotypes. The nomenclature of these races is based on the reaction of a series of differential lines (Gulya et al., 1998). Race specific resistance is controlled by major genes, denoted *Pl*. Tourvieille de Labrouhe et al. (2005) showed that whatever the method of management (mixture, alternation, monoculture) of *Pl* genes, their selection pressure led to appearance of new virulences.

This paper reports studies of levels of aggressiveness in 3 populations of *P. halstedii,* race 710, obtained under different strategies of *Pl* gene management: mixture, alternance and monoculture, in comparison with a population obtained in the absence of any effective *Pl* gene.

MATERIALS AND METHODS

Sunflower genotypes

Four quasi-isogenic hybrids were used, obtained from crosses of 2 forms each of two inbred lines:

- L1a: carrying resistance gene *Pl2,* resistant to race 100 and susceptible to race 710,
- L1b: carrying resistance genes *Pl2* and *Pl8,* resistant to races100 and 710,
- L2a: carrying no known resistance gene,
- L2b: carrying resistance gene *Pl6,* resistant to races 100 and 710.

The four hybrids were produced as follows: H1= L1a x L2a, H2= L1a x L2b, H3= L1b x L2a and H4= L1b x L2b.

P. halstedii strains present in the soil were trapped with a sunflower hybrid (Airelle), carrying no downy mildew resistant gene. To characterise aggressiveness of *P. halstedii* strains, two inbred lines not carrying any *Pl* gene and known to have different levels of non race specific resistance (Vear et al., 2007) were studied: FU and BT.

Experimental protocol

The protocol was developed by Tourvieille de Labrouhe et al. (2005) to determine durability of resistance. Four plots constituted by netting cages were maintained with climate conditions favourable for expression of disease. Plot P1 was planted in four consecutive years with H1 (no effective resistance against race 710). Plot P2 was planted all years with an equal mixture of the four hybrids. Plot P3 was planted in first year with H1, then successively with H2, H3 and H4. Plot P4 was planted with H2, resistant to race 710, in all 4 years.

P. halstedii *strains*

After 4 years, *P.halstedii* strains were collected from soil according to the method described by Tourvieille de Labrouhe et al. (2008) and their virulence profile characterised by the method of Gulya et al. (1998). For plots 1, 2 and 3, four strains were analysed and for P4, 3 strains.

Measurements of aggressiveness

The protocols developed by Sakr et al. (2008) were used, determining:

- Length of period between infection and sporulation on 80% of infected plants = latent period,
- Maximal sporulation density on cotyledons obtained 12 and 13 days after infection = sporulation density,

- Percentage infection = % infection,
- Hypocotyl length 13 days after infection, calculated by a percentage of the hypocotyl length of healthy plants = hypocotyl length.

All tests were carried out in growth chambers respecting European regulations (No 2003/DRAF/70).

Statistical Analyses

All statistical analyses of the phenotypic data were performed using StatBox 6.7® (GimmerSoft) software. To compare strains and genotypes, there were 2 replications for sporulation density and 3 replications for percentage infection, latent period and hypocotyl length. To compare each characteristic in the different plots, the means of each strain were used as replications in one-way analyses of variance (ANOVA). The Newman-Keuls test was used to compare the means at P=0.05

RESULTS

Changes in percentage attack in the 4 plots

Data are presented in Table 1.

Table 1. Changes in downy mildew attack in 4 plots observed over 5 years.

Plots	**2001**[1]	**2002** (*)	**2003** (*)	**2004** (*)	**2005**
P1					
% diseased plants	71.5	37.4	75.4	60.3	
Number of diseased plants	203	125	215	194	
% of race 710	100	100	100	100	84.0
P2					
% diseased plants	13.9	6.5	9.9	15.1	
Number of diseased plants	43	19	33	51	
% of race 710	100	100	81.0	91.3	48.9
P3					
% diseased plants	75.2	1.1	1.5	1.1	
Number of diseased plants	236	4	5	11	
% of race 710	100	100	100	9.1	12.5
P4					
% diseased plants	2.7	1.1	4.9	14.8	
Number of diseased plants	10	4	16	52	
% of race 710	100	100	16.7	30.0	34.5

[1]Tourvieille de Labrouhe et al. 2005

Table 1 shows that total numbers of diseased plants differed between plots (from 737 for plot P1 to 82 for plot P4). There was a continued reduction in percentage of *P.halstedii* samples of race 710 especially in the absence of susceptible sunflower genotypes in plots P3 and P4. Nevertheless this race was present in soil samples taken in 2005 from all plots.

Comparison of aggressiveness of 15 strains of race 710 on inbred lines FU and BT

The two sunflower lines gave a significantly different response (Table 2).

Table 2. Anova on aggressive criteria of 15 strains of *P. halstedii* measured on two sunflower lines.

% infection	Line effect			Strain effect			Interaction		
	BT	FU	Significant	Mini	Maxi	Significant	Min.	Max.	Significant
	100%	99.3%	P<0.001	98.6%	100%	NS	97.2%	100%	NS
Sporulation density (zoosporangia per cotyledon)	963 10^5	788 10^5	P<0.001	677 10^5	1264 10^5	P<0.001	562 10^5	1343 10^5	NS
Latent period (days)	8.1 d.	9.0 d.	P<0.001	8.3 d.	8.9 d.	NS	7.8 d.	9.7 d.	NS
Hypocotyl length (% of length of healthy plants)	33.0%	40.1%	P<0.001	31.1%	40.3%	NS	26.7%	43.7%	NS

The inbred line BT showed a higher percentage infection, a higher sporulation density, a shorter latent period and less reduced hypocotyl length than FU. The 15 strains appeared as being homogeneous for all criteria analysed except spore density. There was no interaction between parasite strains and host genotypes.

Comparison of strain aggressiveness in each plot

Plot P4 was not distinct from P1 whereas P2 presented greater mean sporulation density and reduction in hypocotyl length, and P3 showed a shorter latent period and greater reduction in hypocotyl length (Table 3).

Table 3. Comparison of means observed for isolates from each plot compared with P1 (no effective *Pl* gene).

	% infection		Latent period (days)		Sporulation density (zoosporangia per cotyledon)		Hypocotyl length (% of length of healthy plants)	
	mean	reference[1]	mean	/reference[1]	mean	/reference[1]	mean	/reference[1]
P1 (reference)	99.65		8.83		8.15		40.00	
P2	99.84	NS	8.55	NS	10.89	S	35.11	S
P3	99.86	NS	8.41	S	8.30	NS	35.10	S
P4	99.09	NS	8.71	NS	7.32	NS	38.03	NS

[1]Test of Newman Keuls, P=0.05

DISCUSSION

The presence of strains of race 710 in plots not grown with a susceptible genotype for 3 (P3) or 4 years (P4) trapped by a susceptible genotype in soil tests may be explained by the maintenance of the inoculum in the soil and/or hybrid seed impurities susceptible to isolates sampled in 2005. With the first hypothesis, the evolution of parasitic populations may depend on characters linked to fitness but independent of aggressiveness, such as their capacity to survive for a long time as oospores. With the second hypothesis, the level of susceptible seed impurities would be the important factor which intervenes in the evolution of parasitic populations.

Study of the reaction of two inbred lines to 15 strains underlined their differences in behaviour. The very good resistance of inbred line FU observed in the field was confirmed by the measurements of aggressiveness criteria described by Sakr et al. (2008). These methods can be used to characterise non-race-specific partial resistance since there were no interactions between genotypes and strains. For the 15 strains analysed, only sporulation density varied (from 1 to 2), overall, the *P. halstedii* strains appeared to be quite homogeneous.

Comparison of parasite populations isolated from the 4 plots showed that strains of race 710 from plot P4 (monoculture of *Pl*6) were not different from the population isolated from P1, with no efficient *Pl* gene. This could be explained on one hand by selection of strains which survive in the soil, independently from the factors of aggressiveness measured, or, on the other hand, by a weak level of parasitic multiplication linked to a small number of plants susceptible to race 710, thus giving incomplete expression of parasitic diversity. This second hypothesis appears most likely because the number of plants infected with race 710 was always very low in plot P4. Plot P2 was grown with a mixture of different hybrid forms, giving 25% of plants susceptible to race 710, one third of which contributed to parasitic multiplication (Table 1). Compared with plot P1, and with few infected plants, it is reasonable to suggest that isolates with a high sporulation capacity could have been favoured and may have caused the secondary infections shown by 20% of infested plants in this plot between 2001 and 2004 (Tourvieille de Labrouhe et al., 2005). These secondary infections contributed to the stock of inoculum which may explain why strains isolated from this plot showed a significantly higher sporulation density. In plot P3 (alternation), the abundant downy mildew population created in the first year, from more than 230 diseased plants, was confronted with new resistance genes every year but race 710 remained in 2005, although at a lower level than in the other 3 plots. This population evolved towards increased aggressiveness as measured by latent period. Compared with plot P4, it had a wider genetic base. Differences in aggressiveness, as compared with plot P1, were weak but significant for latent period, suggesting that, from a similar number of diseased plants, different aggressiveness factors could be

selected if the number of diseased plants is small. The 2 plots that significantly differed for either latent period or sporulation density (i.e., P3 and P2) also differed for hypocotyl length.

It is commonly admitted that non-race specific partial resistance applies selection pressure on parasitic populations, which may lead to more aggressive strains. An example was maize resistance against *Cochliobolus heterostrophus* (Kolmer and Leonard, 1986). In contrast, many authors report that use of race specific resistance does not lead to modifications in aggressiveness. Sullivan et al., (2005) showed that race specific resistance in tobacco did not exert a selective effect on aggressiveness of *Phytophthora parasitica* var. *nicotiana*e and in the pathosystem *Venturia inaequalis* / apple, Parisi et al. (2004) found that virulent strains taken from cultivars carrying vertical resistance genes were highly aggressive. Since the four sunflower hybrids in the present study were isogenic except for their *Pl* genes, it appears reasonable to consider that selection pressure was mainly applied on criteria linked to virulence (Tourvieille de Labrouhe et al., 2005). The results obtained showed positive effects of certain modes of *Pl* gene management on aggressiveness factors. This effect no doubt depends more on the number of susceptible plants than on direct selection pressure of monogenic resistances. It could be suggested that management of *Pl* genes which reduce the number of susceptible plants, limits selection pressure for more aggressive strains, but increases the risk of appearance of new virulence. In contrast, management modes which lead to a non negligible number of diseased plants (mixtures and alternation), may slow down the appearance of new virulence (Tourvieille de Labrouhe et al., 2005), but could favour more aggressive strains. This conclusion must be taken into account in the choice of methods to obtain durable control of sunflower downy mildew with both race-specific and non-race-specific resistance.

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REFERENCES

Cowger, C., and C.C. Mundt. 2002. Aggressiveness of *Mycosphaerella graminicola* Isolates from Suseptible and Partially Resistant Wheat Cultivars. Phytopathology 92:624-630.

Flier, W.G., L.P.N.M. Kroon, A. Hermansen, H.M.G.van. Raaij, B. Speiser, L. Tamm, J.G. Fuchs, J. Lambion, J. Razzaghian, D. Andrivon, S. Wilcockson, and C. Leifert. 2007. Genetic structure and pathogenicity of populations of *Phytophthora infestans* from organic potato crops in France, Norway, Switzerland and the United Kingdome. Plant Pathol. 56:562-572.

Gandon, S., and Y. Michalakis. 2000. Evolution of parasite virulence against qualitative or quantitative host resistance. Proc. Royal Soc. London, 267:985-990.

Gulya, T.J., D. Tourvieille de Labrouhe, S. Masirevic, A. Penaud, K. Rashid, and F. Viranyi. 1998. Proposal for standardized nomenclature and identification of races of *Plasmopara halstedii* (sunflower downy mildew). p. 130-136. In: Proc. of the ISA Symposium. III: Sunflower Downy Mildew. Fargo, ND, USA.

McDonald, B.A., and C. Linde. 2002. Pathogen population genetics and the durability of disease resistance. Euphytica 124:163-180.

Kolmer, J.A., and K.J. Leonard. 1986. Genetic selection and adaptation of *Cochliobolus heterostrphus* to corn hosts with partial resistance. Phytopathology 76:774-777.

Parisi, L., V. Fouillet, H.J. Schouten, R. Groenwoil, F. Laurens, F. Didelot, K. Evans, C. Fischer, F. Gennari, H. Kemp, M. Lateur, A. Patocchi, J. Thissen, and C. Tsipouridis. 2004. Variability of the pathogenicity of *Venturia inaequalis* in Europe. Acta Horticulturae 663:107-113.

Sakr, N., M. Ducher, J. Tourvieille, P. Walser, and D. Tourvieille de Labrouhe. 2007. A new method to obtain monozoosporangial isolates of sunflower downy mildew (*Plasmopara halstedii*). Cryptogamie, Mycologie 28:123-131.

Sakr, N., X. Giresse, P. Walser, S. Richard-Cervera, J. Tourvieille, F. Vear, F. Delmotte, M. Ducher, and D. Tourvieille de Labrouhe. 2008. Variation of aggressiveness of different pathotypes of *Plasmopara halstedii* (agent of Sunflower Downy Mildew). Ms submitted.

Sullivan, M.J., T.A. Melton, and H.D. Shew. 2005. Managing the race structure of *Phytophthora parasitica* var. *nicotiana*e with cultivar rotation. Plant Disease 89:1285-1294.

Tourvieille de Labrouhe, D., E. Mestries, and P. Walser. 2005. Quelles perspectives pour la lutte génétique vis-à-vis du mildiou du tournesol? Oleagineux, Corps Gras, Lipides 12:85-93.

Tourvieille de Labrouhe, D., A. Penaud, P. Walser, E. Mestries, J. Moinard, S. Frederic, Thiery, C.B. Garric, and X. Pinochet. 2008. Determine the «sunflower downy mildew» risk by soil analysis. In: Proc. 17th Int. Sunflower Conf., Cordoba, Spain.

Vanderplank, J.E. 1968. Disease resistance in plants. Academic Press, New York, USA.

Vear, F., F. Serre, S. Roche, P. Walser, and D. Tourvieille de Labrouhe. 2007. Recent research on downy mildew resistance useful for breeding industrial-use sunflower. Helia 30:45-54.

Morphological and molecular identification of *Diaporthe helianthi* from *Xanthium italicum*

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ABSTRACT

Up to the present time, *Diaporthe helianthi* has not been reported outside the genus *Helianthus* in Croatia. This pathogen has been recently recovered on *Xanthium italicum* (cocklebur) in Slavonia and Baranja County. Isolates of *Diaporthe* sp. originating from *X. italicum* were studied and compared with *D. helianthi* isolates from sunflower. Phylogenetic analysis of nuclear ribosomal DNA internal transcribed spacer sequences (ITS1 and ITS2) showed that *X. italicum* is a new host for *D. helianthi*.

Key words: *Diaporthe helianthi* – identification – *Xanthium italicum*.

INTRODUCTION

The cultivated sunflower (*Helianthus annuus* L.) is the most important annual crop grown for edible oil in Croatia. Stem canker caused by *Diaporthe helianthi* Munt.-Cvet. et al. (anamorph *Phomopsis helianthi* Munt.-Cvet. et al.) is one of the most important sunflower diseases. The pathogen was identified in former Yugoslavia for the first time in 1980 (Mihaljcevic et al., 1980; Muntanola-Cvetkovic et al., 1981) and nowadays this disease has spread to many other countries. The fungus can cause yield losses up to 40% (Demazure, 1995) and reduce oil content (Franco and Morales, 1997) in the case of environmental conditions being favourable for infection.

The occurrence of fungi from *Diaporthe*/*Phomopsis* genera on weeds has been studied over a long period. Weeds as alternative hosts of *Diaporthe*/*Phomopsis* have a very important role as a potential source of inoculum for cultivated plants (Roy et al., 1997; Mengistu and Reddy, 2005; Vrandecic et al., 2006). Mihaljcevic and Muntanola-Cvetkovic (1985) reported *Phomopsis* spp. on 15 plant species, among which were *Xanthium strumarium* L. and *X. italicum* Moretti. Nikandorow et al. (1990) determined *X. spinosum* L., *X. orientale* L. and *X. occidentale* L. to be hosts of *Phomopsis* species. Muntanola-Cvetkovic et al. (1996) identified two *Diaporthe/Phomopsis* species on *X. italicum*. Until recently, sunflower was the only known host for *D. helianthi*. Piven' et al. (2000) stated that *Cyclachaena xanthiifolia* (family *Astraceae*) are potential alternative hosts for *Phomopsis arctii* (Lasch) Nitschke (*Diaporthe arctii*) and *P. helianthi*. This paper presents results of the study on morphological, cultural and biomolecular characterization to identify *Diaporthe/Phomopsis* isolate obtained from *X. italicum*.

MATERIALS AND METHODS

Isolates used in this study (Table 1) were obtained from naturally infected living plants or overwintered residues of *X. italicum* and sunflower plants from location in Slavonia and Baranja County (Croatia). *X. italicum* plant tissues with symptoms of infection with *Diaporthe*/*Phomopsis* were disinfected and small tissue pieces were placed in Petri dishes on moist filter paper or directly on potato dextrose agar (PDA). Petri dishes were kept at 25°C with 12 h light/dark regime. Isolation of *D. helianthi* isolates was performed by transferring mycelia or pycnidia with conidia exudates on PDA. In order to examine microscopic features and cultural characteristics, pure cultures were kept in a thermostat (25°C, 12 h light/dark). Morphological and cultural characteristics of *Diaporthe/Phomopsis* isolates from *X. italicum* were compared with isolates of *D. helianthi* from sunflower.

DNA extraction was made following Cenis (1992). The standard PCR conditions for ITS475 primers are described in White et al. (1990). Purified PCR products were sequenced in both directions using primers ITS4 and ITS5 (Gene Lab – ENEA, Roma). Sequences were aligned by CLUSTAL W (Thompson et al., 1994) and manually adjusted by Chromas (version 1.45). Additional *Diaporthe/Phomopsis* sequences were obtained from GenBank (http://www.ncbi.nlm.nih.gov) and added to alignment. The *Colletotrichum coccodes* (Wallr.) Hughes and *Colletotrichum dematium* (Pers.) Grove were included as an outgroup. Alignment gaps were treated as missing data. Phylogenetic analysis was

conducted by UPGMA (Kimura 2-parameter model) methods using MEGA version 3.1. (Kumar et al. 2004). Bootstrap analysis for 1000 replicates was done to evaluate tree topologies.

Table 1. Isolates used in this study

Isolate	Species	Host	Origin	Reference	GenBank numbers
CBS592.81	*D. helianthi*	Sunflower	ex Yugoslavia	Rekab et al. (2004)	AY705842
IMI318865	*D. helianthi*	Sunflower	ex Yugoslavia	Rekab et al. (2004)	AJ312363
Dh95004	*D. helianthi*	Sunflower	France	Say-Lesage et al. (2002)	AF358438
Dh95016	*D. helianthi*	Sunflower	France	Say-Lesage et al. (2002)	AF358435
F1	*D. helianthi*	Sunflower	France	Rekab et al. (2004)	AJ312350
A3	*D. helianthi*	Sunflower	Argentina	Rekab et al. (2004)	AJ312364
Xa3	*D. helianthi*	*X. italicum*	Croatia	This study	
Xa5	*D. helianthi*	*X. italicum*	Croatia	This study	
Su5/04	*D. helianthi*	Sunflower	Croatia	This study	
Su12/05	*D. helianthi*	Sunflower	Croatia	This study	
978	*C. coccodes*	Pepper	Italy		AM422215
AR3563	*C. dematium*	*Lirope muscarii*	Mexico	Farr et al. (2006)	DQ286154

RESULTS AND DISCUSSION

After 8 days, colonies of *Diaporthe/Phomopsis* from *X. italicum* on PDA formed less abundant white mycelium, the aerial part plenty of it, sometimes with narrow greenish-yellow areas. Reverse of culture was whitish to beige color and had in the beginning light brown scattered spots, which later turned dark brown. The pycnidia formed in simple stromatic structures usually aggregate, rarely solitary, measuring 240-450 x 230-380 µm. Conidia only of ß-type, 24.4 x 1.8 µm. After 30-40 days isolates from *X. italicum* (Xa3 and Xa5) and isolate Su5/04 from sunflower formed spare globose perithecia. Biometrical values of perithecia (isolates from *X. italicum*) were 290 x 280 µm. Asci hyaline, elongated-elliptical, 8-spored, 37.1-59.8 x 5.8-10.5 µm (av.= 47.3 x 7.8), ascospores irregularly biseriate, subelliptical, slightly constricted at the septum, 1-septate, 9.2-16.2 x 2.2-5.5 µm (av.= 12.5 x 3.2). Comparing cultures characteristics and biometrical values of *Diaporthe/Phomopsis* from *X. italicum* and *D. helianthi* from sunflower, no differences were determined.

Comparing our isolates from Croatia with the *Phomopsis* sp. isolates from *X. italicum* (XIT-2) described by Muntanola-Cvetković et al. (1996), similarities are established in symptoms, pycnidia formation and biometrical values of ß conidia, as well as in development and the characteristics of teleomorphic stage. Our isolates did not form pycnidia containing α conidia either in natural environment or in laboratory. Muntanola-Cvetković et al. (1996) found this type of conidia in the majority of cultures, although always in a small number.

The sequence analyses of rDNA-ITS *Diaporthe/Phomopsis* isolates using UPGMA methods revealed that our isolates from *X. italicum* (Xa3 and Xa5) group together (100% bootstrap support) with the isolates of *D. helianthi* from sunflower (Su5/04 and Su12/05) and with *D. helianthi* isolates from former Yugoslavia (AJ312363 and AY705842), France (AF358438, AJ312350 and AF358435) and one from Argentina (AJ312364) which Rekab et al. (2004) marked as *D. helianthi s. str*. All isolates from France and former Yugoslavia originated from countries where severe epiphytotics of sunflower stem canker were reported. On the basis of morphological and molecular characteristics, the fungi isolated from *X. italicum* were identified as *D. helianthi*.

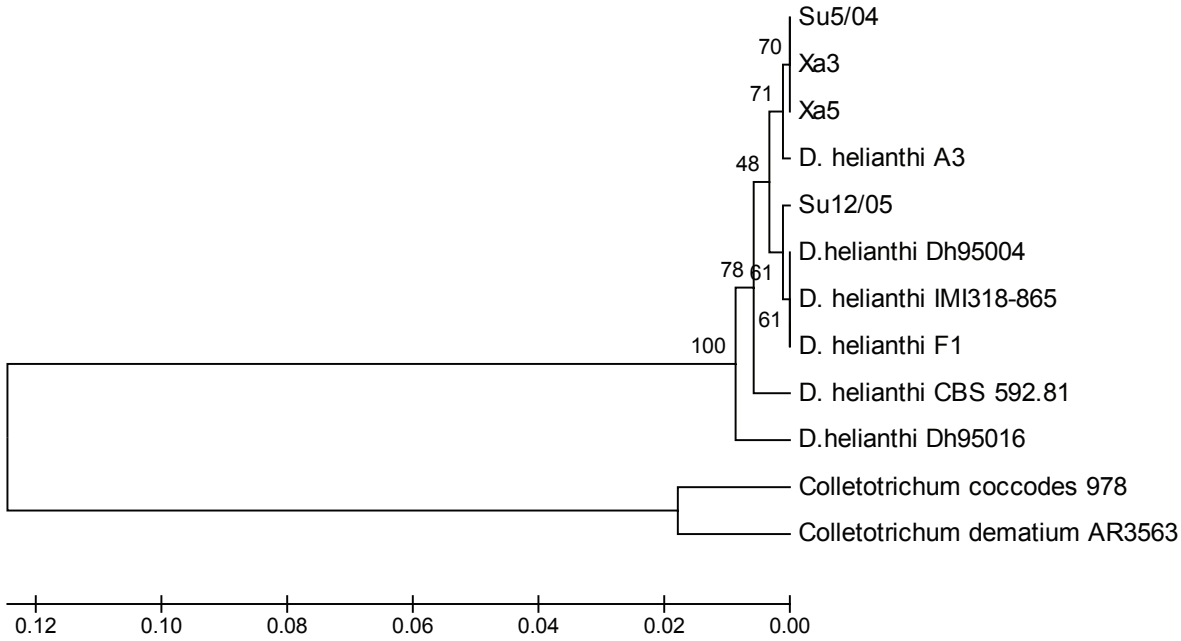

Fig. 1. Molecular phylogenetic tree based on ITS1-5.8S gene-ITS2 sequences using UPGMA -Kimura 2-parameter model. Numbers above each branch represent percentages of 1000 bootstrap repetitions. *C. coccodes* (AM422215) and *C. dematium* (DQ286154) were used as an outgroup. The scale bar shows the number of substitutions per site.

REFERENCES

Cenis, J.L. 1992. Rapid exstraction of fungal DNA for PCR ampification. Nucleic Acids Res. 20:2380.

Demazure, B. 1995. *Phomopsis*: Du progrès dans les varietés. Grande Cult. Infos. 41: 39-41.

Farr, D.F., M.C. Aime, A.Y. Rossman, and M.E. Palm. 2006. Species of *Colletotrichum* on *Agavaceae*. Mycol. Res. 110:1395-1408.

Franco, A.D., and A.O. Morales. 1997. Influence of sunflower stem canker (*Diaporthe helianthi*) on seed quality and yield during seed development. Helia 26:57-62.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Brief. Bioinform. 5:150-163.

Mengistu, A., and N. Reddy. 2005. Detection of *Phomopsis longicolla* T. W. Hobbs and its Pathogenicity on *Glicine max* (L.) Merr. and Weed Hosts. Seed Technol. 27:97-100.

Mihaljcevic, M., and M. Muntanola-Cvetkovic. 1985. Response of sunflower plants to different *Phomopsis* isolates. II. From weeds. p. 419-424. In: Proc. XI Int. Sunf. Conf. Mar del Plata, Argentina.

Mihaljcevic, M., M. Petrov, and M. Muntanola-Cvetkovic. 1980. *Phomopsis* sp., a new parasite of sunflower in Yugoslavia. Savrem. poljop. 28:531-539.

Muntanola-Cvetkovic, M., M. Mihaljcevic, and M. Petrov. 1981. On the identity of the causative agent of a serious *Phomopsis* - *Diaporthe* disease in sunflower plant. Nova Hedwigia 34:417-435.

Muntanola-Cvetkovic, M., J. Vukojevic, and M. Mihaljcevic. 1996. Cultural growth patterns and incompatibility reaction in *Diaporthe* and *Phomopis* population. J. Phytopathol. 144:285-295.

Nikandrow, A., G.J. Weidemann, and B.A. Auld. 1990. Incidence and pathogenicity of *Colletotrichum orbiculare* and a *Phomopsis* sp. on *Xanthium* spp. Plant Disease 74:796-799.

Piven', V.T., E.G. Dolzhenko, and L. Slysar'. 2000. *Cyclahaena xanthifolia* – a potential alternative host of *Phomopsis* species. Zashchita i Karantin Rastenii 5:28-29.

Rekab, D., G. Del Sorbo, C. Reggio, A. Zoina, and G. Firrao. 2004. Polymorphisms in nuclear rDNA and mtDNA reveal the polyphyletic nature of isolates of *Phomopsis* pathogenic to sunflower and a tight monophyletic clade of defined geographic origin. Mycol. Res. 108:393-402.

Roy, K.W., S. Ratnayake, and K. McLean. 1997. Colonization of weeds by *Phomopsis longicolla***.** Can. J. Plant Pathol. 19:193-196.

Says-Lesage, V., P. Roeckel-Drevet, A. Viguié, J. Tourvieille, P. Nicolas, and D. Tourvieille de Labrouche. 2002. Molecural variability within Dia*porthe/Phomopsis helianthi* from France. Phytopathology 92:308-313.

Thompson, J.D., D.G. Higgins, and Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680.

Vrandecic, K., D. Jurkovic, and Cosic, J. 2006. Effect of *Diaporthe/Phomopsis* species isolated from soybean and *Abutilon theophrasti* on soybean seed germination. J. Phytopathol. 154:725-728.

White, T.J., T. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p. 315-322 In: M.A. Innis, D.H. Gelfand, J J. Sninsky, and T J. White (eds), PCR Protocols: A Guide to Methods and Applications, Academic Press, Inc., New York.

Origins of major genes for downy mildew resistance in sunflower

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ABSTRACT

New sources of major gene resistance to sunflower downy mildew were compared with known resistance genes. All genes appear to come from crosses with wild *Helianthus*, and most frequently from wild *H. annuus*. The gene *Pl6* has been found in many different ecotypes but resistances which segregate independently from this gene have also been obtained. Genes considered as different may be the result of intra-cluster recombinations. Only 1 or perhaps 2 genes have been obtained from *H. argophyllus*. Identification of genes from *H. tuberosus*, is not complete, possibly because these sources show downy mildew sporulation on cotyledons. Some other annual species also show major gene type resistances. It is concluded that knowledge of these sources is important, both for their use in breeding and also to distinguish between major gene and quantitative resistance.

Key words: introgression – *Pl* genes – *Plasmopara halstedii* – resistance tests – segregation.

INTRODUCTION

Over 40 years, there has been considerable search for new genes giving resistance to new downy mildew races when these appear. Well known genes have been shown to be clustered: firstly *Pl1, Pl2, Pl6* and *Pl7* (Mouzeyar et al., 1995; Roeckel-Drevet et al., 1997; Mestries et al., 1998) on LG 8 (Cartisol LG1), secondly *Pl5* and *Pl8* (Bert et al., 2001; Radwan et al., 2004), on LG13 (Cartisol LG6) and more recently *Plarg* (Dussle et al., 2004) on LG1 (CartisolLG13). The *Pl2/Pl6* cluster was found to have a structure TIR-NBS-LRR (Bouzidi et al., 2001), typical of specific resistance genes producing hypersensitive reactions whereas the *Pl5/Pl8* cluster was shown to be NBS-LRR-CC (Radwan et al., 2002). *Pl6* has been found to show within-gene segregation leading to separation of resistances to races 703 and 710 from those for races 100 and 304 (Vear et al., 1997). Many other ecotypes of wild *H. annuus* or other wild species, in particular *H. argophyllus* have been tested and resistances introgressed into cultivated sunflower. This requires a large effort, and before being able to say whether a gene is new, it is necessary to have the homozygous form, and then make crosses with known genes. So the question may be asked as what wild species give the greatest probability of finding new and useful genes. This paper compares origins in cultivated sunflower, wild *H. annuus* and *H. argophyllus* and some other species, both from the bibliography and from recent genetical analyses made by INRA.

MATERIALS AND METHODS

Material: All the cultivated sunflower lines: resistance sources, race differentials and susceptible lines used for test crosses are maintained by INRA, together with collections of wild *H. annuus* and other *Helianthu*s species, interspecific pools and introgressions. For genetical analyses, it was necessary for lines to be homozygous for downy mildew resistance and to produce sufficient seed for tests to be made with several downy mildew races. Test cross progenies were obtained by crossing new resistance sources with lines carrying known genes and then crossing the F_1 hybrid with a completely susceptible line. These progenies were then tested to determine whether they segregated for downy mildew resistance (no segregation: same gene or closely linked; 3R/1S: 2 independent genes).

Downy mildew resistance tests: Classic seedling tests on germinated seed (Tourvieille de Labrouhe et al., 2000) were carried out in growth chambers approved for manipulation of downy mildew races observed in France. Large scale tests, with 100-300 seedlings per progeny, to determine genetical segregation were made with races 710, 304, and 703. Test of resistance to other races were made on 20-30 plants/genotype, with races 100, 314, 334, 704 and 714.

RESULTS

Resistance sources: Table 1 presents knowledge of origins and *Pl* genes in the species most commonly used as resistance sources.

Resistance from cultivated sunflower: In these sources, resistance did not come directly from wild *Helianthus*. However, the ancestors reported suggest that downy mildew resistance came from crosses with wild species at some time, although there are some specificities that have not appeared in recent crosses. The lines nearly all appear to be traceable to a Canadian line 953 involving wild *H. annuus* (Fick and Zimmer, 1974), with crosses made either at Morden or by INTA in Argentina. *Pl1* came from 953-102-1-1, and was included in varieties such as Advent, from which Vrânceanu and Stoenescu (1970) obtained AD66, but also Rha265 and RHA266. *Pl2* came from both 953-102 giving RHA274, and 953-88 as HA61. *Pl2* was widely used, but new sources have not been reported, perhaps they have not been retained since they do not provide resistance to recent downy mildew races. Although partly from the same origin, downy mildew resistance from Argentinean populations appears quite specific because, at least in the resources held and lines developed at INRA, they are all resistant to race 710 but susceptible to race 703. According to the ancestry detailed by Romano and Vazquez (2003), resistance in the open pollinated variety Guayacan, and so the pool USDA HAR5 and INRA line QHP1, also came from the Canadian line 953-102. In contrast, resistance in the varieties Charata (which gave HAR4) and Caburé probably came from an Argentinean interspecific pool with Russian open pollinated varieties crossed with *H. annuus*, *H. argophyllus* and *H .petiolaris* in 1955/56. It seems likely that in the multiplication of these open pollinated varieties some intercrossing must have occurred, to have spread the gene which we refer to as "Pl_{QHP1}". In the development of the USDA or INRA lines, resistance to race 710 was certainly retained because that was the most useful resistance, but the combination with susceptibility to race 703 is quite specific and not known for any other resistance source. This resistance segregates as a single dominant gene, giving clear resistance; it also segregates in test crosses with *Pl6* (in spite of apparently having a common origin with *Pl1* and *Pl2)* and also *Pl5*, and *Plarg* (Table 1). Bulk segregant analyses have been made, but this gene has not yet been located on a linkage group.

Resistance directly from wild Helianthus annuus

(i) Pl6: Miller and Gulya (1991) developed HA335 and HA336 from crosses with wild *H. annuus* from Texas with resistance to all known races except those named "xx4", such as 304, 314 and 334, observed in France. Their resistance gene *Pl6* was located in the cluster with *Pl1* and *Pl2*. More recently, in collaboration with J. Miller, we found that 2 other USDA origins probably also carry *Pl6* (Table 1), both being resistance to race 710 and susceptible to race 304: "TP5", from a Californian *H. annuus* and HA459 (from a Texas *H. annuus)*. Some origins from crosses made at Montpellier, with wild *H. annuus* in the 1990s also appeared to carry *Pl6*, for example ecotype MPHE-519 from Arizona More recently, further crosses have been made with a wide range of ecotypes and tests were made with race 710 and then race 304 to search for useful *Pl* genes. The results of these tests are presented in Table 2. Thirty two progenies out of 129 showed some resistance to race 710. Nine of the 22 origins with some resistance and sufficient seed for further tests showed susceptibility to race 304. The ecotypes concerned came from different parts of the US: California, Arizona, Nebraska, New Mexico and Texas. It was concluded that they probably carried *Pl6*, so it seems that this gene is very widespread in wild *H. annuus* populations.

(ii) Not *Pl6*: At Montpellier, 2 lines developed from wild *H. annuus* ecotypes MPHE-361 (from Wyoming) and MPHE-829 (from Iowa) have been shown to be resistant to all races tested. Their resistance segregates independently of the *Pl6*, *Pl5/8* and *Plarg* clusters (Table 1). These genes are being mapped and the lines are available to breeders. J. Miller developed HA458, also resistant to all known races from a cross with a *H. annuus* ecotype from Idaho. The resistance gene appears independent of all known clusters (Table 1), and is also being mapped. It is not yet known whether these 3 sources carry the same or different genes, but they certainly appear different from *Pl6.*

Among the crosses made more recently at Montpellier and continued in research since resistant to both races 710 ands 304, there are origins from 13 ecotypes, from: Texas, Wyoming, Oklahoma, Utah, Kansas, Colorado and California (Table 2). It may be some time before the resistance genes they contain are identified as many show considerable self-sterility and require further crosses to cultivated sunflower to obtain sufficient seed by selfing to be able to demonstrate homozygous resistance and so be able to make test crosses and prepare material for mapping. Among these origins, it would be interesting, and quite logical, to find a "*Pl6+",* which has the whole *Pl6* cluster in resistant form, combining the resistances of *Pl1, Pl2* and *Pl6*. Or there is something not known about the structure of this cluster which would make such a form non-viable.

Table 1. Origins of sunflower downy mildew resistance genes and results of test crosses to determine whether they segregate independently

Source line/pool	Origin	Resistance to main French races	gene	Test cross segregations* or publication				
				HA335	XRQ	RHA419	QHP1	PMI3
Resistance from cultivated sunflower								
AD66	953-102-1-1	100	*Pl1*	Vrânceanu and Stoenescu, 1970				
RHA265/266	953-102-1-1	100	*Pl1*					
HA60	953-102-1-1	100	*Pl1*					
RHA274	953-102-1-1	100,304,334	*Pl2*	Zimmer and Kinman, 1972				
HA61	953-88	100,304,334	*Pl2*					
Guyacan/HAR5	953-102-1-1	100,304,314, 334,710,714	Pl_{QHP1}					
QHP1	HAR5 x PRS7*(Pl1*)	100,304,314, 334,710,714	Pl_{QHP1}	70/310	79/348	83/329	--	70/270
Charata/HAR4/ Caburé	(Russian pool x wilds)	100,304,314, 334,710,714	Pl_{QHP1}					
Resistance directly from wild *H. annuus* (*Pl6*)								
HA335/336	HA89x*H.ann*. 423/432 (Texas)	100,703,710	*Pl6*	Miller and Gulya, 1991; Roeckel-Drevet et al., 1996				
HA458	HA434x*H.ann.* (Texas)	100,703,710	*Pl6*	0/292	78/254			
"TP5"	HA434x*H.ann.* (California)	100,703,710	*Pl6*	0/430	98/376			
"MPHE-519"	MPHE-519 x 90R19	100,703,710	*Pl6*	0/153	13/98			
not *Pl6*								
HA458	HA434x*H.ann.* (Idaho)	100,304,314, 334,703,704, 710,714	*Pl?*	133/523	74/223	22/82	16/94	
MPHE-361	90R19x*H.ann.* (Wyoming)	100,304,314, 334,703,704, 710,714	*Pl?*	76/348	87/330	48/267	36/167	52/275
MPHE-829	RT1B11x*H.ann.* (Iowa)	100,304,314, 334,703,704, 710,714	*Pl?*	42/198	69/181	51/200	50/217	73/259
Resistance from *H. argophyllus*								
RHA340	HA89x *H.arg*415	100,304,314, 334,703,704, 710,714	*Pl8*	Miller and Gulya, 1991; Vear et al., 2000				
RHA419	RHA373x *H.arg*1575	100,304,314, 334,703,704, 710,714	Pl_{arg}	Miller et al., 2002; Vear et al., 2003				
"79ARGMTP"	MPHE-92 x FS20	100,304,314, 334,703,704, 710,714	Pl_{arg}	83/391	50/304	0/260	59/257	80/373
PAA1/OQP7	PBP1xAR22	100,304,314, 334,703,704, 710,714	Pl_{arg}	--	34/109	0/106		
Resistance from *H. tuberosus*								
Progress/DM3/ Rf5566		100,304,314, 703,710,704, 714	*Pl5*	Vrânceanu et al., 1981; Miller and Gulya, 1987				
XRQ	HA89xProgress	100,304,314, 703,710,704, 714	*Pl5*	Bert et al., 2001; Vear et al., 2000				
Novinka/XPQ		100,304,314, 703,710,704, 714	*Pl5?*	Vear et al., 1998				
DM2/PMI3	Novinka	100,304,703, 704	P_{PMI3}	Vear et al., 1998				
HIR34	Armair9343xH.tubD19-6	100,304,314	*Pl4*	Leclercq et al., 1970; Vear et al., 1998				

*numbers of susceptible plants in resistance tests with race 710 (703 with PMI3) on test cross progenies (susceptible x (known resistance cluster x new source)F1

Table 2. Downy mildew resistance of *H. annuus* introgressions, susceptible or resistant to race 304

Pl6 ?				Not *Pl6*			
Genotype	Origin	Resistance		Genotype	Origin	Resistance	
	Wild *H. annuus*	710	304		Wild *H. annuus*	710	304
HAS9	Arizona	Seg	**S**	HAS1	Texas	**R**	seg
HAS20	California	Seg	**S**	HAS6	Wyoming	**R**	**R**
HAS46	Arizona	Seg	**S**	HAS40	Texas	**R**	seg
HAS101	Kansas	Seg	**S**	HAS42	Oklahoma	seg	seg
HAS147	California	Seg	**S**	HAS32	Texas	seg	seg
HAS164	New Mexico	Seg	**S**	HAS54	Oklahoma	seg	seg
HAS186	Texas	Seg	**S**	HAS62	Utah	seg	seg
HAS210	Wyoming	Seg	**S**	HAS85	Wyoming	seg	seg
HAS238	Nebraska	Seg	**S**	HAS94	Wyoming	seg	seg
				HAS103	Kansas	seg	seg
				HAS122	Colorado	seg	seg
				HAS156	California	seg	seg
				HAS171	Texas	seg	seg

Resistance from H. argophyllus: RHA340 was developed by Miller and Gulya from a cross between *H. argophyllus* 415 and HA89. The gene was identified as *Pl8*, resistant to all known races, but with pronounced sporulation on cotyledons, in seedling tests although perfectly efficient in the field. Miller et al. (2002) developed RHA419 from RHA373 x H. *argophyllus* 1575 and its gene was mapped by Dussle et al. (2004) to a different LG from *Pl6* and *Pl8*. At INRA, Montpellier a resistant line, 79ARG was developed from an interspecific pool obtained from crossing *H. argophyllus* (MPHE-92) with cultivated sunflower. This line is also resistant to all known races, showing no segregation with the resistance of RHA419. It was also found to have the same marker linkages (ORS610 and ORS543). In studies of quantitative resistance, it was found that some INRA inbred lines (PAA1, OQP7, OQP8) developed from a cross with *H. argophyllus* made by Leclercq in about 1975, and considered to be susceptible to downy mildew when the presence of the slightest spore was considered to show susceptibility, are resistant to race 710 and also to all the other French races. A test cross with RHA419 showed no segregation (Table 1), so it was concluded that this origin also contains *Plarg*.

Resistance from H. tuberosus: Pl5 was first reported by Vrânceanu et al. (1981) and resistant lines were also developed by Miller and Gulya (1987) from the Russian open pollinated variety Progress, obtained at Krasnodar apparently from an interspecific cross with *H. tuberosus*. This resistance was selected to obtain resistance to race 710 (race 4). Other lines, such as the INRA line XRQ, were developed independently in France, from a sample of Progress provided to Leclercq by Novi-Sad. This source has been widely used since *Pl5* gives resistance to all French races except 334, (which is only observed very rarely). Like *Pl8*, it gives type II resistance (sporulation on cotyledons). Incomplete forms of *Pl5* occur: whereas XRQ is resistant to a Spanish isolate of race 330, the differential D5, PM17 is susceptible. The open pollinated variety Novinka, apparently from the same origin as Progress gave the INRA line XPQ, with resistance not distinguishable from XRQ, but from this variety were also derived USDA pool DM2 and the INRA line PMI3, which is resistant to race 703 but susceptible to 710. It seemed likely that its gene was an incomplete *Pl5*, but genetical analyses with races 703 and 304, to which it is resistant, showed segregation in test crosses with XRQ. Using bulk segregant analysis, it showed no linkage with markers in the region of *Pl5/Pl8* or with the Pl6 cluster. The gene Pl_{PMI3} has still not been mapped.

The other resistance source obtained from *H. tuberosus* was HIR34, with a gene denoted *Pl4*. It has a similar range of resistance to *Pl2*, except that it is susceptible to races 334, 307 and a US isolate of race 330, it has type II resistance and does not map in the *Pl2/Pl6* cluster.

Resistance from other species: HA337, HA338 and HA339 were all developed from *H. praecox* by Miller and Gulya (1991), with a gene designated *Pl7*, but which has not been distinguished from *Pl6* by its resistance to different races or its map position. At the same time as the interspecific pool from *H. argophyllus* was studied, a number of other interspecific pools developed at INRA Montpellier were also tested for their resistance to race 710. All showed some downy mildew resistance. Progenies apparently homozygous for resistance to 710 were obtained from a *H. neglectus* pool but these were susceptible to

races 304, 714 and 334, suggesting that a *Pl6* type gene was present. Interspecific pools from *H. petiolaris fallax, H. resinosus* and *H. debilis*, showed some resistance to 710 but no homozygous lines were obtained. For a pool from *H. occidentalis*, it was concluded more recently (Vear, 2006) that resistance may be under quantitative control rather than *Pl* genes.

In more recent studies, resistance to both races 710 and 304 has been fixed in introgression lines from *H. resinosus, H. strumosus, H. debilis* and *H. tomentosus*. These lines are in the course of study to determine whether they provide new *Pl* genes.

DISCUSSION

Resistance genes all appear to come from quite recent crosses with wild *Helianthus*, and in particular wild *H. annuus*. What is identified depends on the resistance requirement. New sources of *Pl1* and *Pl2* probably exist quite widely but are of little interest in modern breeding and so are not introgressed. In contrast, tests made with races 710 or 730 have shown that *Pl6* is present in many wild *H. annuus* ecotypes, most frequently in southern US but from Texas to California. In addition, the gene *Pl7*, from *H. praecox* and the resistance in a pool from *H. exilis* also appear to be the same. There does appear to be at least a second cluster from wild *H. annuus*, but the results of mapping of resistance derived from MPHE-361, MPHE-829 and HA458 are necessary to conclude whether their resistance genes are indistinguishable. The absence of segregation between *Pl5* (from *H. tuberosus*) and *Pl8* (*H. argophyllus*) was surprising, it was questioned whether these genes, which appear to have the same structure, were the result of natural interspecific crosses, or whether, in the multiple interspecific hybridisation at Krasnodar, the open pollinated variety Progress included a gene from *H. argophyllus*. However, since then, Pl_{arg} has been identified from three completely independent crosses at Fargo, Montpellier and Clermont-Ferrand, over 20 years and with quite different *H. argophyllus* ecotypes. Now it seems that *H. argophyllus* has only one "*Pl* gene" (it may be that new races will show some differences between them). So what is the relation between *Pl8* and Pl_{arg}? It could be that *H. argophyllus* contains the same genes as *H. tubersosus* or that there are sites in the sunflower genome (susceptible alleles) where these resistance genes become integrated so that the cultivated genotype used in the interspecific cross may determine the position of interspecific *Pl* genes.

At present, wild *H. annuus* appears the most fruitful source of *Pl* genes, but *Pl6* is often found, and it is this species with which it has been easiest to work. It is also true that the sources derived from both *H. annuus* and *H. tuberosus* show variation in the numbers of downy mildew races controlled, giving the appearance of more "new" genes than there really are. *Pl1* appears to be a *Pl2-,* having lost resistance to races such as 304*,* and forms of *Pl6* which had lost resistance to races 100 and 300 were obtained experimentally (Vear et al., 1997). For *Pl5*, there appear to be several sources differing slightly in the races they resist, although no within-cluster recombination has been obtained intentionally. It is also true that, with sporulation on cotyledons, individual plants or single progenies with incomplete forms of *Pl5* and *Pl8* may be difficult to identify. In contrast, so far, there do not appear to be any *Plarg-*. This last resistance may be a different structure from the other clusters, but its appearance from 3 different crosses suggests that *H. argophyllus* is not very rich in different *Pl* genes.

In the last 20 years breeders have spent a lot of effort on introducing new *Pl* genes into their best lines following changes in *P. halstedii* races. Since 2003 studies have been made on quantitative, hopefully non-race specific, resistance with levels that could be sufficient alone but which certainly would be of use in combination with *Pl* genes (Tourvieille de Labrouhe et al., 2008). QTL have been identified (Vear et al., 2008) which appear independent of the known *Pl* gene clusters, but it is important to continue identification and mapping of the other sources of complete resistance to check that quantitative resistance is not controlled by incomplete major genes. Overall, if it is found that, among the new sources of complete resistance, there are some new *Pl* genes and that quantitative resistances are different and not race specific, breeders should have the resources necessary to provide durable resistance to downy mildew quite rapidly. In addition, in the long term, if it becomes possible to introgress genes from the perennial *Helianthus* species, the small successes so far from *H. tubersosus* and the apparent resistance in *H. resinosus, H. tomentosus and H. occidentalis*, suggest that new and perhaps different types of downy mildew resistance could become available.

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REFERENCES

Bert, P.F., D. Tourvieille de Labrouhe, J. Philippon, S. Mouzeyar, I. Jouan, P. Nicolas, and F. Vear. 2001. Identification of a second linkage group carrying genes controlling resistance to downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus* L). Theor. Appl. Genet. 103:992-997.

Bouzidi, M.F., S. Badaoui, F. Cambon, F. Vear, D. Tourvieille de Labrouhe, P. Nicolas, and S. Mouzeyar. 2001. Molecular analysis of a locus for resistance to downy mildew in sunflower with specific PCR-based markers. Theor. Appl. Genet. 104:592-600.

Dussle, C.M., V. Hahn, S.J. Knapp, and E. Bauer. 2004. Pl_{arg} from *Helianthus annuus* is unlinked to other known downy mildew resistance genes in sunflower. Theor. Appl. Genet., 109:1083-1086.

Fick, G.N., and D.E. Zimmer. 1974. RHA271, RHA273, RHA274 Sunflower parental lines for producing downy mildew resistant hybrids. Farm. Research 32:7-9.

Leclercq, P., Y. Cauderon, and M. Dauge. 1970. Sélection pour la résistance au mildiou du tournesol à partir d'hybrides topinambour x tournesol. Ann. Amélior. Pl. 20:363-373.

Miller, J.F., and T.J. Gulya. 1987. Inheritance of resistance to race 3 downy mildew in sunflower. Crop Sci. 27:210-212.

Miller, J.F., and T.J. Gulya 1991. Inheritance of resistance to race 4 of downy mildew derived from interspecific crosses in sunflower. Crop Sci. 31:340-343.

Miller, J.F., T.J. Gulya, and G.J. Seiler. 2002. Registration of five fertility restorer sunflower germplasms. Crop Sci. 42:989-991.

Mouzeyar, S., P. Drevet-Roeckel, J. Phillipon, L. Gentzbittel, F. Vear, D. Tourvieille de Labrouhe, and P. Nicolas. 1995. RFLP and RAPD mapping of the sunflower *Pl1* gene for résistance to *Plasmopara halstedii* race 1. Theor. Appl. Genet. 91:733-737.

Radwan, O., M-F. Bouzidi, F. Vear, J. Phillipon, D. Tourvieille de Labrouhe, P. Nicolas, and S. Mouzeyar. 2002. Identification of non-TIR-NBS-LRR markers linked to the *Pl5/Pl8* locus for resistance to downy mildew in sunflower Theor. Appl. Genet. 106:1438-1446.

Roeckel-Drevet, P., G. Gagne, S. Mouzeyar, L. Gentzbittel, J. Phillipon, P. Nicolas, D. Tourvieille de Labrouhe, and F. Vear. 1996. Colocation of downy mildew (*Plasmopara halstedii*) resistance genes in sunflower (*Helianthus annuus* L.). Euphytica 91:225-228.

Romano, A.B., and A.N. Vazquez. 2003. Origin of the argentine sunflower varieties. Helia 26:127-136.

Tourvieille de Labrouhe, D., E. Pilorgé, P. Nicolas, and F. Vear. 2000. Le mildiou du tournesol. INRA-Editions, Versailles, France. 150 p.

Tourvieille de Labrouhe, D., F. Serre, P. Walser, S. Roche, and F. Vear. 2008. Quantitative resistance to downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus*). Submitted to Euphytica.

Vear, F., L. Gentzbittel, J. Philippon, S. Mouzeyar, E. Mestries, P. Roeckel-Drevet, D. Tourvieille de Labrouhe, and P. Nicolas. 1997. The genetics of resistance to five races of downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus* L.). Theor. Appl. Genet. 95:584-589.

Vear, F., J. Millon, J. Philippon, S. Mouzeyar, P. Nicolas, and D. Tourvieille de Labrouhe. 1988. Analysis and location of sunflower downy mildew resistance genes. p. 84-93. In ISA symposium "Sunflower Downy mildew" Fargo, USA.

Vear, F., J. Phillippon, S. Roche, P. Walser, D. Tourvieille de Labrouhe, S. Mouzeyar, and P. Nicolas. 2000. Genetical analyses of the sunflower downy mildew resistance gene *Pl5*. p. J31-J36. In: Proc.15th Int. Sunflower Conf., Toulouse, France.

Vear, F., D. Tourvieille de Labrouhe, and J.F. Miller. 2003. Inheritance of the wide-range downy mildew resistance in the sunflower line RHA419. Helia 26:19-24.

Vear, F., F. Serre, I. Jouan, P.F. Bert, S. Roche, P. Walser, D. Tourvieille de Labrouhe, and P. Vincourt. 2008. Inheritance of quantitative resistance to downy mildew (*Plasmopara halstedii*) in sunflower (*Helianthus annuus* L.) Submitted to Euphytica.

Vrânceanu, V.L., and F.M. Stoenescu. 1970. Immunity to sunflower downy mildew due to a single dominant gene. Probl. Agric. 22:34-40.

Vrânceanu, V.L., N. Pirvu, and F.M. Stoenescu. 1981. New sunflower downy mildew resistance genes and their management. Helia 4:23-27.

Zimmer, D.E., and M.L. Kinman. 1972. Downy mildew resistance in cultivated sunflower and its inheritance. Crop Sci. 12:749-751.

Caracterización de la resistencia genética a podredumbre basal en girasol causada por *Sclerotinia sclerotiorum* en Argentina

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ABSTRACT

Sclerotinia sclerotiorum is an optional parasite fungi causative of stem rot in sunflower and others crops in Central and North Area of Argentina. Planting resistant materials is the most economical and efficient (Fick and Miller, 1997) management strategy. The objectives of this work were to identify differences in behaviour between materials of a wide genetic diversity compared with *S. sclerotiorum* inoculation and to obtain a reliable, simple, repeatable method for an assessment consistent with the genotypes over the years in Manfredi, INTA Experimental Station, Argentina. During 4 years, 384 entries from the INTA collection and improvement program, 21 commercial hybrids and 8 susceptible and tolerant controls were evaluated. The design of the trials was in alpha lattice with two replications. The planting was done manually in an area naturally infested with *S. sclerotiorum* and reinforced with mycelium of the pathogen grown in wheat grains. The evaluation was performed at 30 days of infection, recording the percentage of diseased plants. During the four years, there was a good manifestation of the disease allowing the evaluations. The contrasting behaviour between susceptible and resistant controls over the years was consistent. There were statistical differences between genotypes. Three groups with different levels of resistance to stalk rot between genotypes with a broad genetic variability were identified. High infection stalk rot in sunflower, and consistency in the response of genotypes through the years were achieved. The methodology used in infection and evaluation showed reliability, repeatability and simplicity by allowing the selection of superior genotypes.

Key words: breeding – genetic resistance – genetic resources – *Sclerotinia* – stalk rot – sunflower.

RESUMEN

Sclerotinia sclerotiorum es un hongo polífago agente causal de Podredumbre Basal de tallo en girasol y otros cultivos de importancia económica, principalmente en la zona Central y Norte Argentina. La siembra de materiales resistentes es la estrategia de manejo más económica y eficiente. Los objetivos de este trabajo fueron detectar diferencias de comportamiento entre materiales de amplia diversidad genética frente a inoculación con *S. sclerotiorum* y contar con un método confiable, simple y repetible para una evaluación consistente de genotipos a través de los años en INTA EEA Manfredi, Argentina. Durante 4 años, se evaluaron 384 entradas de la colección y el programa de mejoramiento del INTA, 21 híbridos comerciales y 8 testigos susceptibles y tolerantes. El diseño de los ensayos fue alpha lattice en 2 repeticiones. La siembra se realizó manualmente en infectario de *Sclerotinia sclerotiorum* reforzado con micelio del patógeno vehiculizado en granos de trigo. La evaluación se realizó a los 30 días de infectado determinando la proporción de plantas enfermas. Durante los cuatro años, existió buena manifestación de la enfermedad y el comportamiento entre los testigos susceptibles y resistentes fue contrastante y consistente durante todos los años. Existieron diferencias estadísticas entre los genotipos. Se identificaron tres grupos con diferentes niveles de resistencia a Podredumbre Basal entre genotipos de amplia variabilidad genética. La metodología utilizada en infección y evaluación demostró confiabilidad, repetibilidad y sencillez permitiendo la selección de genotipos superiores.

Palabras clave: girasol - mejoramiento - podredumbre basal - recursos genéticos - resistencia genética – *Sclerotinia.*

INTRODUCCIÓN

Sclerotinia sclerotiorum es un hongo polífago y agente causal de podredumbres en diversos cultivos de importancia económica, entre ellas la Podredumbre Basal del tallo en girasol (Cuk, 1980) y también en soja. En su infección micelial invade desde el suelo la zona radical superior y la base del tallo (Huang y Dueck, 1980).

La Podredumbre Basal puede causar severos daños en el girasol, principalmente en la zona Central y Norte de Argentina, y es una limitante para este cultivo, fundamentalmente en fechas de siembra tardías. En la campaña 2002/03, se reportaron severos daños (9,5 y 32,4% de plantas afectadas) en ensayos de evaluación de híbridos comerciales bajo condiciones de infección natural, fecha de siembra normal, y labranza convencional en la localidad de Serrano, Córdoba, (Alvarez y Guerra, 2005). La creciente expansión de la soja y su inclusión en las rotaciones, constituyen un agravante potencial ante las probabilidades de expansión de esta enfermedad.

La infección natural suplementada con inoculación artificial es considerada un efectivo método para realizar selección entre materiales (Miller, 1996). La siembra de materiales resistentes es la estrategia de manejo más económica y eficiente.

Los objetivos de este trabajo fueron: 1) Detectar diferencias de comportamiento entre materiales de la Colección de Germoplasma de Girasol, del Programa de Mejoramiento del INTA y de Híbridos Comerciales frente a inoculación asistida con micelio de *S. sclerotiorum*. 2) Contar con un método confiable, simple y repetible que permita una evaluación consistente del comportamiento de los genotipos posibilitando la detección de resistencia.

MATERIALES Y MÉTODOS

Durante 4 años (2002/03, 2004/05, 2005/06 y 2006/07), se evaluaron 423 genotipos compuestos por 384 entradas de la colección de Recursos Genéticos del INTA y 21 híbridos comerciales, de los cuales se utilizaron 8 como testigos en base a su comportamiento sanitario diferencial (4 susceptibles y 4 tolerantes) frente a Podredumbre Basal y de capítulo (Trogia et al., 2002).

Los ensayos se dispusieron en diseños Alpha lattice en 2 repeticiones. Las parcelas eran de 1 hilera de 5,10 m de largo, distanciadas a 0,70 m. La siembra se realizó en un infectario natural de *S. sclerotiorum* en forma manual a 0,30 m entre sí. El análisis estadístico mediante ANOVA, consideró Genotipo como fuente de variación. LSMeans se calculó por el procedimiento GLM de SAS (SAS 2002).

Cuando las plantas alcanzaron el estado V4 se realizó el raleo, dejando 1 planta por golpe (Schneiter y Miller, 1980). En el estado de prefloración, se reforzó la presencia de patógeno introduciendo micelio vehiculizado en granos de trigo en la zona de suelo próxima al cuello de la planta a una profundidad de 2 a 3 cm y parcialmente cubierto. El inóculo se preparó cultivando esclerotos de *S. sclerotiorum* en APG (2%) e incubándolos a 25ºC. Posteriormente a los 4 días se vehiculizaron en granos de trigo. Este vehículo se preparó remojando trigo durante 24 horas, posteriormente se escurrió el exceso de agua y fraccionó en frascos de 500 ml de capacidad. Se esterilizaron en autoclave secuencialmente en 3 oportunidades a 1 atm durante 30 min, dejando enfriar. En condiciones de asepsia se inoculó con colonias de *S. sclerotiorum* (aislado a partir de esclerotos recolectados en el campo) y se incubó a 25ºC durante 25 a 30 días, agitando periódicamente para permitir un crecimiento homogéneo. La evaluación se realizó en dos oportunidades: a los 15 y a los 30 días de infectado, considerando planta afectada a toda aquella que presentara lesión en la base del tallo (Pereyra y Escande, 1994). Se determinó la proporción de plantas enfermas como el cociente entre plantas con síntomas y plantas totales x 100. Los genotipos se clasificaron en resistentes, intermedios y susceptibles.

RESULTADOS Y DISCUSIÓN

Durante los cuatro años de evaluación, existió una buena manifestación de la enfermedad que permitió realizar las evaluaciones. En la Fig. 1 se observa un comportamiento contrastante entre los testigos susceptibles (S1, S3, S5 y S7) y resistentes (S2, S4, S6 y S8), el cual fue consistente durante todos los años de evaluación, siendo la proporción de plantas enfermas de los susceptibles siempre mayores a los de los resistentes. Durante los tres primeros años de evaluación (2002/03, 2004/05 y 2005/06), el nivel de enfermedad en todos los testigos fue superior a la de 2006/07, lo cual se debería a las condiciones ambientales con un período de sequía y altas temperaturas posterior a la inoculación, que podrían haber afectado el proceso de infección de las plantas. Sin embargo, esta situación natural no afectó significativamente los parámetros estadísticos ni la discriminación de los genotipos por su grado de resistencia.

De acuerdo a la Tabla 1, durante los 3 primeros años se obtuvo una alta incidencia (superior a 80%) de las plantas infectadas para el promedio de todos los genotipos, mayor a la registrada en el 4º año, (18.2% en 2006/07). Los Coeficientes de Variación (C.V), se encontraron dentro de los valores esperados para este tipo de evaluaciones. Las Diferencias Mínimas Significativas (DMS) permitieron diferenciar estadísticamente los genotipos por su comportamiento frente a la enfermedad.

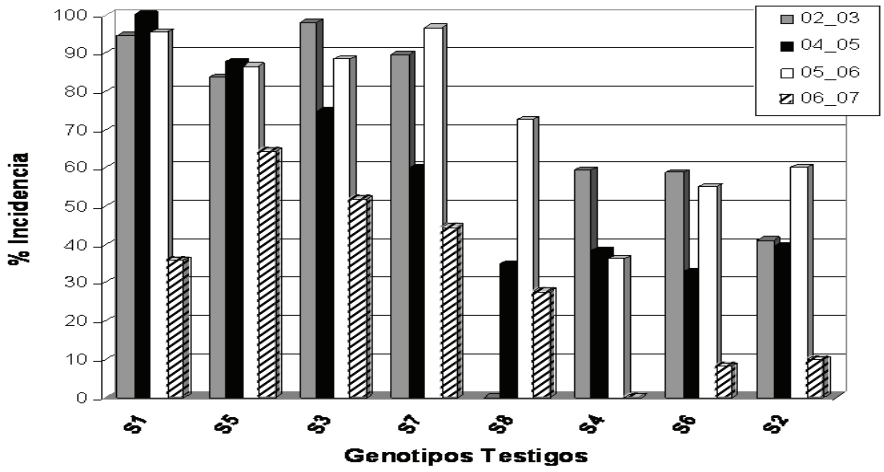

Fig. 1. Incidencia de podredumbre basal en testigos susceptibles (S1, S3, S5 y S7) y resistentes (S2, S4, S6 y S8), en cuatro años de evaluación.

Tabla 1. Número y porcentaje de genotipos con diferente grado de incidencia de Podredumbre Basal. Promedio, Coeficiente de Variación y DMS (α=0,1), durante cuatro años de evaluación.

Incidencia	2002/03		2004/05		2005/06		2006/07	
	Nº	%	Nº	%	Nº	%	Nº	%
Alta	5	4,5	21	20,2	1	1,0	74	71,2
Media	34	30,6	7	6,7	32	30,8	18	17,3
Baja	72	64,9	76	73,1	71	68,3	12	11,5
Promedio (%)	80,3		80,1		86,2		18,2	
C.V. (%)	24,2		20,2		12,6		34,7	
DMS (0,1)	22,8		37,6		18,2		25,7	

En la Fig. 2, la severidad del ataque y la reacción de los genotipos permitieron identificar tres grupos de materiales con diferente comportamiento según grado de resistencia. Resistentes: genotipos con valores menores a la suma del valor mínimo del ensayo y la DMS (Negros); Medios: con valores que difieren del valor mínimo y/o máximo del ensayo (Blancos) y Susceptibles: valores mayores a la diferencia entre el valor máximo del ensayo y la DMS (Grises).

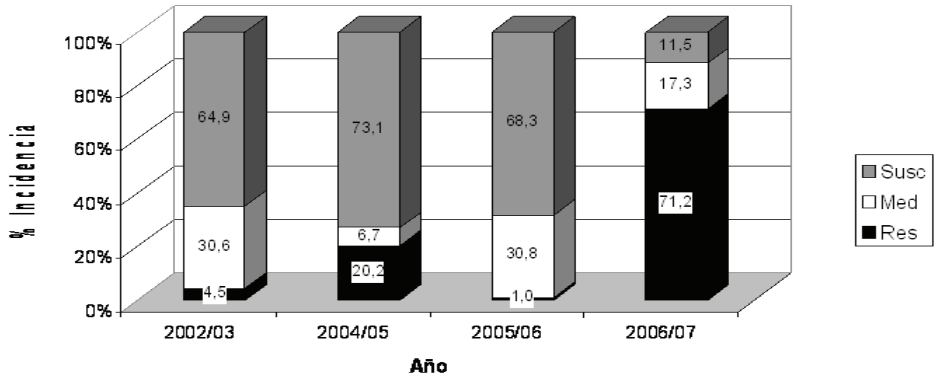

Fig. 2. Proporción de genotipos con diferente grado de resistencia frente a podredumbre basal (susceptibles, medios y resistentes) en cuatro años de evaluación.

Las proporciones entre los tres grupos varió con el año y los materiales evaluados en cada año, de 11.5 (2006/07) a 73.1 % (2004/05) para los genotipos de susceptibles, y de 1.0 (2005/06) a 71.2 % (2006/07) para los genotipos de resistentes. Comparando los diferentes años de evaluación, la alta proporción de entradas con mayor nivel de resistencia en 2006/07, se debería a que un alto número de genotipos participantes fueron seleccionados por su buen comportamiento frente a la enfermedad por esta metodología, en años anteriores.

De acuerdo a la Fig. 3 en el año 2002/03, 4 genotipos se comportaron como resistentes (barras negras), y 1 sola entrada perteneciente a la Colección de Recursos Genéticos de INTA (CGGI 491, PROCISUR-B-C0, datos no publicados) superó al mejor testigo resistente (rayas horizontales).

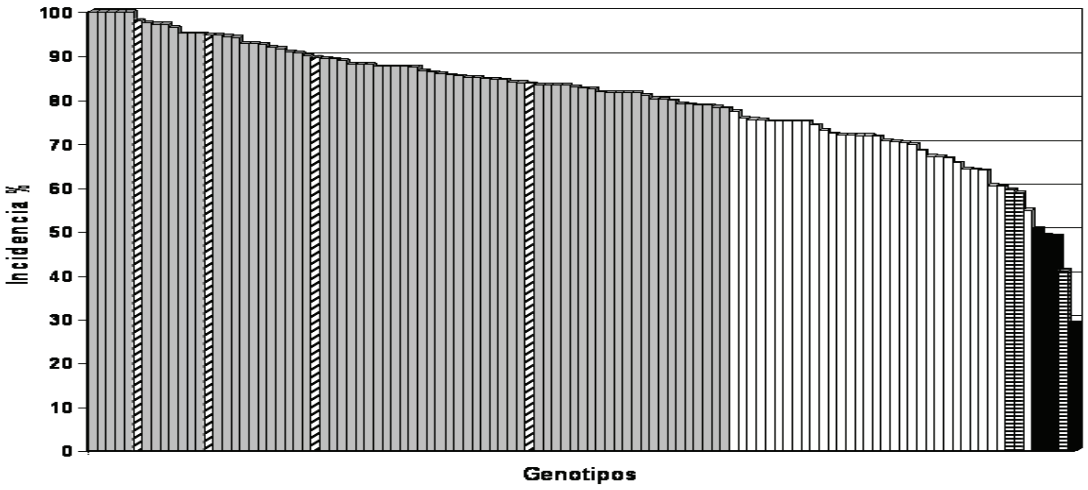

Fig. 3. Respuesta de 111 genotipos frente a *Sclerotinia sclerotiorum*, año 2002/03, INTA Manfredi. Susceptibles: barra color gris, Medios: barra color blanco, Resistentes: barra negra, Testigos Resistentes: rayas horizontales, Testigos Susceptibles: rayas diagonales.

En las evaluaciones correspondientes a los años 2004/05 y 2005/06, de acuerdo a Fig. 4 y 5, ninguna entrada de Colección demostró mejor comportamiento que el mejor testigo resistente (rayas horizontales). 16 entradas (2004/05) y ninguna (2005/06), integraron el grupo resistente (barras negras).

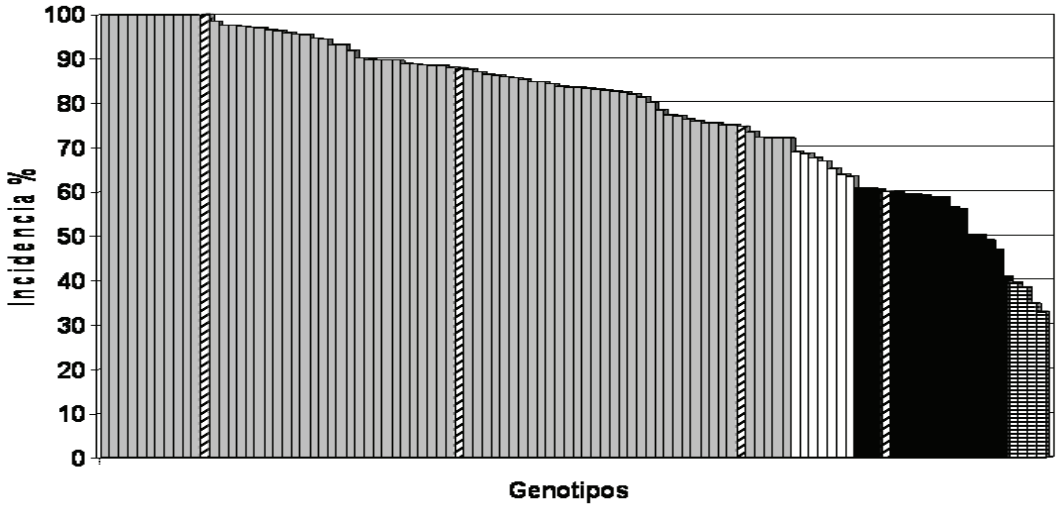

Fig. 4. Respuesta de 104 genotipos frente a *Sclerotinia sclerotiorum*, año 2004/05, INTA Manfredi. Susceptibles: barras color gris, Medios: barras color blanco, Resistentes: barras color negro, Testigos Resistentes: rayas horizontales, Testigos Susceptibles: rayas diagonales.

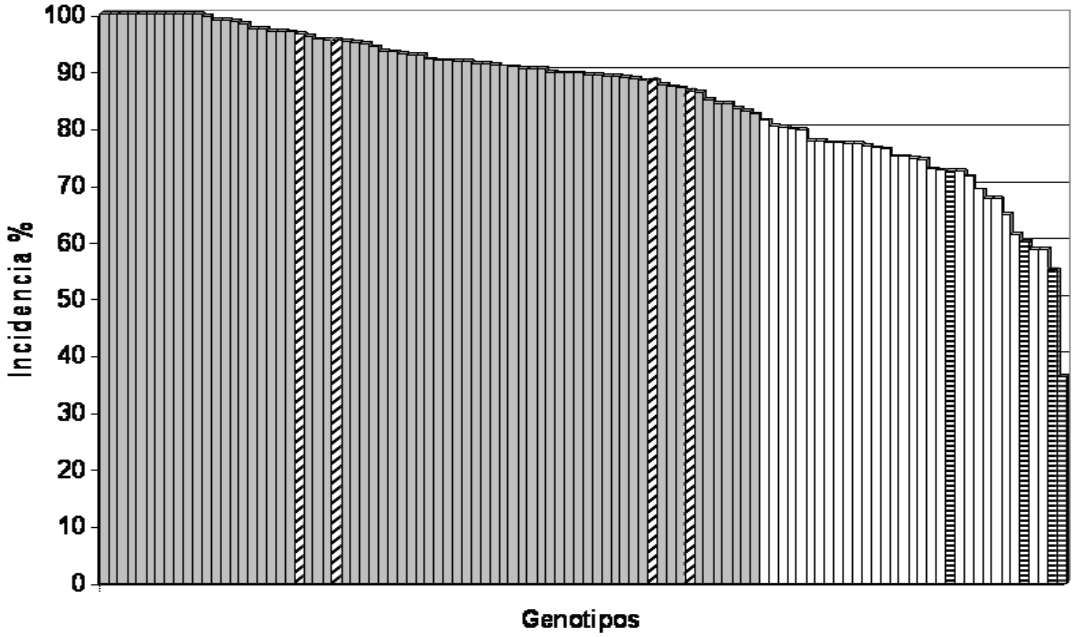

Fig. 5. Respuesta de 104 genotipos frente a *Sclerotinia sclerotiorum*, año 2005/06, INTA Manfredi. Susceptibles: barras color gris, Medios: barras color blanco, Resistentes: barras color negro, Testigos Resistentes: rayas horizontales, Testigos Susceptibles: rayas diagonales.

La Fig. 6, muestra que en el año 2006/07, 61 genotipos integraron el grupo resistente (barras negras), 26 entradas superaron el valor del mejor testigo resistente (rayas horizontales), 25 de ellos corresponden al programa de mejora seleccionados años anteriores por esta metodología y sólo 1 (CGGI 13_3, IDANOV 8281-3), pertenece a la Colección de Recursos Genéticos de INTA (datos no publicados).

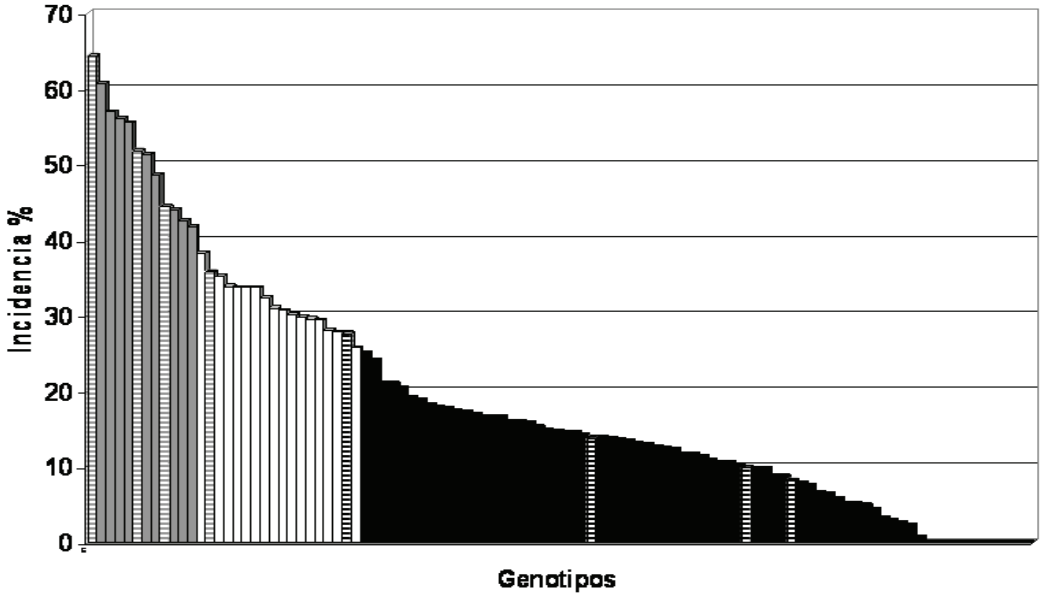

Fig. 6. Respuesta de 104 genotipos frente a *Sclerotinia sclerotiorum*, año 2005/06, INTA Manfredi. Susceptibles: barras color gris, Medios: barras color blanco, Resistentes: barras color negro, Testigos Resistentes: rayas horizontales, Testigos Susceptibles: rayas diagonales.

Como resultado del presente trabajo, se puede concluir que:

1. Es posible lograr elevados niveles de infección y enfermedad de Podredumbre Basal del tallo en girasol, determinando a la vez consistencia en la respuesta de los genotipos a través de diferentes años y distintos niveles de infección general.
2. La metodología utilizada en la infección y evaluación demostró confiabilidad, repetibilidad y una aceptable sencillez permitiendo la utilización de la variabilidad en los procesos de mejora.
3. Existen diferentes niveles de resistencia a Podredumbre Basal en condiciones de infectario con inoculación micelial entre genotipos de amplia variabilidad genética como poblaciones de la Colección de Germoplasma de Girasol del INTA, líneas e híbridos comerciales.
4. La colección de Recursos Genéticos de Girasol de INTA es una fuente valiosa para selección de genotipos por caracteres de resistencia genética a la PodredumbreBasal.
5. Mediante mejoramiento genético, es posible la obtención de genotipos superiores con altos niveles de resistencia frente a *S sclerotiorum*.

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BIBLIOGRAFIA

Alvarez, D., and G. Guerra. 2005. Evaluación de los Recursos Genéticos de Girasol por Podredumbre Basal *Sclerotinia sclerotiorum*. Segundo Congreso Nacional de Girasol, Buenos Aires, Argentina.

Cuk, L. 1980. Resistance evaluation of sunflower genotypes naturally infected by *Sclerotinia sclerotiorum*. (Lib.) de Bary. p. 182-186. In: Proc 9th Int. Sunflower Cong., Torremolinos, Spain.

Huang, H.C., and J. Dueck. 1980. Wilt of sunflower from infection by micelial germinating sclerotia of *Sclerotinia sclerotiorum*. Can. J. Plant Pathol. 2:47-52.

Miller, J. F. 1996. Development of sunflower germplasm with rResístance to *Sclerotinia* stalk rot. p. 56-63. In: Proc. ISA Symp. Sunflower Disease Tolerance in Sunflower, 13 June, Beijing, P.R.China.

Pereyra, V., and A. Escande. 1994. Enfermedades de girasol en la Argentina. Manual de reconocimiento. INTA. Unidad Integrada Balcarce, Argentina.

SAS 2002. SAS Institute Inc 9.1.3. SAS Institute Inc, Cary, NC, USA.

Schneiter, A., and J.F. Miller. 1981. Description of sunflower growth stage. Crop Sci. 21:901:903.

Molecular characterization of a novel *Sunflower chlorotic mottle virus* (SuCMoV) strain

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ABSTRACT

Sunflower plants showing chlorotic ring spot symptoms were observed during the 2005/2006 crop season in the southeast of the Province of Buenos Aires in Argentina. Preliminary studies, including host range symptoms, serological tests and electron microscopy, had identified this virus isolate as a potyvirus closely related to *Sunflower chlorotic mottle virus* (SuCMoV). The nucleotide sequence of the genomic 3´ terminal region of this potyvirus was determined and characterized. The sequence consisted of 1304 nucleotides (nt) includingthe C-terminal region of the nuclear inclusion b protein gene (NIb), the capsid protein gene (CP) and the 3´non-coding region (3´-NCR). The partial putative NIb gene (240 nt) encoded a protein of 80 amino acids (aa) residues and the CP gene (807 nt) encoded a protein of 269 aa residues. The 3´-NCR was 257 nt in length excluding the poly (A) tract. Sequence comparisons of the predicted CP aa and 3´-NCR were analyzed separately in order to determine the relationship between this potyvirus and SuCMoV, and other reported potyviruses. The CP of this potyvirus isolated from sunflower shared 94.8% aa identity with SuCMoV (Argentina) and 89.2% with SuCMoV-Zi (Brazil). The 3´-NCR shared 94.2% nt sequence identity with SuCMoV. These data indicate that the potyvirus causing chlorotic ring spot (CRS) symptoms in sunflower is closely related to SuCMoV and it is provisionally referred to as SuCMoV strain CRS.

Key words: coat protein sequence – molecular assays – SuCMoV – sunflower – strain.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops in Argentina, with a total planted area of 2,380,000 ha and a total yield of 3,500 million tons in the 2006/2007 growing season. It has been a strategic revenue crop for this country since Argentina is the main exporter of edible sunflower oil in the world. *Sunflower chlorotic mottle virus* (SuCMoV) is one of the most widely distributed potyviruses on cultivated and wild sunflowers in this country and was reported in several provinces. Achene yield was significantly reduced by SuCMoV infections occurring at early ontogenetic stages (Lenardon et al., 2001). Recently, SuCMoV has been recognized as a new PVY strain by the ICTV (Dujovny et al., 2000; Berger et al., 2005), however some inconsistencies of its taxonomic status need to be clarified. During the 2005/2006 growing season, SuCMoV broke out in commercial sunflower hybrids in the southeast of the province of Buenos Aires with an unusual increase in disease incidence. At the same time, a whole commercial sunflower field showing chlorotic ring spot symptoms (CRS) on leaf blades (Fig. 1) was detected in the same geographical area. Preliminary biological, serological experiments, and electron microscopy studies had previously identified the virus as a potyvirus related to SuCMoV (Lenardon et al., 2005).

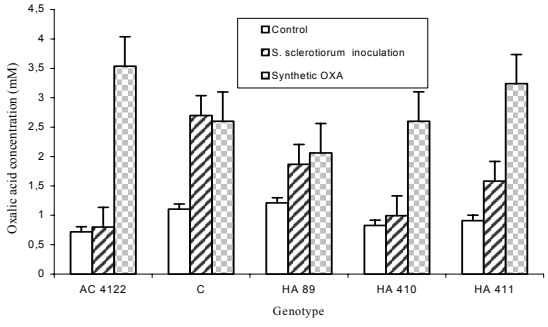


Fig. 1. Sunflower leaves showing chlorotic ringspot symptoms.

The genus Potyvirus, in the plant family Potyviridae, is by far, the largest virus genus known in the plant kingdom, with nearly 200 members, which accounts for almost 25% of known plant viruses. Its members share similar morphology, particle structure, host range, and modes of transmission (Berger et al., 2005). The virions contain a single molecular linear, positive-sense 8.2 to 9.7 kb ssRNA that has a VPg structure at its 5´terminus and a poly (A) tract at its 3´terminus. The coding ORF is translated to one polyprotein, which is subsequently processed into 10 different proteins by virus-encoded proteinases (Allison et al., 1986). The potyvirus genomic RNA is encapsidated by a single type of coat protein (CP). Genomic sequence data have become useful for demarcating virus strains and species (Fauquet et al., 2003; Berger et al., 2005). Sequences within the 3´ proximal portion of the genome are commonly used for species demarcation (Shukla et al., 1994; Adams et al., 2005), including the nucleotide or amino acid sequences of CP and the nucleotide sequence of the 3´ non-coding region (3´-NCR).

This report was undertaken to determine molecular properties (genome organization, amino acid sequence and phylogenetic analyses) of SuCMoV-CRS.

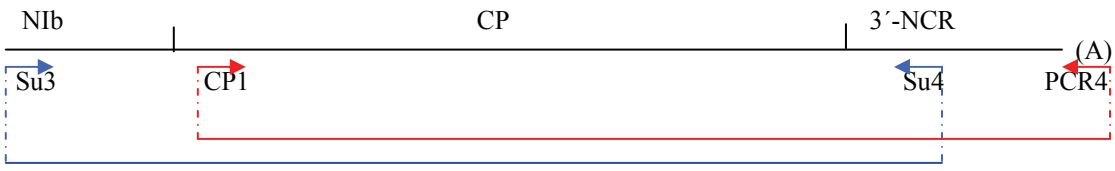
MATERIALS AND METHODS

Virus source and maintenance

Sunflower plants showing leaf blades with isolated and confluent chlorotic ring spots were collected from commercial sunflower fields located in Pieres county, in the southeast of the province of Buenos Aires. Single chlorotic ring spots were mechanically inoculated onto sunflower hybrid CF-7 and *Nicotiana occidentalis* L., in which they were maintained. The mechanical inoculations were conducted according to our standard procedure: leaves from infected plants were ground in 0.01M phosphate buffer, pH 7, containing 0.1% Na_2SO_3 (1:5 w/v) with a mortar and pestle. Extracted juice was mixed with 600 mesh carborundum before being rub-inoculated on the hosts and plants were kept under greenhouse conditions (22ºC + 5ºC) for symptom expression.

RT-PCR

Total RNA was extracted from sunflower fresh leaf tissue (100 mg) using the RNeasy Plant Mini Kit (Qiagen, California, USA) according to the manufacturer's instructions. To clone the 3´ end of the NIb and the CP cistrons, DNA was synthesized using the Access RT-PCR system (Promega, Wisconsin, USA) and specific primers Su3 and Su4 (Fig. 2). To clone the CP cistron and the 3´-NCR a first strand cDNA was made with M-MLVreverse transcriptase (Promega) and Eco/Not as initial primer (Tsuneyoshi et al., 1998). PCR was carried out using Taq DNA Polymerase (Promega) and the primers CP1, and PCR4 (Fig. 2). The amplified products were visualized by electrophoresis on a 1.4% agarose gel stained with ethidium bromide.



Su3: 5´-GAGGCGTGGGGCTATCC-3´
Su4: 5´-AAAAGTAGTACAGGAAAAGCC-3´
CP1: 5´-GGTGACAACATAGATGCAGG-3´
PCR4: (Tsuneyoshi et al., 1998)

Fig. 2. Cloning strategy of the SuCMoV-CRS 3´end. The position of the different PCR-generated cDNA clones is shown below the viral genomic map.

Cloning and sequencing of PCR products
PCR products were cloned using the pGEM T-easy vector system (Promega), following the manufacturer's instructions and subsequently subjected to DNA sequencing at Macrogen Inc. (Seoul, Korea).

Sequence analysis
The nucleotide (nt) and predicted amino acid (aa) sequences of the whole CP coding region and the nt sequence corresponding to the 3´- NCR of the SuCMoV-CRS were compared with 38 potyvirus sequences deposited in GenBank, EMBL, DDBJ and PDB databases using pair-wise Align program (Table 1).

Sequence assembly and analysis were performed utilizing the Lasergene software package, including Editseq, Seq Man and MegAlign programs (DNASTAR, Inc., Madison, Wi, USA). Multiple sequence alignments produced by Clustal W algorithm were used as input data for reconstructing phylogenetic trees by the Neighbor-Joining method using the software MEGA version 4 (Tamura et al., 2007). Statistical significance was estimated by performing 500 replications of bootstrap resampling of the original alignment using the bootstrap option of the phylogenetic tree menu.

RESULTS

Following mechanical inoculation sunflower and *N. occidentalis* plants became infected, developing symptoms similar to those seem in the field collected plants.

A 1304 nt fragment of the 3´ terminal region genome of the SuCMoV-CRS was cloned and sequenced (GenBank accession number EU418771). Sequence analysis of this virus genome portion revealed putative proteins and a 3´- NCR similar in size and arrangement to those of representative potyviruses. This sequence covered part of the NIb coding region (nt 1 to 240), the whole CP coding region (nt 241 to 1047) and the 3´-NCR (nt 1048 to 1304). The first predicted 80 aa belonged to the C-terminus of the NIb and the dipeptide at the putative NIb/CP junction was Q/G. The CP gene encoded 269 aa residues with the Asp-Ala-Gly (DAG) motif presented at the N-terminus of CP (4 aa from the cleavage site). Also, the following consensus motifs have been found in the putative CP: MVWCIENGTSP, AFDF, QMKAAAL at 117, 200 and 220 aa from the cleavage site. The 3´-NCR consisted of 257 nt excluding the polyadenylated tract.

The percentage of identity between the nucleotides of the SuCMoV-CRS CP and selected potyviruses ranged from 39.8% to 87.3%. Comparisons of the predicted CP aa ranged from 47.6% to 94.8% identity (Table 1). SuCMoV-CRS shared 94.8% aa identity with the CP of an Argentinian SuCMoV isolate which caused chlorotic mottling on sunflower, 89.2% aa identity with a Brazilian SuCMoV-Zi isolated reported from zinnia plants and 84.1% with *Bidens mosaic virus* (BiMV) (Table 1). Comparisons of the CP core aa (Lys^{32} to $Prol^{184}$) between this virus and SuCMoV (Argentina) showed identity of 96.1%. Additionally, the identity of the CP of SuCMoV-CRS was less related with other potyviruses-infecting sunflower such as *Sunflower chlorotic spot virus* (syn *Bidens mottle virus*) (72.8%) and *Sunflower mosaic virus* (66.7%) (Table 1).

Nucleotide comparisons of the 3´-NCR between SuCMoV-CRS and several potyvirus species showed the highest degree of sequence identity with SuCMoV (94.2%) and BiMV (76.8%). The other potyviruses showed a lower degree of sequence identity ranging from 34.4% (TEV) to 57.9% (PepMoV) (Table 1).

In the phylogenetic analysis the high bootstrap values confirmed grouping of the SuCMoV-CRS with SuCMoV, SuCMoV-Zi and BiMV, which are clustered with high confidence to PVY isolates and PepSMV (Fig. 3).

Table 1. Percentage of nucleotide and amino acid identities between the coat protein and the 3´NCR of SuCMoV - CRS with those of selected potyviruses, respectively

Virus acronym	Accession number	CP nucleotide % identity	CP amino acid % identity	3´-NCR nucleotide % identity
SuCMoV	AF255677	**87.3**	**94.8**	**94.2**
SuCMoV-Zi	AY344048	82.4	**89.2**	without data
BiMV	AY960151	77.4	**84.1**	**76.8**
PVY-LYE	AJ439545	77.0	80.4	57.7
PVY-H	M95491	76.8	80.1	54.8
PVY-NTN	AJ890347	76.5	80.0	56.2
PVY-T	D12570	77.6	79.6	51.7
PVY-O	EF026074	75.5	79.3	56.2
PVY-US	M81435	76.7	79.3	55.3
PVY-MN	AF463399	76.7	79.2	56.5
PVY	U09509	77.0	78.9	55.3
PVY-N	D00441	77.4	78.9	51.7
PVY-N:O	EF026076	75.9	78.9	56.8
PVY-Wilga	AJ889867	75.6	78.8	56.3
PepSMV	NC_008393	72.5	77.4	40.1
PepMoV	AY748921	70.0	76.6	**57.9**
PepYMV	EF488081	70.0	74.5	56.8
PTV	AJ437280	69.9	73.9	41.8
SuCSV	AF538686	69.3	**72.8**	48.4
WPMV	AJ437279	70.6	72.2	40.4
PVV	AJ243766	68.4	71.1	44.6
SuMV	AF465545	60.9	**66.7**	37.8
LMV	AJ278854	61.9	63.7	45.4
TEV	M11458	63.4	63.2	**34.4**
TuMV	AB105134	61.0	61.1	41.7
ChiVMV	AJ237843	54.3	59.6	40.7
ZYMV	AB369279	59.6	59.6	41.7
CDV	AM113761	59.3	59.5	45.8
PetFMV	AF030689	47.6	59.5	45.8
SMV	D00717	58.7	59.1	42.3
PRSV	AY162218	60.0	59.0	37.6
PVA	Z21670	59.7	58.9	47.3
BCMV	AM258976	59.1	56.6	44.4
MDMV	D00949	58.9	55.3	45.9
TVMV	U38621	**39.8**	54.1	38.3
ClYVV	AB011819	58.0	53.0	40.6
PPV	NC_001445	55.1	51.4	45.0
SCMV	EU196455	**52.1**	**47.6**	45.4

DISCUSSION

Molecular characteristics of the sunflower-potyvirus inducing CRS, such as nucleotides, predicted amino acid sequence identities and the arrangement of the 3´ end of the genome clearly showed that it is closely related to SuCMoV. This research confirms previous findings based on biological and serological properties and virion morphology (Lenardon et al., 2005).

The 3´-NCR of SuCMoV-CRS showed a higher sequence identity with SuCMoV (94.2%) and BiMV (76.8%) and a lower one with other potyvirus members than usually reported for strains (34.4% to 57.9%). According to Frenkel et al. (1989), the nt sequence of the 3´-NCR of potyvirus strains is highly conserved (83-99%), while distinct potyviruses have only 30-53% nt sequence similarity. Furthermore, Shukla et al. (1994) proposed that species of the same virus should have a 3´-NCR sequence identity of >75%. Considering this criterion, SuCMoV-CRS should remain as a SuCMoV strain.

The CP of SuCMoV-CRS showed a sequence identity of 94.8 % with SuCMoV and most of its differences in aa residues between them were confined to the N-terminal part of this protein. This region is known to be highly variable and to contain mayor virus specific epitopes, due to its localization at the surface of the virions (Shukla et al., 1988). The essential DAG triplet for aphid transmission in the potyvirus genus was found in the N-terminal region together with other consensus aa motifs for CP coding regions common in other potyviruses (Shukla et al., 1994; Atreya et al., 1990, 1991).

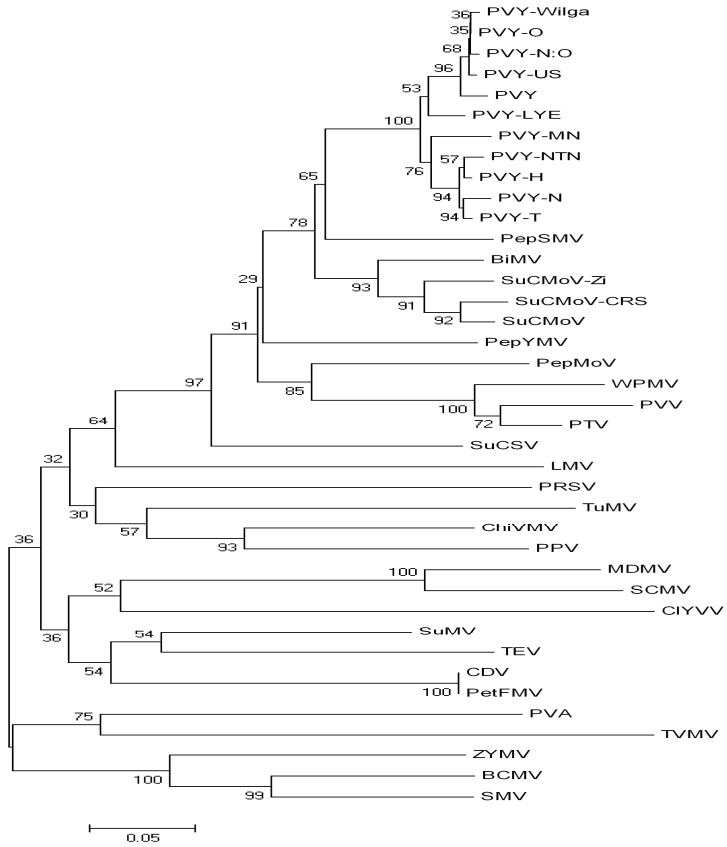


Fig. 3. Phylogenetic tree illustrating the position of the SuCMoV-CRS among the members of the *Potyviridae* family. Neighbor-joining trees were constructed by the program Mega-4 from multiple sequence alignments of CP aa using Clustal W. The bootstrap values of 500 replications are shown in each node.

Comparisons within the SMV/BCMV and the SCMV subgroups demonstrated that the discrimination between strains of the same species and isolates of different virus species occurred at about 83% aa identity (Chen et al., 2004), whereas Adams et al. (2005) consider that a value of 82% aa identity would reliably distinguish between most species except for the PPV, WPMV and PTV group. More recently, species demarcation criteria for the genus *Potyvirus* included CP nt identity of less than 76% and aa identity of less than about 80% (Berger et al., 2005) based on earlier studies of several virus species and strains.

The comparisons between the CP aa among potyviruses have shown that SuCMoV is regarded as a PVY strain (Berger et al., 2005), and BiMV is also considered a strain of PVY (Inoue-Nagata et al., 2006). Nevertheless, other propositions were made recently for species demarcation based on nt and aa identity within ORFs and the CI gene have been proposed as being the best region for diagnosis and taxonomy studies if only a sub portion of the genome is to be sequenced, rather than the CP usually used, because it most accurately reflects the taxonomic status according to the complete ORF (Adams et al., 2005).

The phylogenetic analysis based on the CP aa sequence identities confirmed the taxonomic relatedness of this sunflower-potyvirus inducing chlorotic ring spot symptoms to SuCMoV, SuCMoV-Zi and BiMV, which could be clustered into a new subgroup among PVY isolates.

The denomination of SuCMoV- (strain) CRS is proposed for this potyvirus closely related with SuCMoV in the light of its association with the symptoms on naturally-infecting sunflower and its ability to reproduce the same systemic symptoms on healthy sunflower plants mechanically inoculated under greenhouse conditions. Research about the identification of the viral genomic region involved in symptom expression is under way and may provide insight of the virus-host interaction.

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REFERENCES

Adams, M.J., J.F. Antoniw, and C.M. Fauquet. 2005. Molecular criteria for genus and species discrimination within the family. *Potyviridae*. Arch. Virol. 150:459-479.

Allison, R.F., R.E. Johnston, and W.G. Dougherty. 1986. The nucleotide sequence of the coding region of tobacco etch virus genomic RNA: evidence for the synthesis of a single polyprotein. Virology 150:9-20.

Atreya, C.D., R. Raccah, and T.P. Pirone. 1990. A point mutation in the coat protein abolishes aphid transmissibility of a potyvirus. Virology 178:161-165.

Atreya, P.L., C.D. Atreya, and T.P. Pirone. 1991. Amino acids substitutions in the coat protein result in loss of insect transmissibility of a plant virus. Proc. Natl. Acad. Sci. USA 88:7888-7891.

Berger, P.H., M.J. Adams, A.A. Brunt, J.H. Hill, J. Hammond, R.L. Jordan, R.J. Morales, S.T. Ohki, E. Rybicki, I. Uyeda, and H.J. Vetten. 2005. *Potyviridae.* p. 819-841. In: C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, L. and A. Ball (eds), Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, CA, USA.

Chen, J., H.Y. Zheng, L. Lin, M.J. Adams, J.F. Antoniw, M.F. Zhao, Y.F. Shang, and J.P. Chen. 2004. A virus related to *Soybean mosaic virus* from *Pinellia ternata* in China and its comparison with local soybean SMV isolates. Arch. Virol. 149:349-363.

Dujovny, G., T. Sasaya, H. Koganezawa, T. Usugi, K. Shohara, and S.L. Lenardon. 2000. Molecular characterization of a new potyvirus infecting sunflower. Arch. Virol. 145:2249-2258.

Fauquet, C.M., D.M. Bisaro, R.W. Briddon, J.K. Brown, B.D. Harrison, E.P. Rybicki, D.C. Stenger, and J. Stanley. 2003. Revision of taxonomic criteria for species demarcation in the family *Geminiviridae*, and an updated list of begomovirus species. Arch Virol. 148:405-421.

Frenkel, M.J., C.W. Ward, and D.D. Shukla. 1989. The use of 3´non-coding nucleotide sequences in the taxonomy of potyviruses: application to watermelon mosaic virus 2 and soybean mosaic virus-N. J. Gen. Virol. 70:2775-2783.

Inoue-Nagata, A.K., P.A. Oliveira, L.S. Dutra, and T. Nagata. 2006. *Bidens mosaic virus* is a member of the *Potato virus Y* species. Virus Genes 33:45-49.

Lenardon, S.L., F. Giolitti, A. León, M.E. Bazzalo, and M. Grondona. 2001. Effect of *Sunflower chlorotic mottle virus* infection on sunflower yield parameters. Helia 24:55-66.

Lenardon, S.L., F. Giolitti, and M. Rodríguez. 2005. Anillos cloróticos del girasol: raza del *Sunflower chlorotic mottle virus* (SuCMoV). p. 596. In: Actas del XIII Congreso Latinoamericano de Fitopatología, Villa Carlos Paz, Argentina.

Shukla, D.D., P.M. Strike, S.L. Tracy, K.H. Gough, and C.W. Ward. 1988. The N and C termini of the coat protein of potyviruses are surface-located and the N terminus contains the major virus-specific epitopes. J. Gen. Virol. 69:1497-1508.

Shukla, D.D., C.W. Ward, and A. Brunt. 1994. The *Potyviridae*. CAB International, Wallingford, UK.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24:1596-1599.

Tsuneyoshi, T., T. Matsumi, T.C. Deng, I. Sako, and S. Sumi. 1998. Differentiation of Allium carlaviruses isolated from different parts of the world based on the viral coat protein sequence. Arch. Virol. 143:1093-1107.

Estimation of sunflower breeding material tolerance to *Diaporthe/Phomopsis helianthi*

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ABSTRACT

Phomopsis of sunflower [caused by *Phomopsis helianthi* Munt.-Cvet. et al. (teleomorph *Diaporthe helianthi* Munt.-Cvet. et al.)], is one of the principal sunflower diseases in the Republic of Croatia and Europe, and has a great influence on grain and oil yield. Hence, in the framework of the sunflower breeding program at the Agricultural Institute Osijek, one of the main objectives is to work on the resistance to this and other principal pathogens. Although sunflower (*Helianthus annuus* L.) has a narrow genetic variability, the source of genetic resistance to this pathogen is found among wild *Helianthus* species, and differences among cultivated genotype tolerance are observed as well. This paper presents only one segment of the work on tolerance by artificial infection under field conditions with the aim to investigating the level of tolerance to this pathogen of a wide range of breeding materials (e.g. cms and restorer lines). The most tolerant material will be used in the creation of new commercial sunflower hybrids.

Key words: artificial infection – *Diaporthe/Phomopsis helianthi* – sunflower – tolerance.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops in world production and the area under sunflower is in constant increase. Same trend is in Europe, where area under sunflower increased in the period 1995-2005 by 2.3 million ha (FAOSTAT, 2005). Major sunflower producers in Europe are: Russia, Ukraine, Romania, France, Bulgaria, Spain, Hungary and Moldova.

In the Republic of Croatia, sunflower production is characterized by significant oscillations in areas, grain and oil yield. These oscillations significantly depend on the occurrence and intensity of diseases, which, in some years, lead to significant decreasing in grain and oil yields. Different diseases are dominant in different production areas and significantly depend on agroecological conditions. It is known that over 30 different pathogens (among them fungi are predominant) attack sunflower and cause diseases which can produce important economic damage (Škorić et al., 2002.).

Phomopsis helianthi Munt.-Cvet. et al. (teleomorph *Diaporthe helianthi* Munt.-Cvet. et al.), is one of the most important sunflower pathogens in Europe. It causes a disease named gray stem spot (stem canker). It was first described in the former Yugoslavia in 1981 (Mihaljčević et al., 1982.) and from then on it spread all over the world and became one of the most prevalent diseases of cultivated sunflower (Degener et al., 1999). In environmental conditions favorable to disease development (Laville, 1986), it could cause significant grain yield losses (10-50%) and oil content decrease.

Growing resistant hybrids is the most effective measure for disease control. However, there are no completely resistant genotypes and the main challenge to the breeders represents searching for sources of resistance and introducing them into genotypes with valuable agronomic traits. Sources of resistance could be found in some wild species, first of all in some populations of *H. tuberosus* (Škorić et al., 2002). According to Deglene et al. (1999), sunflower resistance in breeding programs could be improved by using inbred lines, which have high values of general combining abilities. In sunflower breeding aimed at disease tolerance, artificial infection in controlled (laboratory) or uncontrolled (field) conditions is essential. There are a few methods of artificial infection and some authors use the least aggressive ones, which are closer to natural infection. Also, there are differences regarding a place of infection (Vear et al., 1997). Sunflower breeding programs in Croatia have a long tradition and have been carried out through scientific projects and programs in the framework of The Agricultural Institute Osijek (Vratarić and Sudarić, 2004; Mijić et al., 2004; Krizmanić et al., 2006). The main goal is the creation of new, superior hybrids, with a high grain yield (above 5 t/ha), oil content (above 50%), and high, stable oil yield. Special attention is given to creation lines with an emphasized tolerance to predominant pathogens. Sunflower

breeding with resistance/tolerance to main diseases is the best way to control them and represents the most ecologically acceptable way to do so (Fick and Miller, 1997; Miller and Fick, 1997; Škorić et al., 2002; Vratarić and Sudarić, 2004).

The aim of the investigation was to estimate the tolerance to the pathogen *D. helianthi* of a wide spectrum of inbred lines, including cytoplasmic male sterile (cms, A lines), male fertile (mf, B lines), restorers of fertility (rf, R lines) and two-way sterile hybrids or single cross (SC), by artificial infection method in the field. Inbred lines of good combining abilities for the most important agronomic traits (grain yield, oil content), which show the lowest level of susceptibility, will be considered as potential parents for hybrid development in the framework of the Agricultural Institute Osijek sunflower breeding program.

MATERIALS AND METHODS

The research was conducted during two consecutive years (2006 and 2007) at the experiment field of The Agricultural Institute Osijek (Croatia). Tested breeding material involved 19 different sunflower genotypes, 5 of which were cytoplasmatic male sterile (cms) inbred lines (L-301 A, L-271 A, L-G/04 A, L-205 A, L-101 A), four male fertile lines (L-302 B, L-14 B, L-190 B, L-272 B), 6 sterile single-crosses (female component for three-way hybrids, G/04 A x L-104 B, G/04 A x L-14 B, G/04 A x L-282 B, G/04 A x L-272 B, G/04 A x L-190 B, G/04 A x L-302 B), and four restorer-fertility lines (PI 12/99 R, O3G R, L-Š 89 R, O3 MR). Tested material was developed at the Agricultural Institute Osijek sunflower breeding program. Each genotype was sown in two 5-m long rows, in three replications. One row of each genotype represented the control, while the other was artificially infected. In each replication, 7 plants of each genotype were artificially infected in full button stage (R2, according to Schnieter and Miller, 1981). Sunflower stems were infected on 11th July 2006 and 15th July 2007, with fungal mycelium grown in the laboratory. Previously, during 2004 and 2005, the patogenicity was tested in a considerable number of strains in the location of Osijek, a location of large-scale sunflower production in Croatia. The most aggressive one was used for this investigation. Circular plug of mycelia was placed on a leaf stalk intercept (2-3 cm long) from one of mid-stem leaves. Infection spot was covered with a piece of wet cotton wool and aluminum foil to prevent mycelial dryness and create favorable micro-climate conditions for pathogen development. Susceptibility estimation was performed by weekly measurements of the length of lesions during three weeks after infection. Analysis of variance (ANOVA) and LSD test were processed by Statistical Analysis System for Windows software (SAS Institute, 2003).

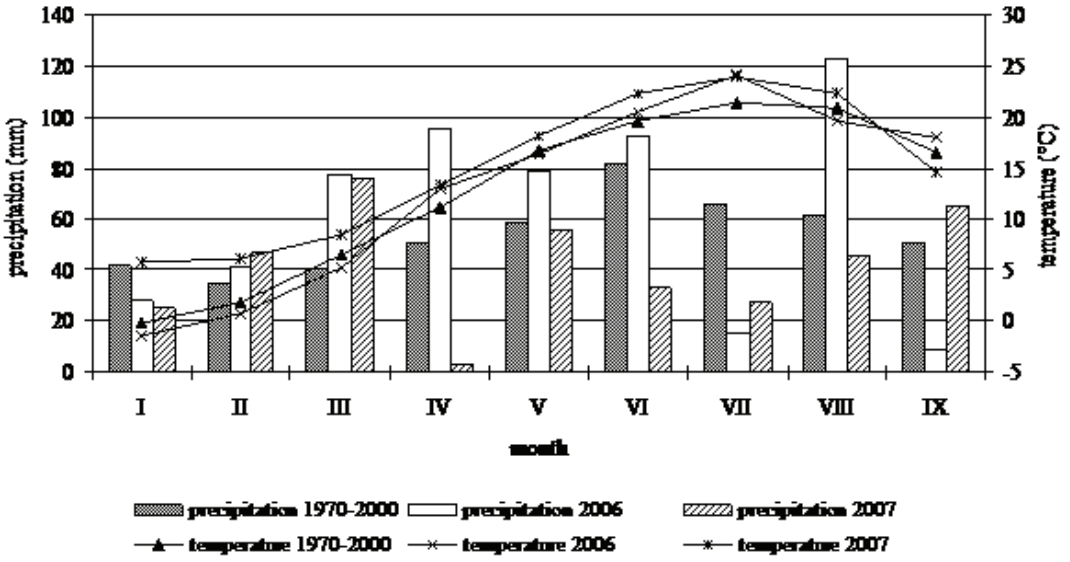

Fig. 1. Monthly air temperatures (°C) and precipitations (mm) for investigated years (2006-2007) and 30-year average (1970-2000), Osijek.

RESULTS AND DISCUSSION

During the first lesion measurement, artificial infection success was clearly visible. Most of the infected plants showed disease symptoms. Occurrence of symptoms was perceived clearer in the second and particularly in the third measurement in both years. Lesion length per measurement as well as the average length of lesions for both years is shown in Table 1. On average, the highest tolerance to the pathogen was shown in SC, then A lines, and B lines, while the lowest resistance recorded was in fertility restorer lines (R). The lowest average value was recorded for the single cross hybrid L-G04 A x L-14 B (2.48), and the highest average value for mf (B) line L-302 B (5.06). The lowest susceptibility to the pathogen corresponded to the cms lines L-101 A and L-205 A. From mf (B) lines, L-272 B and L-190 B were more tolerant. The most tolerant two-way hybrids to artificial infection in this investigation were L-G/04 x L-14 and L-G/04 x L-282. These results should be examined in a further investigation, particularly after developing hybrids from tolerant lines. Although the procedure of creating three-way hybrids is longer and more complex, some authors (Giriraj et al., 1988; Bochkovoy et al., 2000) give these hybrids a certain advantage regarding grain yield stability. Fertility restorers L-O3 M R and L-O3 G R showed the lowest susceptibility to infection (Table 1).

Table 1. Average lesion length (cm) of sunflower inbred lines after infection with *D. helianthi* at Osijek in 2006-2007.

No	Lines	Lesion length (cm)		
		2006	2007	Average
1	L- 271 A	3.94	5.87	4.91
2	L-G/04 A	3.72	5.23	4.48
3	L-301 A	1.84	6.53	4.19
4	L- 205 A	2.58	3.20	2.89
5	L-101 A	2.01	3.39	2.70
	Average	3.88	3.80	3.83
6	L-302 B	4.87	5.25	5.06
7	L-14 B	3.76	4.03	3.90
8	L-190 B	2.33	4.31	3.32
9	L-272 B	2.04	4.23	3.13
	Average	3.41	4.30	3.90
10	L-G/04 x L-104 SC	3.66	5.48	4.57
11	L-G/04 x L-272 SC	1.79	5.29	3.54
12	L-G/04 x L-190 SC	1.51	5.51	3.51
13	L-G/04 x L-302 SC	2.10	4.19	3.14
14	L-G/04 x L-282 SC	0.92	4.43	2.68
15	L-G/04 x L-14 SC	0.97	3.99	2.48
	Average	1.83	4.82	3.32
16	L- 12/99 R	3.52	5.59	4.56
17	L-Š 89 R	1.65	7.07	4.36
18	L-O3 G R	1.44	6.08	3.76
19	L-O3M R	2.22	4.97	3.59
	Average	2.90	5.24	4.10
	LSD 0.05	0.89	1.17	0.72

Legend: A – cytoplasmatic male sterile lines; B – male fertile lines; SC – single cross sterile hybrids; R – restorer of fertility

It is important to emphasize that, besides their genetic potential, the environment has a strong influence on genotype tolerance level. Regarding the fact that these results were obtained in field trials, all data should be observed through climate conditions during investigation (Fig 1). During a two month period (July, August) the amount of precipitation in a 30-year average recorded at the Agricultural Institute Osijek experimental field was 128.2 mm. In 2006, the same period of time recorded a little more than the 30-year average (134.9 mm), while in 2007 this amount was significantly lower (72.4 mm). Observing only July, the month when the artificial infection was carried out, the amount of precipitation was lower in 2006 (15.3 mm) in comparison with 2007 (27.4 mm) and the 30-year average (66.3 mm). In August 2006, this value was 122.6 mm, significantly above the 30-year average (61.9 mm) or the same month in 2007 (45 mm). However, artificial infection and measurements were, in both years investigated,

conducted during the second and the third ten day's period of July, when only 15.3 mm of precipitation (2006) was measured, which makes this period drought and unsuitable for artificial infection and pathogen development. In 2007, the rainfall in July was almost double (27.4 mm), but still under the 30-year average (66.3 mm). Air temperatures for 2006 (21.8 °C) in these two months were on average on the same level as the 30-year average (21.1 °C). In 2007, the two month average was 23.1 °C, which made that period more suitable for pathogen development.

Comparing these meteorological data with the results of lesion length for investigated sunflower lines given in Table 1, it could be concluded that precipitation and air temperatures in July are most important for artificial infection as well as for pathogen development. Regarding that fact, in 2007 all investigated lines have longer lesions in comparison with 2006. Also, it can be concluded that, in this investigation, the precipitation had a stronger influence than temperatures on artificial infection as well as on pathogen and disease development. It is known that years with lower air temperatures and a higher precipitation are extremely suitable for white head rot development (Vratarić and Sudarić, 2004; Jurković and Ćosić, 2004; Duvnjak et al., 2006), while higher temperatures and moisture are suitable for Stem canker development.

Although these results were obtained in two-year trials, they could be a good indicator and guideline in further sunflower breeding work related to disease resistance on *D. helianthi.* The research should be continued in following years, including new genotypes. Additionally, testing important agronomic traits in combination with resistance to this pathogen will give a more objective estimation of selecting material for new sunflower hybrid development.

REFERENCES

Bochkovoy, A.D., V.P. Brazhnic, N.I. Bochkaryov, and V.D. Savchenko. 2000. Three-way sunflower hybrids: promising directions of investigations. p. E99-E105. In: Proc. 15th Int. Sunflower Conf., 12-15 June, Toulouse. France.

Degener, J., A.E. Melchinger, and V. Hahn. 1999. Resistance in the leaf and stem of sunflower after infection with two isolates of *Phomopsis*. Plant Breed. 118:405-410.

Deglene, L., G. Alibert, P. Lesigne, D. Tourviell de Labrouhe, and A. Sarrfi. 1999. Inheritance of resistance to stem canker (*Phomopsis helianthi*) in sunflower. Plant Pathol. 48:559-563.

Duvnjak, T., M. Krizmanić, M. Vratarić, A. Mijić, A. Sudarić, I. Liović, M. Bilandžić, and K. Vrandečić. 2006. Sunflower inbred line testing on resistance to *Sclerotinia sclerotiorum.* p. 103-105. In: Proc. 12th Congr. Mediterranean Phytopathological Union. 11-15 June. Rhodes Island. Greece.

FAOSTAT, Database 2005 (www.faostat.fao.org).

Fick, G.N., and J.F. Miller. 1997. Sunflower breeding. p. 395-440. In: A.A. Schneiter (ed.) Sunflower Technology and Production. Agron. Monogr. 35. ASA. CSSA. SSSA. Madison, WI, USA.

Giriraj, K., Shanta, R. Hiremath, and K. Seenappa. 1988. Stability of sunflower genotypes for seed yield. p. 537-541. In Proc. 12th Int. Sunflower Conf., 25-29 July, Novi Sad, Yugoslavia.

Jurković, D., and J. Ćosić. 2004. Bolesti suncokreta. U Suncokret (*Helianthus annuus* L.). Urednik Marija Vratarić. Poljoprivredni institut Osijek p. 283-328.

Laville, J. 1986. Cahier technique Tournesol: maladies. CETIOM, Paris

Krizmanić, M., A. Mijić, I. Liović, M. Bilandzić, and T. Duvnjak. 2006. Sunflower breeding at the Agricultural Institute Osijek. Helia 29(44):153-159.

Mihaljčević, M. 1997. Promene sortimenta suncokreta kao posledica promena u populaciji parazita. Zbornik radova. Sveska 29, p. 555-568.

Mihaljčević, M., M. Muntanola-Cvetković, and M. Petrov. 1982. Further studies on the sunflower disease caused by *Diaporthe (Phomopsis) helianthi* and possibilities of breeding for resistance. p. 157-159. In: Proc. 10th Int. Sunflower Conf., Surfers Paradise, Australia.

Mijić, A., M. Vratarić, A. Sudarić, and T. Duvnjak. 2004. Trendovi u oplemenjivanju suncokreta u R. Hrvatskoj i svijetu. XXXIX znanstveni skup hrvatskih agronoma s međunarodnim sudjelovanjem. Opatija 17.-20.2.2004. Priopćenja, p. 175-178.

Miller, J.F., and G.N. Fick. 1997. The Genetics of Sunflower. p. 441-495. In: A.A. Schneiter (ed.) Sunflower Technology and Production. Agron. Monogr. 35. ASA. CSSA. SSSA. Madison. WI. USA

Muntanola-Cvetković, M., M. Mihaljčević, and M. Petrov. 1981. On the identity of the causative agent of a serious *Phomopsis – Diaporthe* disease in sunflower plant. Nova Hedwigia 34:417-435.

SAS Institute Inc. (2003). SAS for Windows (r) 9.1. Cary, NC. USA.

Schneiter, A.A., and J.F. Miller. 1981. Description of Sunflower Growth Stages. Crop Sci. 21:901-903.

Škorić, D., R. Marinković, S. Jocić, D. Jovanović, and N. Hladni. 2002. Dostignuća i dalji pravci u oplemenjivanju suncokreta i izbor hibrida za setvu u 2002. godini. Zbornik radova Naučnog instituta za ratarstvo i povrtlarstvo Novi Sad, 36:147- 159.

Vear, F., M. Garreyn, and D. Tourviell de Labrouhe. 1997. Inheritance of resistance to phomopsis (*Diaporthe helianthi*) in sunflower. Plant Breed. 116:277-281.

Vratarić, M., and A. Sudarić. 2004. Oplemenjivanje i genetika suncokreta. U Suncokret (*Helianthus annuus* L.). Urednik Marija Vratarić. Poljoprivredni institut Osijek, p. 69-162.

Study of the expression of shikimate dehydrogenase activity in sunflower genotypes treated with *Sclerotinia sclerotiorum*

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ABSTRACT

The expression of shikimate dehydrogenase in cotyledons of five sunflower inbred lines treated with *Sclerotinia sclerotiorum* was compared with exogenous application of synthetic oxalic acid. Normally, shikimate dehydrogenase becomes enzymatically active in sunflower at seed germination stage, and it reaches its maximum during the cotyledon stage, gradually decreases and disappears after four leaf stage. We found that shikimate dehydrogenase activity was very faint in control plant protein extract whereas its intensity greatly increased in samples derived from seedlings inoculated with *S. sclerotiorum* as well as with synthetic OXA at the same stage. The expression of shikimate dehydrogenase at the first phase of growth may serve as a tool for rapid screening and selection of resistant genotypes of sunflower to *S. sclerotiorum.* Some agronomy parameters in terms of plant dry and fresh weight and the total chlorophyll concentration were assessed for both treatments compared with their untreated controls. Exogenous oxalic acid treatment caused more deleterious effects in comparison with its endogenous production of the pathogen, considering stem rot and eliciting photosynthesis reduction. The excessive toxicity of exogenous treatment suggests that *S. sclerotiorum* infection triggers a more complex metabolic pathway involvings oxalic acid secreted by the pathogen.

Key words: dehydrogenase activity – *Helianthus annuus* – resistant genotypes – *Sclerotinia sclerotiorum* – screening – shikimate.

INTRODUCTION

Sclerotinia root, stem, and head rot are major diseases of sunflower caused by the pertotrophic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary (Rönicke et al., 2005). The general inability of economically important crops to develop germplasm resistant to this pathogen has focused attention on the need for a more detailed understanding of the pathogenic factors involved in disease development (Cessna et al., 2000). Oxalic acid secretion by *Sclerotinia* appears to be an essential determinant of its pathogenicity (Maxwell and Lumsden, 1970; Noyes and Hancock, 1981; Marciano et al., 1983; Godoy et al., 1990; Dutton and Evans, 1996; Zhou and Boland, 1999). Evidence for such involvement is based on the recovery of millimolar concentrations of oxalate from infected tissues (Bateman and Beer, 1965; Maxwell and Lumsden, 1970; Marciano et al., 1983; Godoy et al., 1990) and from the manual injection of oxalate, or of culture filtrate containing oxalate, into plants and observation of the development of *Sclerotinia* disease-like symptoms independent of the pathogen (Bateman and Beer, 1965; Noyes and Hancock, 1981).

Speculation regarding the mechanism or mechanisms by which oxalate secretion might enhance *Sclerotinia* virulence currently centers on three modes of action (reviewed in Dutton and Evans, 1996). First, because several of the fungal enzymes secreted during invasion of plant tissues (e.g., polygalacturonase) have maximal activities at low pH, various researchers have postulated that oxalate might aid *Sclerotinia* virulence by shifting the apoplastic pH to a value better suited for enzymatic degradation of plant cell walls (Bateman and Beer, 1965). Second, because oxalate may be directly toxic to host plants, presumably because of its acidity, the secretion of oxalate has been suggested to weaken the plant, thereby facilitating invasion (Noyes and Hancock, 1981). Finally, chelation of cell wall Ca^{2+} by the oxalate anion has been proposed both to compromise the function of Ca^{2+}-dependent defense responses and to weaken the plant cell wall (Bateman and Beer, 1965). Although each of these hypotheses has its logical appeal, evidence supporting them is incomplete, and arguments against their validity have also been made (Dutton and Evans, 1996).

Shikimate dehydrogenase (EC 1.1.1.25) (SKDH) is an important biochemical marker produced by plants for investigation of *S. sclerotiorum* infection that catalyzes the fourth step in the shikimate pathway, which is essential for biosynthesis of aromatic amino acids and aromatic compounds. The increase in SKDH activity occurring after *Sclerotinia* infection affects the biosynthesis of shikimic acid, which is involved in the synthesis of lignin for cell walls (Buiatti, 1993; Carrera and Poverene, 1995) and is considered to be the most interesting component in relation to the plant resistance to *S. sclerotiorum* (Quillet, 1990).

The aim of this study was to determine the effects of OXA treatment in *Helianthus annuus* L. either when OXA was endogenously produced by *S. sclerotiorum* or, alternatively, when it was exogenously treated as synthetic moiety. Study of the expression of SKDH activity may help to develop a fast and reliable screening technique in breeding sunflowers for resistance to *S. sclerotiorum*.

MATERIALS AND METHODS

Fungal and plant material

Black sclerotia of *S. sclerotiorum* collected from stems of infected plants were germinated and grown on potato dextrose agar (PDA) at 25 °C. After several passages on PDA and controlling the proper hyphae by observation under optical microscope 400 X, sclerotia were subcultured on PDA (Becton Dickinson, Sparks, MD, USA) under light (24 h/day). After 3 days, 0.2 cm agar plugs were removed with a sterile cork borer from the leading edge of the mycelia and were subcultured on PDA agar plate, 0.5 cm agar plugs were removed from the leading edge of the second two-day old mycelia and used for inoculation.

Five inbred lines of sunflower of different origins were used in the experiments: AC 4122 and C are maintainer inbred lines, developed at University of Udine from an Italian open pollinated population ALA, HA 89 is a maintainer inbred line and HA 410 (Reg. no. GP-227) and HA 411 (Reg.no.GP-228) are inbred lines released by USDA-ARS, Fargo, ND, North Dakota. AC 4122 and HA410 are resistant inbred lines, C and HA 89 are susceptible inbred lines, and HA 411 is an intermediate inbred line. Seeds of five genotypes were surface sterilized as described by Burrus et al. (1991) and germinated in sterile test tubes (130 x 25 mm) on a solid MS medium (Murashige and Skoog, 1962).

Plugs of PDA prepared as described earlier were placed on the leaves which were wounded slightly. Leaves of uninoculated, control plants were treated similarly with PDA agar plugs without the mycelia. The inoculated parts of plants were then washed by sterilized water and transferred to Hoagland solution (H2395, Sigma Chemical Co. prepared according to manufacture's directions, autoclaved and stored at room temperature) and maintained at 20-25°C, relative humidity about 40-50% and light intensity about 500 mM. m^{-2} s^{-1}.

Preparation of synthetic oxalic acid

A stock solution of 1 M oxalic acid (Sigma Chemical Co.) was prepared, then it was diluted to obtain the same toxin concentration of culture filtrate (toxin concentration was estimated using Oxalate kit 591C followed by spectrophotometer assessment).

The recovery of vegetal extraction

Five days after exposure to *S. sclerotiorum* and synthetic OXA, the plant tissue above the cotyledons (also from untreated control plants) was collected and homogenized in a mortar and placed in a sealed tube containing buffer 50 mM Tris-HCl, (pH 7.4), 0.25 M sucrose, 1 mM EDTA (ethylenediaminetetraacetic acid), 1 mM PMSF (phenylmethanesulfonyl fluoride), 2.5% v/v β-mercaptoethanol. After homogenization and centrifugation at 2000 *g for 5 min, the supernatant was used for OXA determination, total protein determination, and SKDH activity assay.

Oxalic acid measurement

Ten µl of extracted plant material as previously described was used for measuring toxin (OXA) concentration by oxalate kit 591 C followed by spectrophotometer assessment at 590 nm wave lengths.

Determination of total soluble protein and Shikimate dehydrogenase activity assays

The extracted plant material was employed to determine the total protein content, using the Bio-Rad protein assay kit with BSA as standard (Bradford, 1976) followed by spectrophotometer assessment at 595 nm wave length.

Native-PAGE

Native Polyacrylamide Gel Electrophoresis was performed using 12% (W/V) polyacrylamide slab gel in 0.2 M Tris, 2 mM EDTA and 0.15 M boric acid (pH 8.5) as electrode buffer (Guries and Ledig, 1978). Staining of SKDH was done by fixing for an hour in buffer solution containing tetrazolium salt as described by Tanksley and Rick (1980).

Experimental design and statistical analysis

The treatments corresponded to five genotypes inoculated with *S. sclerotiorum* culture filtrate, treated with synthetic OXA and the controls, which were grown in hydroponics with Hoagland solution under similar conditions. The analyses of total fresh weight, total dry matter per plant at the end of the experiment, OXA concentration (mM), and chlorophyll concentration (mg m^{-2}) 5 days after treating and the expression of SKDH were carried 48 h after treating with either *S. sclerotiorum* or exogenous OXA.

The experiment was carried out following a bifactorial completely randomized block design with three replicates and four plants for each replication. The first factor, genotype, was constituted by the five inbred lines, and the second factor, toxin treatment, was constituted by endogenous OXA produced by *S. sclerotiorum* and exogenously applied synthetic OXA. Statistical analyses of triplicate determinations of OXA contents and enzymatic activity of SKDH from five genotypes were subjected to Analysis of Variance. Significant differences were expressed as $P<0.01$, and the least significant difference procedure was used to compare means of treatments. Correlation coefficients and regression analysis were calculated between the variables having significant differences between genotype means.

RESULTS AND DISCUSSION

The effects of two treatments, inoculation with *S. sclerotiorum* and exogenous oxalic acid, on plant growth were compared by measuring fresh weight and dry weight (plants were dried at 60 ºC in an oven for 3 days), which were the only growth parameters that could be calculated in early growth phase. Significant differences were recorded between genotypes for dry and fresh weight. In samples treated with culture filtrate, the fresh weights (considered as a percentage of controls) were significantly higher in HA 410, intermediate in HA 411 and AC 4122, and low in C and HA 89. These differences revealed an individual variability of responses to toxin penetrated into the cells, which confirms the polygenic nature of this disease (Mestries et al., 1998). The dry matter of these samples did not show significant differences between genotypes (Table 1). These data indirectly suggest that *S. sclerotiorum* manipulates the metabolism of host-derived carbohydrates and consequently increases in cell water content.

In the cases of samples treated with synthetic OXA, fresh weight of HA 411, AC 4122 and C had higher values whereas other genotypes followed them with lesser significant differences. Concerning dry matter, there were no significant differences except in resistant line AC 4122 with the lowest dry weight. These results provide an alternative explanation for oxalate-induced wilting. It seems that synthetic OXA induces an equal effect of destruction on all resistant or susceptible genotypes. This firstly causes a great reduction in plant growth, then self-reconstruction of the plant happens and it continues its growth.

Table 1. Growth characters of five sunflower genotypes analysed 5 days after inoculation with *S. sclerotiorum* and treated with synthetic OXA

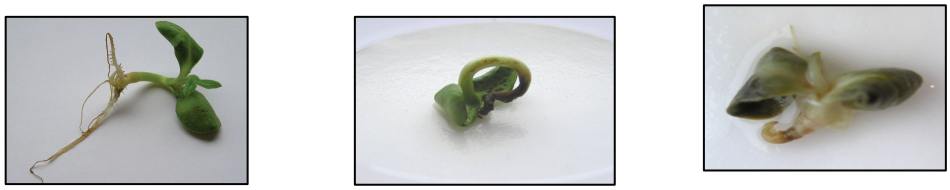
Genotypes	Synthetic OXA		*S. sclerotiorum* inoculation	
	Dry Weight Plant (%)[1]	Fresh Plant Weight (%)[1]	Dry Weight Plant (%)[1]	Fresh Plant Weight (%)[1]
AC 4122	58.6 b[2]	43.91 a	65.6 a	36.8 b
C	76.4 a	39.3 ab	64.9 a	27.7 c
HA 89	71.1 a	30.9 c	64.4 a	28.6 c
HA 410	71.1 a	36.9 bc	71.4 b	47.8 a
HA 411	75.5 a	43.4 a	68.4 ab	36.2 b

[1]Values are reported as percentage of the controls,

[2]Means followed by the same letter are not significantly different at 1% level as indicated by Duncan's Multiple Range Test.

The toxic metabolite of pathogen causes a decrease in chlorophyll (chl) concentration and this reduction is clearly associated with other symptoms of phytopathogenicity, i.e. stem rot. Chl

concentration data will provide information on a plant's photosynthetic potential (Raymond et al., 2004). The effect of both treatments on plant metabolism was revealed as a reduction in Chl concentration (data not shown). This phenomenon for the samples treated with synthetic OXA was not accompanied by any signs of stem rot and basal stalk rot, which implies the different nature and effect of OXA on the plant (Fig 1).



a **b** **c**

Fig. 1. Comparison of growth in plants a) uninoculated control, b) inoculated with *S. sclerotiorum* and c) treated with synthetic OXA.

The intracellular OXA content in sunflower seedlings was determined five days after inoculation with *S. sclerotiorum* or synthetic oxalic acid treatment to verify whether the metabolic response to OXA could be correlated with disease resistance.

As reported in Fig. 2, in uninoculated lines C (1.10 mM) and HA 89 (1.21 mM) a higher OXA content was observed, which demonstrates that they are more susceptible to fungal disease as compared to the other lines. This confirms previous observations by Tahmasebi Enferadi et al. (1998 b) about the different thresholds of OXA concentration between different genotypes.

Concerning samples inoculated with *S. sclerotiorum*, OXA concentration values were the highest in susceptible HA 89 (1.81 mM) and C (2.6 mM) when compared to their untreated controls. On the contrary, it was observed that OXA intracellular concentration in samples treated with synthetic acid was lowest in HA 89 (2 mM) whereas AC 4122 had the highest content.

Since OXA concentration increases in pathogen-infected plants, our data demonstrate that the more resistant the plants, the more they were able to control catabolism of this acid, as shown in HA 410 and AC 4122. This is probably due to an intercellular mechanism which inhibits abnormal increases in their pH. Therefore, specific macromolecules are produced by a pathogen that can be recognized by the plant (Buiatti, 1993) and lead to the activation of a host defense response. These signals were absent after synthetic OXA treatment, causing the plant to be unable to manage OXA.

Other studies showed that phenolic compounds play an important role in plant defense responses against pest and pathogens (Nicholson and Hammerschmidt, 1992). In sunflower the induction and accumulation of phenolic compounds, their deposition on cell walls and lignification is a well-characterized mechanism of disease resistance against *S. sclerotiorum* (Prats et al., 2003). A higher content of phenolic compounds in resistant varieties was observed as compared to susceptible ones (Prats et al., 2003; Rodríguez et al., 2004). Conceivably, resistant plants had higher activity levels of phenylalanine ammonia–lyase (PAL), which provides the biosynthesis of important phenolic derivatives such as lignin.

Similar to phenolic compounds and PAL, shikimic acid and the related enzymatic activity of SKDH are used in order to find out a biochemical paradigm, which provides a clear correlation with disease resistant genotypes. SKDH is an intermediate step in aromatic amino acid biosynthetic pathway, essential to lignin production, and is considered as a resistance mechanism against *S. sclerotiorum* related to its chemical and/or physical cell barriers.

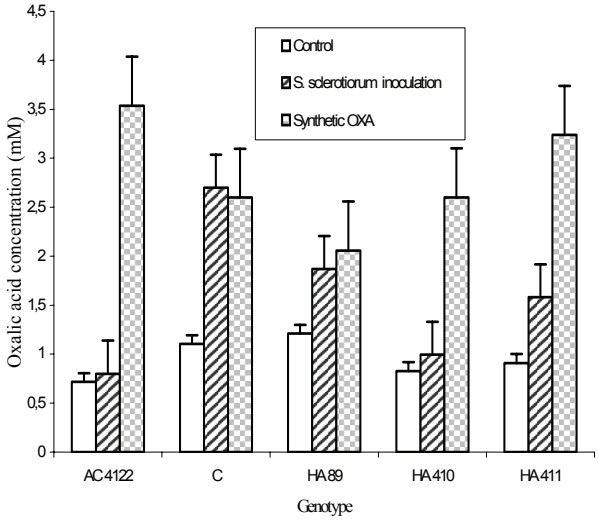


Fig. 2. OXA concentration of different sunflower genotypes 5 days after inoculation with *S. sclerotiorum* and synthetic OXA and untreated plants (control). Bars represent L.S.D. for $P < 0.01$

SKDH that becomes enzymatically active in sunflower at seed germination stage, and it reaches its maximum during the cotyledon stage, gradually decreases and stops after 4-leaf stage as reported by Diaz et al. (1997). SKDH reactivates treating the plant either with pathogen or synthetic OXA. Parental inbred lines have a single band with identical mobility (Carrera and Poverene, 1995), indicating the presence of the same allele in all genotypes, as reported by Ledoux (1992). Enzyme SKDH has a monomeric structure, encoded by a single gene and a single locus with two different co-dominant alleles in heterozygous plants, *skdh*-a and *skdh*-b, with the molecular weight of 64.5 kDa and 58.9 kDa, respectively (Tahmasebi et al., 1998a). The increase in SKDH activity for both homozygous and heterozygous individuals following the attack of *S. sclerotiorum* is accompanied by the expression of only skdh-b. The lack of the expression of skdh-a in homozygous individuals confirms the hypothesis by which skdh-a is considered a null allele, as described by Goodman et al. (1980). Both alleles have most likely the same domains with a few changes in the variable regions, which concerns regions interacting with OXA. In Fig. 3, the domain family of SKDH is shown. It is suggested that the interaction between OXA and reactivation of skdh-b relates to the third domain, Shikomate_DH. This domain involves the biosynthesis of aromatic amino acids and is related to the mechanism of resistance. It seems that other domains have a structural role.

Fig. 3. Domain pattern of SKDH along its polypeptide

A study of the expression of SKDH on Native-PAGE 48 h after treatment of the studied inbred lines demonstrated that only a single allele, *skdh*-b, expressed and its mobility was identified. Conceivably, to data dealing with the enzymatic activity dosage, SKDH was very faint in control plant protein extract whereas its intensity greatly increased in samples derived from seedlings inoculated with *S. sclerotiorum* as well as with synthetic OXA (Fig 4.)

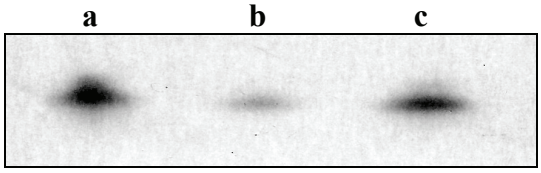


Fig. 4. Expression of skdh-b at the end of cotyledons in homozygous lines of sunflower on Native-PAGE 48 h after treating with a) Synthetic OXA, b) untreated plant, control, and c) *S. sclerotiorum* inoculation.

CONCLUSIONS

In conclusion, the differences observed between symptoms generated by OXA produced by pathogen and OXA originating from a synthetic source can be related to the different nature of biochemical pathway elicited by each treatment, both in resistant and in susceptible inbred lines. Subsequently, this prevents the use of synthetic OXA instead of direct inoculation of plants in rapid screening methods for identification of genotypes resistant to *S. sclerotiorum*. Other advantages of measuring SKDH activity as a rapid and reliable method of screening, are the early discrimination of resistant genotypes in the first growth stage, at the laboratory, on many individuals (since *S. sclerotiorum* is of a polygenic nature, it needs to provide a resistant mass individual) and its cost effectiveness.

Furthermore, our results indicate that SKDH may be a promising biochemical marker that could be used in breeding programs to discriminate between sunflower genotypes resistant and susceptible to *Sclerotinia* infection.

Although different disease resistance mechanisms can be activated simultaneously during defense response, SKDH levels could be directly evaluated to identify resistant lines or, possibly, related to other molecular markers such as total content of phenolic compounds and PAL activity.

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REFERENCES

Bateman, D.F., and S.V. Beer. 1965. Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *Sclerotiorum rolfsii*. Phytopathology 55:204–211.

Buiatti, M. 1993. Basi biochimiche della resistenza a patogeni e loro controllo genetico. p. 65-78. In: P. Crino, M. Buiatti, and A. Sonnino (eds), Strategie di miglioramento genetico per le resistenze a patogeni. Ed agricole, Bologna, Italy.

Carrera, A., and M. Poverene. 1995. Isozyme variation in *Helianthus petiolaris* and *H. annuus*. Euphytica 81:251-257.

Cessna, S.G., V.E Sears, M.B. Dickman, and P.S. Low. 2000. Oxalic acid, a pathogenicity factor for *Sclerotinia sclerotiorum*, suppresses the oxidative burst of the host plant. Plant Cell 12:2191-2200.

Dìaz, J., A. Rosbrcelò, and M.F. de Cáceres. 1997. Changes in shikimate dehydrogenase and the end products of the shikimate pathway, chlorogenic acid and lignin's during the early development to seedlings of *Capsicum annumm* L. New Phytol. 136:183-188.

Dutton, M.V., and C.S. Evans. 1996. Oxalate production by fungi: Its role in pathogenicity and ecology in the soil environment. Can. J. Microbiol. 42:881-895.

Godoy, G., J.R. Steadman, M.B. Dickman, and R. Dam. 1990. Use of mutants to demonstrate the role of oxalic acid in pathogenicity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris*. Physiol. Mol. Plant Pathol. 37:179-191.

Goodman, M.M., C.W. Stuber, C.N. Lee, and F.M. Johnson. 1980. Genetic control of malate dehydro genase isozymes in maize. Genetics 94:153-168.

Guries, R.P. and F. Ledig. 1978. Inheritance of some polymorphic isoenzymes in pitch pine (*Pinus rigida* Mill). Heredity 40:27-32.

Ledoux, A.1992. Utilization des marqueurs isoenzymatiques dans une selection genealogique chez le tournesol. Stage realizé à la Station d'Ameliorement des plantes de l'INRA de Clermond-Ferrand, p. 30-32.

Marciano, P., P. DiLenna, and P. Magro. 1983. Oxalic acid, cell wall degrading enzymes and pH in pathogenesis and their significance in the virulence of two *Sclerotinia sclerotiorum* isolates on sunflower. Physiol. Plant Pathol. 22:339-345.

Maxwell, D.P., and R.D. Lumsden. 1970. Oxalic acid production by *Sclerotinia sclerotiorum* in infected bean and in culture. Phytopathology 60:1395-1398.

Nicholson, R.L. and R. Hammerschmidt. 1992. Phenolic compounds and their role in disease resistance. Annu Rev Phytopathol. 30:369-389.

Noyes, R.D., and J.G. Hancock. 1981. Role of oxalic acid in the sclerotinia wilt of sunflower. Physiol. Plant Pathol. 18, 123–132.

Prats, E., M.E. Bazzalo, A. Leon, and J.V. Jorrin. 2003. Accumulation of soluble phenolic compounds in sunflower capitula correlates with resistance to *Sclerotinia sclerotiorum*. Euphytica 132:321-329.

Quillet, M.C. Etude du polymorphisme enzymatique et proteique chez le tournesol, application à la charactérisation de ligneés. Stage realizé à la Station d'Ameliorement des plantes de l'INRA de Clermond-Ferrand, 1990, p. 30-33.

Raymond, H.E., J.E. McMurtrey, A.E.P. Williams, and L.A. Corp. 2004. Spectral characteristics of leafy spurge (*Euphorbia esula*) leaves and flower bracts. Weed Sci. 52:492-497.

Rodríguez, M.A.**,** N. Venedikian**,** M.E. Bazzalo**,** and A. Godeas. 2004. Histopathology of *Sclerotinia sclerotiorum* attack on flower parts of *Helianthus annuus* heads in tolerant and susceptible varieties. Mycopathologia 157:291-302.

Rönicke, S.,V. Hahn, A. Vogler, and W. Friedt. 2005. Quantitative Trait Loci Analysis of Resistance to *Sclerotinia sclerotiorum* in Sunflower. Phytopathology 95:834–839.

Steven, H.D. 2000. pH regulation of gene expression in fungi. Fungal Genet. Biol. 29:61-71.

Tahmasebi Enferadi, S., G.P. Vannozzi, D. Gómez-Sánchez, and M. Baldini. 1998a. Results of screening sunflower for resistance to toxic metabolites produced by *Sclerotinia sclerotiorum* (Lib) de Bary and prospects for the use of biochemical markers in breeding. Agr. Med. 128:47-58.

Tahmasebi Enferadi, S., Gomez-Sanchez, D., Baldini, M. and G.P. Vannozzi. 1998b. Effect of *Sclerotinia sclerotiorum* (Lib) de Bary culture filtrate on sunflower characters, oxalic acid content and shikimate dehydrogenase activity. Helia 28:81-96.

Tanksley, S.D., and C.M. Rick. 1980. Isozymic gene linkage map of the tomato: applications in genetics and breeding. Theor. Appl. Genet. 57:161-170.

Zhou, T., and G.J. Boland. 1999. Mycelial growth and production of oxalic acid by virulent and hypovirulent isolates of *Sclerotinia sclerotiorum*. Can. J. Plant Pathol. 21:93–99.

Molecular changes in downy mildew-infected sunflower triggered by resistance inducers

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ABSTRACT

Benzothiadiazole (BTH), a plant defense activator, has recently been found to restrict downy mildew development in sunflower. To elucidate the background of this phenomenon, a research programme was started and some of our preliminary results are reported in this paper. The gene expressions of glutation S-tranferase (GST), defensin (PDF) and catalase (CAT) were the subject of investigation using compatible, incompatible and partially resistant sunflower – *Plasmopara halstedii* interactions, respectively. The accumulation of all three gene transcripts were found to be increased in the susceptible sunflower genotype following BTH treatment. Furthermore, in case of the resistant sunflower, HA 335, BTH enhanced GST and PDF accumulation, whereas with the partially resistant RHA 340 the results were ambiguous. It is hoped that our findings may contribute to a better understanding of the plant's own defense system triggered by chemical inducers.

Key words: benzothiadiazole – catalase – defensin – glutathion S-transferase – *Plasmopara halstedii* – sunflower.

INTRODUCTION

Although *Plasmopara halstedii* can be effectively controlled by using genetic resistant plants and seed treatment with fungicides, protection can be hindered by the genetic variability of the fungus (Albourie et al., 1998; Gulya, 2007). Thus, besides the traditional control strategies, there was a need to look for alternative methods to provide effective disease control. One solution can be the use of systemic induced resistance, i.e. the activation of the defense system of plants.

The plant activator BTH (benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester) has already been shown to induce activated resistance in many crops against a broad spectrum of diseases (Cohen et al., 1994; Kogel et al., 1994; Pajot et al., 2001). BTH appears to be able to restrict downy mildew symptoms in sunflower under greenhouse conditions (Bán et al., 2004). Microscopic observations show that BTH treatment significantly decreased the development of fungal structures associated with cell necrosis and H_2O_2 accumulation in the BTH-treated susceptible sunflower hypocotyls.

Glutathione S-transferase (GST) has a well defined role in plant detoxification reactions. It is capable of catalyzing the binding of various xenobiotics, like pathogens. Various abiotic stressors are the inducers of GST activity in plants (Dean et al., 1990). GST is also considered one of the antioxidative enzymes, because it plays an important role in the protection against oxidative membrane damage and necrotic disease symptoms. Enhanced GST activity has been found in plants after pathogen infection, for example in barley plants infected by powdery mildew (El-Zahaby et al., 1995), and tobacco plants infected by TMV (Fodor et al., 1997).

To protect themselves against pathogenic attacks, plants evolve diverse strategies, for example the synthesis of antimicrobial peptides, like defensin. Defensin is a small, cysteine-rich antimicrobial peptide, existing in a wide range of plants and animals. Urdangarin et al. (2000) described full length sunflower cDNA from *Helianthus annuus* flowers encoding for defensin, and the authors supposed there was a relationship between enhanced expression of a defensin gene and decreased susceptibility to *Sclerotinia sclerotiorum*. Solis et al. (2006) isolated a defensin gene from *Lepidium meyenii*, having activity against *Phytophthora infestans*.

Catalase is one of the main antioxidant enzymes; it catalyzes the dismutation of H_2O_2 into water and dioxygen. This enzyme is located in peroxisomes and glyoxisomes. Catalase activity is affected by abiotic stressors, like boron (Karabal et al., 2003), light and chilling (Gechev at al., 2003), and acid rain (Gabara et al., 2003). In sunflower, catalase activity was increased by UV-B radiation (Costa et al., 2002) and cadmium treatment (Azpilicueta et al., 2007). Niebel et al. (1995) demonstrated induction of catalase in potato upon nematode and bacterial infection as well. Several plants have multiple CAT isoenzymes. For

example, in sunflower at least eight isoforms (CAT1-CAT8) have been described (Azpilicueta et al., 2007).

MATERIALS AND METHODS

The USDA sunflower inbred lines RHA 274, RHA 340 and HA 335, as well as *Plasmopara halstedii* pathotype 700 were used to get one compatible, and two incompatible combinations. While HA 335 is characterized by total resistance, RHA 340 exhibits HLI (hypocotyl-limited) resistant type (Virányi and Gulya, 1996).

Pre-germinated seeds were soaked in an aqueous solution of BTH (160 mg/L) for at least 6 hours (first day), followed by their inoculation with *P. halstedii* sporangia (50000 sporangia/ml) using the whole seedling inoculation technique (Cohen and Sackston, 1973). Germlings were subsequently planted into pots filled with a commercial soil mixture and grown in the greenhouse (18/24ºC, 60 % RH, 16h light) for 3 weeks.

Samples were taken 3, 9, 13, 16 days after infection (dpi). The whole seedlings were frozen in liquid nitrogen and ground with mortar and pestle. Total RNAs were extracted using the Qiagen Plant Mini kit, and then the extracted RNA treated with RNase inhibitor to protect the extracted RNA and with DNase I to remove genomic DNA contamination. The extracted RNAs were measured with spectrophotometer and the RNA concentration of 1µg/µl adjusted. One µg of RNA was reverse transcribed using iScript cDNA Synthesis Kit (Bio-Rad).

Primers for PCR amplifications were applied according to Radwan et al. (2005) and Azpilicueta et al. (2007) as shown in Table 1. Twenty-five µl of the PCR reaction mixture contained 1µl RNA, 1 unit of Taq DNA polymerase (Fermentas), 2.5 µl 10X Taq polymerase buffer, 1 µl 2.5mM dNTP mix, 1.5 µl 25mM $MgCl_2$, 2.5 µl 5 µM primers and 13.8 µl PCR water. PCR reactions were performed using a Gene Amp PCR System 2700 PCR machine. The amplification program included an initial step at 94°C for 3 min and 25-32 cycles (Ha-EF1a: 25; Ha-GST: 26; Ha-PDF: 30; CATA2: 31) of 15 sec at 94 °C, 15 sec at Tm °C (Ha-EF1: 58; Ha-GST, Ha-PDF: 61; CATA2: 50), 20sec at 72°C.

The PCR products were electrophorized through 1% agarose gel, visualized with ethidium bromide and photographed in a Molecular Imager Gel Doc system (Bio-Rad). The signals from gels were quantified using a Quantity One program with molecular mass ruler (Bio-Rad), and normalized over the signals from Ha-EF1α.

Table 1. Primer sequences and accession numbers used in this study

Gene[1]	Primer sequences	Accession number
Ha-EF-1α	Forward 5'-AGGCGAGGTATGATGAAATTGTCA-3' Reverse 5'-GTCTCTTGGGCTCATTGATTTGGT-3'	AAM19764
Ha-GST	Forward 5'-CCTCAGGATGCTTACGAGAAGG-3' Reverse 5'-GCAGAAATATCAACCAGGTTGATG-3'	AY667502
Ha-PDF	Forward 5'-ATGGCCAAAATTTCAGTTGCTTTCA-3' Reverse 5'-AAGACTTGCACTGGTCATCACAG-3'	AF364865
CATA2	Forward 5'-TTCCCGCTTGAATGTGAAG-3' Reverse 5'-CCGATTACATAAACCCATCATC3'	AF243517

[1]Ha-EF-1a: constitutive elongation factor 1a, Ha-GST: glutathione S-transferase, Ha-PDF: defensin, CATA2: catalase isoenzymes.

RESULTS

In general, Ha-GST transcript accumulation was higher in the untreated resistant sunflowers than in the susceptible ones. At 3 and 9 dpi the highest transcript accumulation was detected in the HA 335 plants. At 13 and 16 dpi, however, this accumulation was higher in the 'HLI resistant' RHA 340 plants as compared to HA 335. The BTH treatment increased Ha-GST transcript level in both the susceptible and totally resistant plants throughout the experiment. The effect of BTH treatment on this transcript accumulation in the HLI resistant plant was contradictory, because the treatment increased the transcript accumulation at 3 and 16 dpi, but appeared to reduce it at 13 dpi (Fig1).

HA-PDF transcript accumulation was found to be higher in the resistant sunflower lines than in the susceptible one, similar to the HA-GST transcript accumulation. The effect of BTH treatment on PDF activity was detectable in both the susceptible and totally resistant sunflowers. In case of the 'HLI resistant plants, Ha-PDF transcript accumulation was increased by BTH treatment on the second and the

last sampling days only. Among the untreated plants, the totally resistant plants showed Ha-PDF transcript accumulation at 3 dpi, whereas in all the BTH treated plants this transcript accumulation could be detected on the first sampling day. In the second sampling day the increase in transcript accumulation was observed in all plants examined, and there were no differences between the two resistant genotypes. At 13 and 16 dpi the maximum accumulation was evident in the 'HLI resistant' plants. It is interesting to note that there were no differences detected between the untreated 'HLI resistant' and the BTH-treated susceptible plants at 9, 13 dpi (Fig. 1).

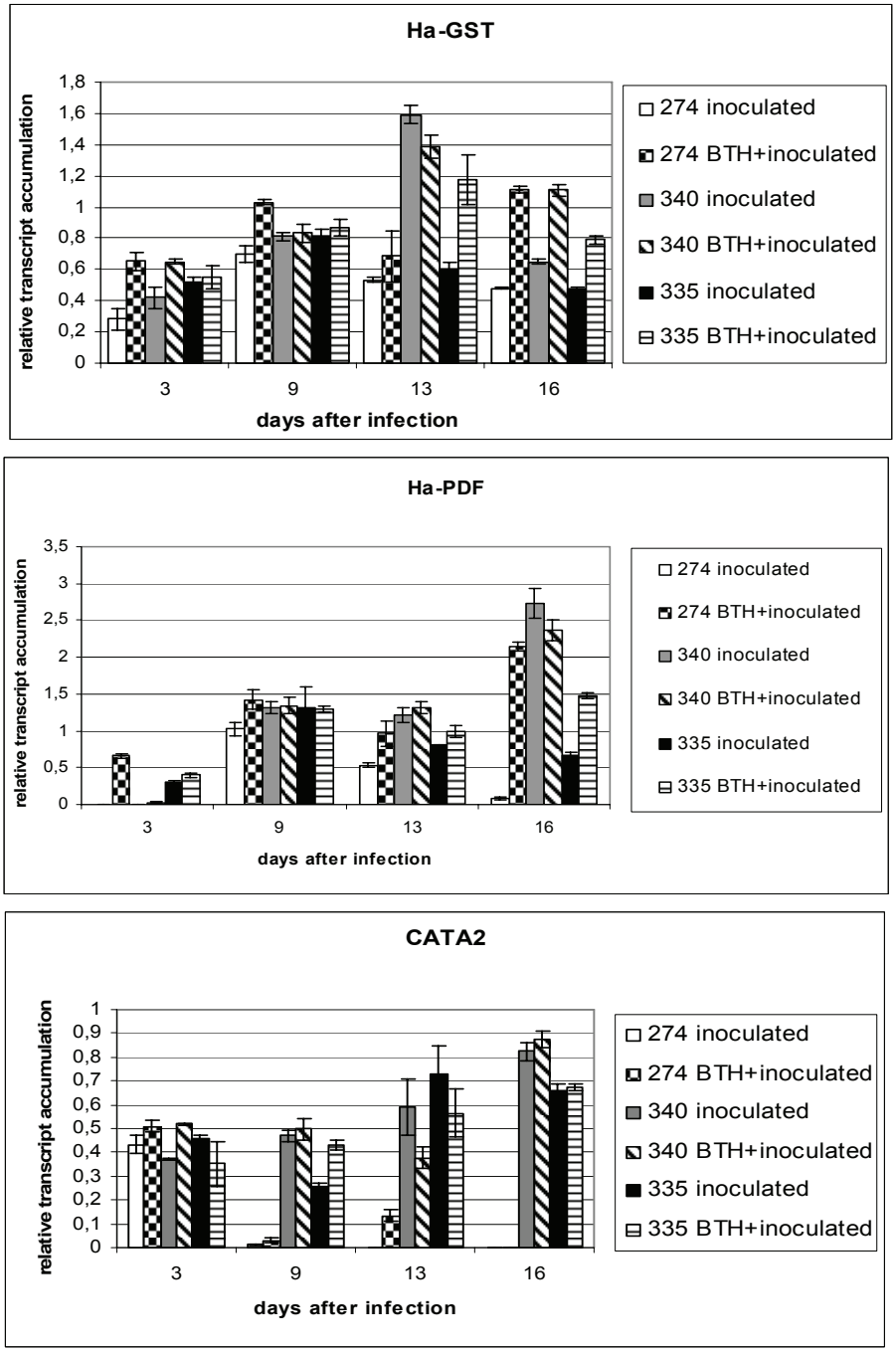

Fig. 1. Accumulation of gene transcripts in sunflower plants after BTH treatment and *Plasmopara halstedii* inoculation. 1. glutathione S-transferase (Ha-GST); 2. defensin (Ha-PDF) and 3. catalase (CATA2) gene expression in a susceptible (RHA 274), partially (HLI) resistant (RHA 340) and totally resistant (HA 335) sunflower line. Each value represents three replicates (±S.D.)

As for catalase activity, both resistant sunflowers exhibited higher CATA2 transcript level, than did the susceptible one, except for 3 dpi. After the first sampling day, catalase activity was not detectable in the untreated susceptible sunflower plants, but BTH-treatment considerably increased the level of CATA2 transcript. In case of untreated 'HLI resistant' plants, a continuous increase in transcript accumulation of CATA2 was found to reach its maximum at 16 dpi. With the exception of the third sampling day, the BTH treatment increased the transcript accumulation in the 'HLI resistant' plants as well. In the totally resistant untreated sunflowers the accumulation of CATA2 transcript reached its maximum at 13 dpi (Fig. 1).

DISCUSSION

In this study molecular changes in BTH-treated sunflowers were the subject of investigations associated with infection by *P. halstedii*. PCR was used in an attempt to describe induced resistance events in different sunflower genotypes.

Glutathione S-transferase usually detoxifies xenobiotics in plant tissues. We found an increased level of GST activity in the BTH-treated, susceptible sunflower and this increased activity resembled that detected in the 'HLI resistant plants'. Fodor et. al (1997) reported similar results with tobacco either treated or non-treated with salicylic acid. In contrast, El-Zahaby et al. (1995) found a significantly higher level of GST activity in susceptible barley plants than in resistant ones after powdery mildew inoculation. They assumed that the fungus itself contained GST enzyme, so that both the host and the pathogen might contribute to this increase in GST activity.

Defensins are a class of antimicrobial peptides found in several plants, including sunflower. In our experimental conditions defensin gene expression was induced by BTH treatment in the susceptible sunflower plants, and this enhanced level was equally found in the 'HLI-resistant' plants. Similar to Radwan et al. (2005), Ha-PDF transcript accumulation was lower in the non-treated susceptible plants, than in the resistant ones.

Catalase is usually considered to be one of the most important antioxidant enzymes. BTH treatment increased CATA2 transcript level in the susceptible sunflower plants but this effect was not evident in the resistant sunflowers.

In conclusion, the plant activator BTH had a positive effect on the natural defense system of sunflower by enhancing the expression of three genes that are considered to be associated with the chemically-induced host resistance to *P. halstedii*.

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REFERENCES

Albourie, J.M., J. Tuorvieille, and D.T. Labrouhe. 1998. Resistance to metalaxyl in isolates of the sunflower pathogen *Plasmopara halstedii*. Eur. J. Plant Pathol. 104:235-242.

Azpilicueta, E.C., P.M. Benavides, L.M. Tomaro, and S.M. Gallego. 2007. Mechanism of CATA3 induction by cadmium in sunflower leaves. Plant Physiol. Biochem. 45: 589-595.

Bán R., F. Virányi, K. Körösi, and S. Nagy. 2004. Indukált rezisztencia a napraforgó-peronoszpórával szemben. Növényvédelem 40:545-550.

Cohen, Y., T. Niderman, E. Mösinger, and R. Fluhr. 1994. β-aminobutyric acid induces the accumulation of pathogenesis-related proteins in tomato (*Lycopersicon esculentum* L.) plant and resistance to late blight infection caused by *Phytophthora infestans*. Plant Physiol. 104:59-66.

Cohen, Y., and W.E. Sackston. 1973. Factors affecting infections of sunflowers by *Plasmopara halstedii*. Can. J. Bot. 52:15-22.

Costa, H., S.M. Gallego, and M.L. Tomaro. 2002. Effect of UV-B radiation on antioxidant defense system in sunflower cotyledons. Plant Sci. 162:939-945.

Dean, J.V., J.W. Gronwald, and C.V. Eberlein. 1990. Induction of glutation S-transferase isoenzymes in sorghum by herbicide antidotes. Plant Physiol. 92:467-473.

El-Zahaby, H. M., G. Gullner, and Z. Király. 1995. Effects of powdery mildew infection of barley on the ascorbate- gluthathione cycle and other antioxidants in different host-pathogen interaction. Phytopathology 85: 1225-1230.

Fodor, J., G. Gullner, A.L. Ádám, B. Barna, T. Kőmíves, and Z. Király. 1997. Local and systemic responses of antioxidants to tobacco mosaic virus infection and salicylic acid in tobacco. Plant Physiol. 114:1443-1451.

Gabara, B., M. Sklodowska, A. Wyrwicka, W. Glinska, and M. Gapinska. 2003. Changes in the ultrastructure of chloroplasts and mitochondria and antioxidant enzyme activity in *Lycopersicon esculentum* Mill. leaves sprayed with acid rain. Plant Sci. 164:507-516.

Gechev, T., H. Willekens, M. Van Montagu, D. Inzé, W. Van Camp, V. Toneva, V., and I. Minkov. 2003. Different responses of tobacco antioxidant enzymes to light and chilling stress. J. Plant Physiol. 160:509-515.

Gulya, T. 2007. Distribution of *Plasmopara halstedii* races from sunflower around the world. Adv. Downy Mildew Res. 3:121-134.

Karabal, E., M. Yücel, and H.A. Öktem. 2003. Antioxidant responses of tolerant and sensitive barley cultivars to boron toxicity. Plant Sci. 164:925-933.

Kogel, K.H., U. Beckhove, J. Dreschers, S. Münch, and Y. Romme. 1994. Acquired resistance in barley. Plant Physiol. 106:1296-1277.

Niebel, A., K. Heungens, N. Barthels, D. Inze, M. Van Montagu, and G. Gheysen. 1995. Characterization of a pathogen-induced potato catalase and its systemic expression upon nematode and bacterial infection. Mol. Plant Microbe Interact. 8:371-378.

Pajot, E., D. Le Corre, and D. Silué. 2001. Phytogard and DL-b-amino butyric acid (BABA) induces resistance to downy mildew (*Bremia lactucae*) in lettuce (*Lactuca sativa* L.). Eur. J. Plant Pathol. 107:861-869.

Radwan, O., S. Mouzeyar, J.S. Venisse, P. Nicolas, and M.F. Bouzidi. 2005. Resistance of sunflower to the biotrophic oomycete *Plasmopara halstedii* is associated with a delayed hypersensitive response within the hypocotyls. J. Exp. Bot. 56:2683-2693.

Solis, J., G. Medrano, and M. Ghislain. 2007. Inhibitory effect of a defensin gene from the Andean crop maca (*Lepidium meyenii*) against *Phytophthora infestans*. J. Plant Physiol. 164:1071-1082.

Urdangarín, M.C., N.S. Norero, W.F. Broekaert, and L. de la Canal. 2000. A defensin gene expressed in sunflower inflorescence. Plant Physiol. Biochem. 38:253-258.

Virányi, F., and T. Gulya. 1996. Expression of resistance in *Plasmopara halstedii*- sunflower pathosystem. p. 14-21. In: ISA Symposium I. Disease Tolerance in Sunflower, 13 June, Beijing, China.

Effectiveness of the genetic resistance to *Plasmopara halstedii* under natural conditions and diversity of the pathogen within sunflower fields

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ABSTRACT

Genetic and chemical strategies are effective ways for controlling the downy mildew caused by *Plasmopara halstedii* in sunflower. Although genetic resistance is very frequent in sunflower hybrids, new races of the pathogen that overcome the genes of resistance appear. The chemical treatment with the systemic fungicide metalaxyl-M is mandatory in Spain for sowing susceptible sunflower, but resistance of *P. halstedii* to metalaxyl-M has been reported. Four experiments were conducted under dryland conditions in four locations of Andalusia. Ten resistant hybrids were grown in three of the experiments with the objective of assessing the effectiveness of the genetic resistance against natural infections by *P. halstedii*. Values of downy mildew incidence and also sunflower production depended significantly on the cultivar in the three fields. However, lowest productions did not correspond to the most susceptible hybrids, showing the efficiency of the genetic resistance. Aiming at assessing the frequency of resistance of *P. halstedii* to metalaxyl-M within one sunflower field and the race of the resistant isolates, metalaxyl-M treated seeds of 19 commercial sunflower hybrids were sown in the fourth experiment. Thirty four isolates with resistance to metalaxyl-M were recovered from plants of different hybrids and different positions within the field, and all of them were characterized as race 310. These results show that isolates with resistance to the fungicide can be widely spread within the field and that resistance to the fungicide can happen in races with a high virulence.

Key words: downy mildew – genetic control – *Helianthus annuus* L. – natural infections – resistant sunflower hybrids.

INTRODUCTION

Sunflower downy mildew is a disease caused by the Oomycete *Plasmopara halstedii* Farl. Berl. & de Toni, that was reported for the first time in Spain in the 1970's. Favourable conditions for disease development are temperatures of 14-16ºC and high moisture in the soil during crop emergence (Gulya et al., 1997). Dwarfing of the plants and chlorosis of the leaves are typical symptoms in sunflower. An effective method of controlling the disease is the incorporation of genetic resistance into the host, but more virulent races of the pathogen that overcome the genes of resistance can appear (Molinero-Ruiz et al., 2002). The treatment with the systemic fungicide metalaxyl-M (or mefenoxam) is being widely used in Spain as a chemical way of controlling the pathogen. The fungicide is applied as a seed dressing because of the early infection of sunflower. However, this method of control may not be effective, since resistance of *P. halstedii* to metalaxyl-M has been recently reported in this country (Molinero-Ruiz et al., 2008). In this work, we assessed the efficacy of the incorporation of genetic resistance into sunflower hybrids when natural infections by *P. halstedii* occur and we also studied the diversity of the pathogen in sunflower fields as far as races and reaction to metalaxyl-M are concerned.

MATERIALS AND METHODS

Aiming at assessing the performance of genetically resistant sunflower cultivars and of metalaxyl-M treated sunflower cultivars under natural infections by *P. halstedii*, four experiments were conducted under dryland conditions in different locations of Andalusia: two in Ecija, Sevilla (Casilla Tejada and La Palmera), one in Carmona, Sevilla (Tomejil) and one in Santa Cruz, Córdoba (El Alcaparro). Plants were sown in March, 2007, in fields where there were previous records of infections by downy mildew. In each location the experiment was designed as a randomised complete block with four replications. Experimental unit consisted of four 10-m-long rows 0.7 m apart. In three of the experiments, those in La Palmera, Tomejil and El Alcaparro, 12 sunflower genotypes were sown: the susceptible control (the open

pollinated variety Peredovik), 10 hybrids commercialized by different seed companies as genetically resistant to *P. halstedii*, and two resistant controls (two hybrids whose seed was treated with metalaxyl-M). The fourth experiment was conducted in Casilla Tejada, a field where the existence of populations of *P. halstedii* resistant to metalaxyl-M was suspected. Nineteen commercial varieties from the Andalusian Network of Agricultural Trials (RAEA) and seed treated with metalaxyl-M at the commercial dose of 2 g a.i./kg seed were tested.

Downy mildew symptoms considered were chlorosis of the leaves and/or dwarfing of the plants. Evaluation of symptoms and harvest were performed on the two central rows of each experimental unit. Disease incidence (percentage of symptomatic plants), and yield (kg of seed per hectare) were calculated and analyzed by means of ANOVA and Tukey comparisons ($P = 0.05$).

Samples from diseased plants were collected in the four fields. In each of them, samples were independently collected from different genotypes in order to determine the diversity of *P. halstedii* as far as races and reaction to metalaxyl-M (sensitivity or resistance) were concerned. Sixty-two samples were processed: 7 from Tomejil, 3 from El Alcaparro, 18 from La Palmera and 34 from Casilla Tejada. Also, 62 isolates of *P. halstedii* were recovered after incubation of samples in a humid chamber kept in darkness. The race of each of the isolates was determined with the methodology internationally used for racial characterization of sunflower downy mildew (Gulya et al., 1998; Molinero-Ruiz et al., 2002). Each isolate was inoculated to nine sunflower lines (differentials) that were grown in a chamber under controlled conditions of temperature (15-18ºC) and photoperiod (14 h of light). After two weeks, sporulation of the pathogen in the plants was induced by means of incubation at 100% relative humidity. Resistant or susceptible reactions were noted and considered to determine the race (numeric code) of the isolates. Similarly to race characterization, the reaction of each isolate of *P. halstedii* to the fungicide metalaxyl-M was determined after its inoculation to 40 treated (2 g a.i./kg seed) and 40 non treated Peredovik seeds. After inoculation, growth, and induction of symptoms, as explained, sensitive or resistant reaction of the pathogen to the fungicide was noted as the percentage of sporulated treated plants. When resistance was observed, the inoculation was repeated in order to verify the results.

RESULTS AND DISCUSSION

The incidence of the disease in the susceptible control Peredovik (DMI), ranged between 2.4 and 18.7% in Santa Cruz and Tomejil respectively, and depended significantly on the sunflower cultivar in the three fields ($p \leq 0.005$). Peredovik and Midi were the most susceptible varieties, with DMI values of between 2.5 and 18.7% and 1.8 and 9.9% respectively (Fig. 1). The remaining varieties, with the exception of Leila, showed DMI values not significantly different from zero in the three fields. Leila showed an intermediate DMI (2.1%) in Tomejil (Fig. 1). First downy mildew infections were observed between 6 and 7 weeks after sowing and the incidence of the disease reached its highest values between 9 and 12 weeks after sowing (Fig. 1). All the isolates of *P. halstedii* recovered from Tomejil, Santa Cruz and La Palmera were race 310 (Table 1). Although Molinero-Ruiz et al. (2002) suggested that a diversity of races of *P. halstedii* can exist in one sunflower field, we only found one race.

The disease in the trial of commercial varieties also depended significantly on the sunflower variety ($p = 0.0049$) and the DMI ranged between 0.4% (Kardan) and 17.2% (F-101) (Table 2). Amira was the only variety which did not show symptoms of downy mildew, what could be due to genetic resistance of the hybrid. Since high incidences of disease happened, no records on yield were obtained. Thirty-four populations of *P. halstedii* were recovered from this field. All of them were characterized as race 310 and all of them were resistant to metalaxyl-M at the commercial dose tested (Table 2). The resistance to metalaxyl-M has already been reported in Spain (Molinero-Ruiz et al., 2008), as well as in USA and France.

Yield was only analyzed in the three fields of genetically resistant cultivars and it depended significantly on the cultivar in the three cases ($p \leq 0.0005$) (Fig. 2). Peredovik was the only cultivar with a significantly lower production in Tomejil, with a little more than one third of the average production of the rest of the varieties (Fig. 2). It showed the highest DMI, but it is also an open pollination variety and not a hybrid, and, consequently, its potential of production may be lower. On the other hand, both resistant controls PR64A14 and Olimpia were highly productive, and their yields did not differ from those of the most productive varieties of each of the experiments (Fig. 2). Fig. 2 shows that although the highest DMI was observed in Tomejil, the average yield in this field was twice times higher than those in Santa Cruz and in La Palmera. These differences were due to very high infections by broomrape (*Orobanche cumana* Wallr.) in these two fields compared to those in Tomejil (Table 3). The highest yields not

significantly different to those of the resistant controls in the case of Es Isabella and Leila in La Palmera seem to be due to the good production potential of the hybrids, since incidence of downy mildew was recorded in both of them.

Our results show that the effectiveness of the genetic resistance to downy mildew depends on the race of *P. halstedii* that is present in the field, since races not controlled by the genes of resistance may easily exist. Therefore, a good knowledge of the genetic resistance in sunflower hybrids is advisable. On the other hand, this work also shows that when resistance of *P. halstedii* to metalaxyl-M happens, treatment with the fungicide at the commercial dose is ineffective, and infections can result in a complete loss of sunflower production. As a conclusion, it seems important to analyze the advantages and disadvantages of both the genetic and the chemical strategies for the control of sunflower downy mildew.

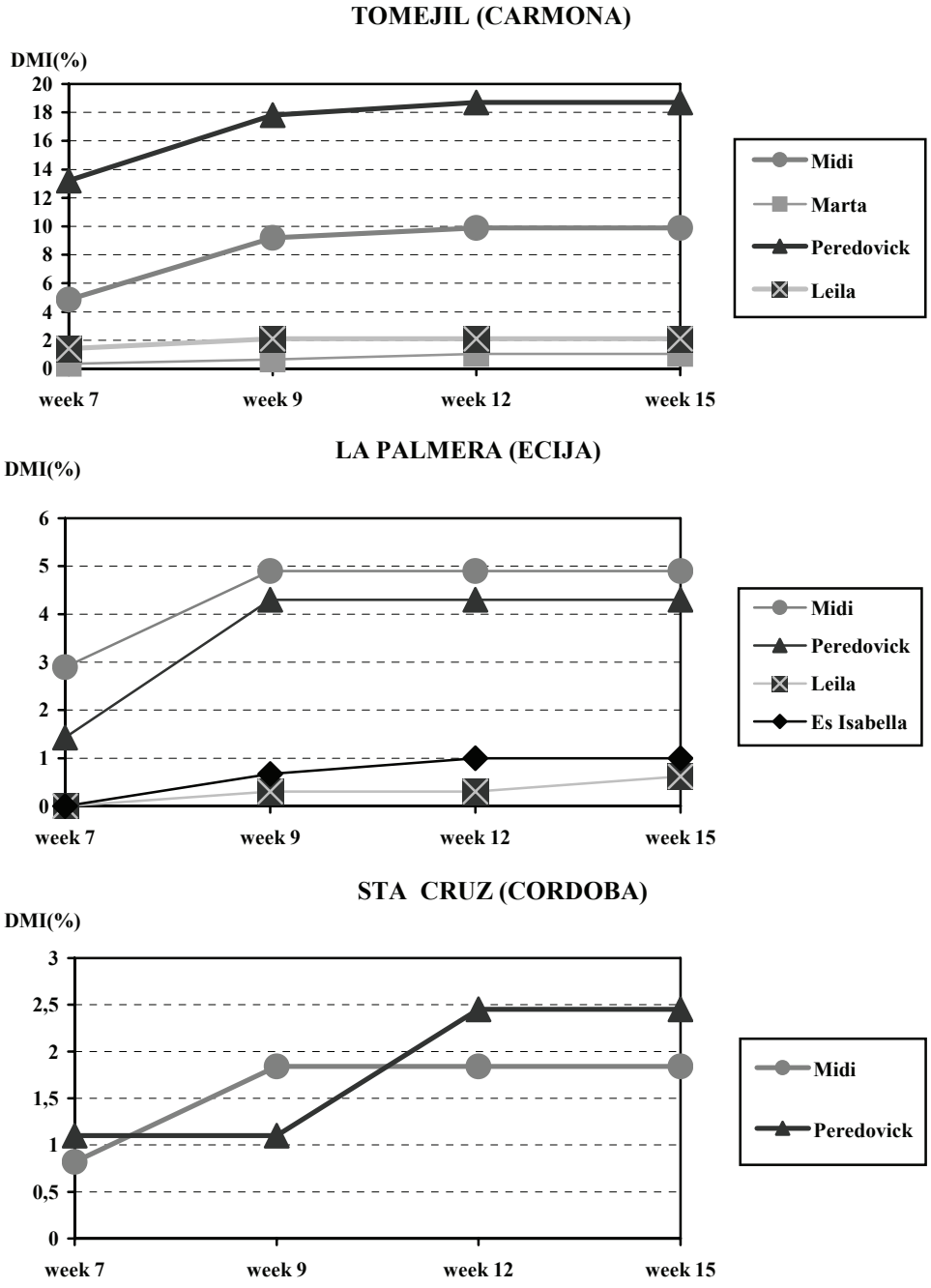

Fig. 1. Downy mildew incidence (DMI) in susceptible sunflower cultivars grown in three different fields of Seville and Córdoba in 2007.

Table 1. Race of 28 isolates of *Plasmopara halstedii* recovered onto different cultivars with resistance to the disease collected in three different sunflower fields

Field	Cultivar	Race
Tomejil	Marta (1) [a]	310
Tomejil	Leila (2)	310
Tomejil	Midi (2)	310
Tomejil	Peredovik (2)	310
Santa Cruz	Midi (1)	310
Santa Cruz	Peredovik (2)	310
La Palmera	Leila (1)	310
La Palmera	Midi (10)	310
La Palmera	Peredovik (5)	310
La Palmera	Es Isabella (2)	310

[a] Parentheses show the number of isolates from each cultivar that were recovered and characterized.

Table 2. Race and reaction to metalaxyl-M showed by 34 isolates of *Plasmopara halstedii* recovered in Casilla Tejada from different commercial sunflower hybrids

Cultivar	Incidence (%)	Race	metalaxyl-M reaction
NX 35607	6.8	310 (1)[a]	R [b]
Quisol	6	310 (1)	R
F-103	4.6	310 (2)	R
PR64A71	9	310 (1)	R
F-104	3.8	310 (1)	--
Voraz	7.5	310 (1)	R
Imigen	5.4	310 (3)	R
F-101	17.2	310 (2)	R
Es AMIRA	0	-- [c]	--
PR64A14	4.1	310 (2)	R
Masoli	3.2	310 (1)	R
PR63A76	10.5	310 (2)	R
Solnet	7.6	310 (2)	R
Transol	6.2	310 (5)	R
Kardan	0.4	310 (6)	R
Olimpia	6	310 (4)	R

[a] Parentheses show the number of isolates from each cultivar that were recovered and characterized.
[b] R= resistant.
[c] -- Not characterized.

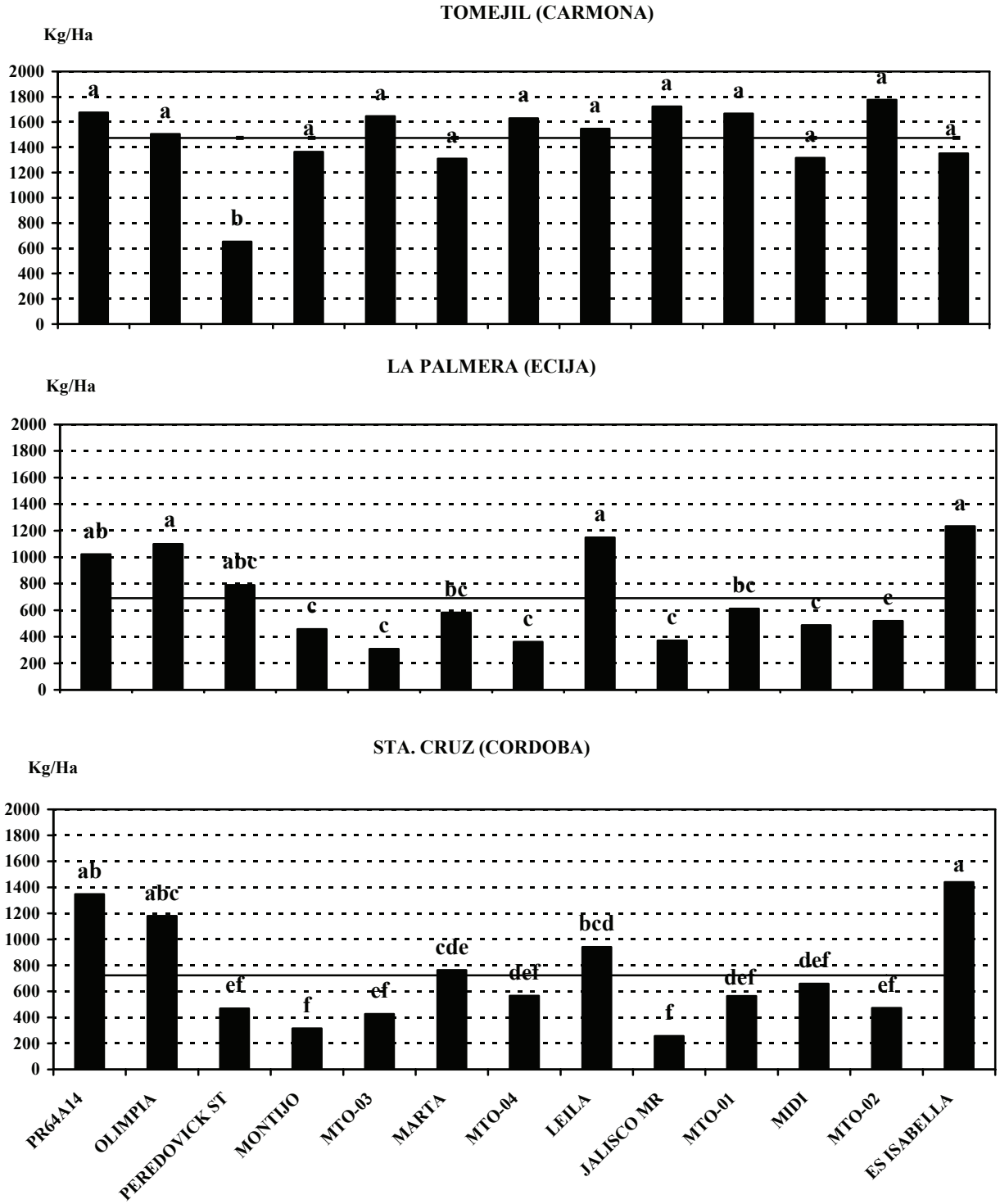

Fig. 2. Yields of 12 sunflower hybrids genetically resistant to sunflower downy mildew and one susceptible control (Peredovik) obtained in three different fields of Córdoba and Seville.

Table 3. Incidence of broomrape in downy mildew experiments in three different locations of Andalusia in 2007

	Incidence of broomrape (%)		
Cultivar	La Palmera	Santa Cruz	Tomejil
Montijo	100	98.7	13.5
MTO-03	100	100	32.25
Marta	100	93	4.25
MTO-04	100	100	25.75
Leila	100	46.6	1
Jalisco MR	35.25	100	20.5
MTO-01	100	100	22.25
Midi	100	96.3	3.75
MTO-02	100	95	24.25
Peredovik	93.75	89.2	3.5
Es Isabella	9.5	11.2	0
PR64A14	71.5	66.4	1
Olimpia	18.75	20.4	0.75

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REFERENCES

Gulya, T.J., K.Y. Rashid, and S. Masirevic. 1997. Sunflower diseases. p. 263-379. In: Sunflower Technology and Production. A.A. Schneiter (ed.). ASA, CSSA and SSSA, Madison WI, USA. 834 p.

Gulya, T.J., D. Tourvieille de Labrouhe, S. Masirevic, A. Penaud, K. Rashid, and F. Viranyi. 1998. Proposal for standardized nomenclature and identification of races of *Plasmopara halstedii* (sunflower downy mildew). p. 130-136. In: Proc. ISA Symposium III, Fargo, USA. Int. Sunfl. Assoc., Paris, France.

Molinero-Ruiz, M.L., M.M. Cordón-Torres, J. Martínez-Aguilar, J.M. Melero-Vara, and J. Domínguez. 2008. Resistance to metalaxyl and to metalaxyl-M in populations of *Plasmopara halstedii* causing downy mildew in sunflower. Can. J. Plant Pathol. (in Press).

Molinero-Ruiz, M.L., J. Domínguez, and J.M. Melero-Vara. 2002. Races of isolates of *Plasmopara halstedii* from Spain and studies on their virulence. Plant Disease 86:736-740.

Determining the sunflower downy mildew risk by soil analysis

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ABSTRACT

A bioassay using a soil sample was developed for assessing downy mildew risk at the field level. The results were correlated with the rate of infected plants when no other limiting factors were observed. The first tests carried out in fields in 2007 allowed the evaluation of soil infestation and seemed to confirm that inoculum could usefully be reduced by crop rotation. Moreover, the bioassay was used to follow the evolution of soil infestation during the spring. It reached a maximum around April 15th under the French conditions. The test conditions had very little effect on the results, so, hopefully. a large-scale use could easily be developed. For less infested soil, the direct characterization of the pathogen population's virulence profiles was not reliable. However, this protocol allows us to obtain fresh inoculum even when susceptible species are not present in the field, which makes it possible to achieve the characterization of the races after isolate multiplication. The interest of this protocol for the management of control methods is discussed.

Key words: bioassay – downy mildew – epidemiology – *Helianthus annuus* – *Plasmopara halstedii* – risk analysis – soil infestation.

RESUME

Afin d'évaluer le risque «mildiou du tournesol» en parcelles agricoles, un biotest réalisé sur un échantillon de terre a été mis au point. Les résultats obtenus sont corrélés avec les taux de plantes malades observés en absence d'autres facteurs limitants. Les premiers essais de ce protocole en parcelles agricoles en 2007 ont permis d'évaluer le potentiel infectieux du sol et semblent confirmer l'intérêt d'un allongement des rotations pour limiter l'inoculum. Ce biotest a également montré son utilité pour suivre l'évolution du potentiel infectieux durant le printemps. Ce potentiel passe par un maximum qui se situe, dans les conditions françaises, autour du 15 avril. Les résultats obtenus sont relativement peu influencés par les conditions de réalisation du test, ce qui laisse espérer une généralisation aisée. Pour les terres peu contaminées, la caractérisation directe du profil de virulence de la population parasitaire n'est pas fiable. Cependant, ce protocole permet l'obtention d'inoculum frais, même en absence d'espèce sensible sur la parcelle, ce qui permet ensuite de mettre en œuvre la caractérisation des races présentes après multiplication de l'isolat. L'intérêt de ce protocole pour la gestion des méthodes de lutte est discuté.

Mots clés: analyse de risque – biotest – épidémiologie – *Helianthus annuus* – mildiou –*Plasmopara halstedii* – potentiel infectieux

INTRODUCTION

Plasmopara halstedii is mainly a soilborne plant pathogen which can survive as oospores from one year to the next (Tourvieille de Labrouhe et al., 2000). This kind of conservation, which results from the sexual reproduction, allows the survival of the pathogen for several years waiting for a susceptible culture. Among arable crops, only sunflower is susceptible to *P. halstedii*. However, some *Asteraceae* known as weeds could harbor the pathogen and enhance the inoculum reservoir. Under favourable conditions, oospores in the soil can germinate and give rise to a zoosporange which releases mobile zoospores in free water. These zoospores are responsible for the primary infection, which is the most harmful form of the disease. If the level of risk depends on the weather conditions, in parallel, quantitative and qualitative (pathotypes) aspects of the inoculum are essential to explain the severity of attacks. In order to understand the evolution of downy mildew risk and also be able to make a diagnosis

of fields before sowing, we have developed a bioassay based on soil sampling. The principle has already been published (Tourvieille and Walser, 2005) and it has served to show the relationship between the presence of downy mildew in a field and the risk for the next sunflower crops. Moreover, this device seems to be of interest for predicting the behaviour of various sunflower hybrids against the endogenous pathogen population. The article presents experiments using this protocol and whose aims were to specify: i) the link between level of soil infestation and downy mildew risk; ii) the evolution of the infestation level of the soil during spring and iii) the possibility of using this protocol in a regional management of the downy mildew risk. It is not certain that downy mildew finds favourable conditions for its expression because of environmental conditions and/or absence of susceptible plants. For this reason, with a large scale study, on the level of a pilot site, we wanted to know if the protocol of soil bioassay could be a decision-making aid in the management of control methods.

MATERIALS AND METHODS

Plant material: The open-pollinated line Peredovik, without any known resistance gene, was used to quantify the infestation level of the soil or to estimate the disease incidence in fields. The virulence profiles of *P. halstedii* populations were determined using a set of nine international differential host lines (D1 to D9) (Gulya et al., 1998).

Test in culture: Experiments were carried out in plots of calcareous clayey loam soil located in Limagne (Centre of France) under a continental moderate climate. To assess the downy mildew risk independently of the climatic conditions, a contamination of plants before emergence was performed ensuring a very important irrigation (≈ 100 mm) when the root of the seedlings reached a size ranging between 0.5 and 1.0 cm length (Vear et al., 2007). The number of infected plants was observed at the stage "appearance of the second pair of leaves". Plants with systemic symptoms of downy mildew resulted from a telluric primary infection.

Soil bioassay: Experimental soils were collected in the seed bed at the sowing period in each field by focusing on low ground locations or headlands. The soil samples were directly placed in pots (30 cm x 30 cm x 6 cm). Two hundred seeds of a trap genotype Peredovik or 10 seeds for each of the nine differentials were sown in each pot, covered by 1 cm of soil and grown at 18°C. After 48 hours, which was the time required for obtaining germs from 0.5 to 1.0 cm in length, each pot was separately immersed in water during 8 to 12 hours. Then the pots were maintained at 18°C with a 16 h photoperiod (12 000 Lux) per day. After 12 days, sporulation was induced by covering the infected seedlings with a plexiglass cap or a transparent plastic bag (PEBD 50µm) for 48 hours to provide a saturated humidity (Tourvieille de Labrouhe and Walser, 2005).

Choice of the fields for the study in a pilot site: Fields were chosen according to 3 factors: i) downy mildew history: the whole of the fields had already expressed downy mildew during the last 3 years or were located in an area where downy mildew was usually observed, ii) there was a delay between two sunflower crops (1 year, 2 years, 3 years and more) and iii) the type of soil (calcareous clay, clayey silt, silt like “boulbène”). Ten fields located in the departments of Gers and Tarn-et-Garonne (South-western France) were finally selected. A soil sampling was performed in each field. In 3 field plots, the same bioassay was carried out in a ring-test between 3 laboratories under variable conditions (Table 1), and in 4 field plots, two methods of sampling were studied: 4 independent samples of 4 liters taken from 4 points in the field were compared with a pooled sample made with 1 liter of soil taken from the same 4 points.

Table 1. Experimental conditions in the different laboratories carrying out the soil bioassay.

	Lab 1	Lab 2	Lab 3
Delay between soil sampling and seed sowing	72 h	48 h	120 h
Light	12 000 lux (neon)	day light	day light
Temperature	18°C ± 1°C	uncontrolled (-)	uncontrolled (-)
Time of immersion	17 h	8 h	8 h
Time of saturated humidity	48 h	66 h	45 h

In addition, we tried to characterize the virulence of the pathogen population by carrying out the bioassay directly with the 9 differential host lines in 4 fields. Results were confronted with the

characterization of the virulence profile according to the classical method using an infected plant collected in the field as the inoculum source (Tourvieille de Labrouhe et al., 2000).

RESULTS

Relationship between the response of the soil bioassay and the disease incidence in the field: On small plots (≈ 70 m²) known to be infested by *P. halstedii,* soil samples were analyzed and downy mildew incidence was observed *in situ* in 2006 and 2007. Observations showed a close relationship between the number of seedlings with symptoms of downy mildew from the soil bioassay and the number of infected plants observed in the year of sampling (Fig. 1).

The relationship between the disease incidence in the field and the rate of infected seedlings as a result of the soil bioassay was quite similar in spite of the differences in the disease pressure, which was 54.5% in 2006 and 16.3% in 2007. The correlation coefficient of the 12 data pairs was highly significant (r=0.917).

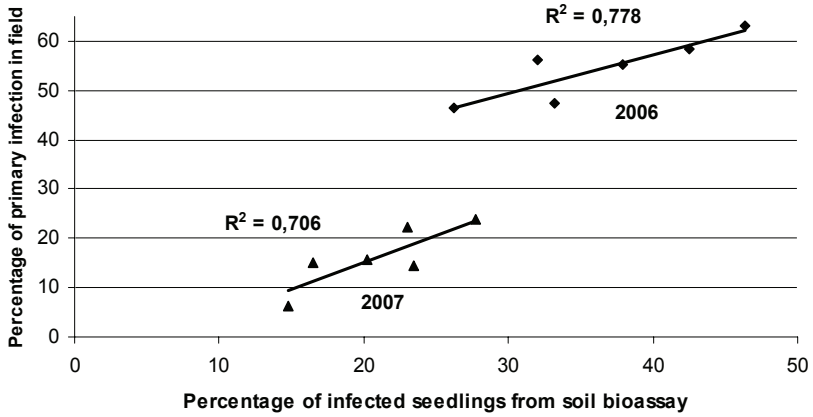

Fig. 1. Relationship between primary infection of downy mildew in the field and infection of seedlings from the soil bioassay.

Infestation of a field plot according to the farming past: On small plots followed for many years, the rate of infected seedlings given by the bioassay was correlated with the number of infected plants observed the previous years. The best correlation was obtained when infected plants were grown the previous year (y-1) or 2-years before (y-2) (Fig. 2).

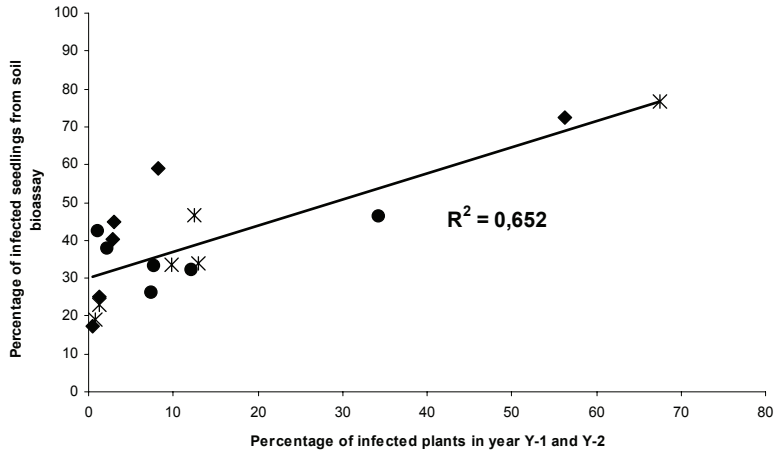

Fig. 2. Relationship between downy mildew incidence in the field observed 1 and 2 years before the soil bioassay and the rate of infected seedlings from the soil bioassay (◆ 2004, ❄ 2005, ● 2006**).**

The rates of infected seedlings assessed by the soil bioassay varied from 14.8% to 76.8% and were on average 43.0% in 2004, 38.7% in 2005 and 36.4% in 2006. These rates were highly correlated with the

downy mildew incidence observed in the field the two previous years (y-1 and y-2). The downy mildew incidences varied from 0.4% to 67.5% and were on average 12.0% in 2004, 17.5% in 2005 and 10.8% in 2006. So the relationship between the soil infestation measured by the soil bioassay and the presence of infected plants the previous years is confirmed in this experiment.

Use of the soil bioassay for measuring the evolution of the soil infestation: To appreciate the evolution of the soil infestation during the whole period of sunflower sowing, soil samples were collected once per week, from March to May. This experiment was carried out in the site of Clermont-Ferrand in 2006 (April-May: mild and humid conditions with soil average temperature ~15°C and sum of precipitations = 178 mm) and in 2007 (April-May: warm and dry conditions). Ten micro-plots were analyzed weekly. Results and the adjusted curves are presented in Fig. 3.

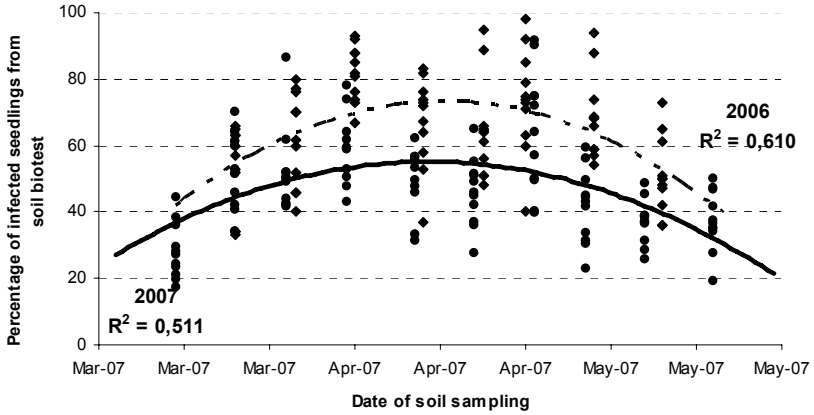

Fig. 3. Evolution of the proportion of infected seedlings given by the soil bioassay according to the date of soil sampling (10 samples per date) in 2006 (◆) and 2007 (●).

Under the environmental conditions of the Centre of France, the best sampling date for assessing the soil infestation appeared to be in mid-April. If the levels of infection seemed to be dependent on the weather conditions of the year (65.1% on average in 2006 and 46.7% on average in 2007), the data corresponding to the maximum of primary infection appeared to be fairly constant in both years.

Application of methodology in a pilot site: The 10 field plots of the pilot site can be classified in 3 classes (Table 2):

- slightly infested: Les Mariettes, Le Carascau and Sarrault with less than 10% of infected seedlings.
- moderately infested: Utaut and Janicaut with less than 25% of infected seedlings.
- strongly infested: La Poëte, Le Rauy, Bordeneuve and La Plèche with more than 30% of infected seedlings.

The comparison of the results from different laboratories showed a very good repeatability for 2 field plots, “La poëte” and ‘Utaut”. In contrast, the two analyses of the plot "Le Rauy" appeared to be rather contrary (Table 2). Moreover, information on the soil of the field plot "Les Barbès" could not be given due to the absence of the emergence of sunflower during the bioassay.

In the 4 field plots where two methods of sampling were tested, the levels of response varied from slightly infested (Le Rauy) to very strongly infested (La Poëte). Differences between the individual samples suggest variability in the soil infestation of the field plot (Table 3). It must be noted that the pooled sample did not correspond to the mean of the 4 independent samples and the mean rate was always the weakest.

Table 2. Percentage of seedlings of a susceptible genotype presenting symptoms of downy mildew in soil bioassay according to the location of sampling and analysis

Location	Type of soil	Laboratories		
		Lab 1	Lab 2	Lab 3
Les Barbes	Silt «Boulbène»	?	-	-
Les Mariettes	Silt clay	6.8%	-	-
La Poëte	Calcareous clay	59.4%	65.6%	62.6%
Utaut	Calcareous clay	21.4%	21.6%	22.2%
Le Rauy	Silt clay	37.7%	8.5%	-
Bordeneuve	Calcareous clay	31.3%	-	-
Le Carascau	Calcareous clay	6.3%	-	-
La Plèche	Calcareous clay	36.4%	-	-
Sarraut	Calcareous clay	3.6%	-	-
Janicot	Calcareous clay	14.3%	-	-

Table 3. Percentage of infected seedlings from soil bioassay according to the method of sampling

Location	Pooled sample	Point 1	Point 2	Point3	Point 4
Les Mariettes	2.4%	2.4%	13.8%	12.1%	3.1%
La Poëte	33.3%	67.7%	80.1%	50.0%	82.1%
Utaut	16.7%	18.8%	22.2%	14.3%	39.3%
Le Rauy	3.8%	12.8%	5.6%	15.4%	5.1%

When the virulence profile was determined by using the soil bioassay, rates of infected seedlings were very low, although the results were not in contradiction with results given by the classic test (Table 4)

Table 4. Characterization of virulence profile of the population of *P. halstedii* in the soil (soil bioassay = a) and of a sample taken on an infected plant (classic test = b).

Location	Test	For each differential: Number of infected seedlings / Number of emerged seedlings									Profile
		D1	D2	D3	D4	D5	D6	D7	D8	D9	
Les Barbes	a [(1)]	*0/2*	*0/3*	*0/2*	*0/1*	*0/0*	*0/8*	*0/2*	*0/0*	*0/1*	?
	b	**7/10**	**7/10**	**8/10**	*0/10*	*0/10*	*0/10*	**5/10**	**7/10**	*0/10*	703
Utaut	a	**8/19**	**16/29**	**1/19**	*0/16*	*0/22*	*0/18*	**2/20**	**1/19**	**14/29**	707 [(2)]
	b	**3/10**	**7/10**	1/10	*0/10*	*0/10*	*0/10*	*0/10*	*0/10*	**2/10**	304 ?
La Poëte	a	**6/18**	**26/30**	**4/21**	*0/22*	*0/19*	*0/20*	**10/31**	**9/29**	**11/20**	707 [(2)]
	b	**1/9**	**6/10**	*0/10*	*0/10*	*0/10*	*0/10*	*0/10*	*0/10*	**4/10**	304
Janicot	a	**3/12**	**2/11**	**2/13**	*0/11*	*0/10*	*0/12*	**1/12**	**3/10**	*0/10*	703
	b	**9/9**	**10/12**	**6/13**	*0/10*	*0/8*	*0/2*	**5/7**	**3/6**	*0/5*	703

[(1)] asphyxia. [(2)] or mixture of pathotypes (304 + 703).

DISCUSSION

Results obtained on small plots clearly showed the interest of analysing the soil infestation using a soil bioassay since its response was well correlated with downy mildew risk observed in the absence of limiting weather factors. The soil bioassay also allowed us to confirm the close relationship between the mildew history of a field plot and the level of infestation. This is easily explained by the fact that the pathogen is maintained from one year to the next by oospores, which are produced in infected tissues of sunflower (Sackston, 1981). The quantity of oospores is therefore directly related to the number of infected plants. Consequently, short crop rotations are prohibited by recommended measures for control of downy mildew, especially when the presence of the disease is detected (Moinard et al., 2006).

The soil bioassay also allowed us to follow the evolution of soil infestation during the sunflower sowing period in spring. It was demonstrated that soil infestation reached a maximum in mid-April under French conditions. This evolution has to be connected with the weather conditions. These become favourable to the pathogen at the end of winter, inducing a break in soil infestation due to the short lifetime of the zoospores after germination (Goossen and Sackston, 1968). These results could lead to two interesting prospects: i) sowing as soon as possible so that sunflower emergence can escape the favourable periods to downy mildew infection; but this would mean selecting hybrids resistant to cold temperatures and ii) carrying out all experiments of selection (Vear et al., 2007) or screening of molecules (Délos et al., 2000) in April under conditions favourable to the pathogen.

It is always difficult to assess soil infestation of a field plot. When it was possible to analyze either several samples of the same plot or a pooled sample representative of the plot, the pooled sample was always less infected than independent samples. This could be explained by the quantity of sampled soil.

Indeed, this quantity from each sampling point for a pooled sample is less important than the soil quantity which is necessary for an independent analysis. Also, in the first case, soil is not always taken from the whole horizon corresponding to the seed bed. But it is also possible that the lifetime of inoculum could be very low in the upper layer of the soil where more drastic climatic conditions could occur. This leads to recommending sampling soil from the -2cm to -8cm horizon for a more effective soil analysis.

In 2007, the use of resistant sunflower hybrids in the field plots did not enable us to confirm the relation between soil infestation and disease incidence, despite quite favourable weather conditions for downy mildew. Neither did we notice links between type of soil and level of infestation. Nevertheless, it was demonstrated that the protocol was not adapted in the case of silt loam like "Boulbène" because immersion caused a packing of soil and a lack of seed germination. In this case, it would be possible to recover inoculum by percolation and to use this more or less infested water to perform the watering of seeds in uninfested substrate. For the other types of soil, bioassay results indicated that the different test conditions in the three laboratories had little influence, but this should be confirmed under less favourable conditions (e.g. higher temperatures).

Direct characterization of the virulence profile has been seen to have its limits. Its sensitivity depends on the level of soil infestation, which must be high enough to guarantee the infection of susceptible differential hosts. Its specificity is also limited because it uses only nine differentials, which is not enough to determine the virulence profile of a mixture of pathotypes. Moreover, variability in a field plot could only be measured by numerous independent analyses. However, the soil bioassay could potentially be used to investigate the downy mildew risk of the variety to be sown in the field plot.

In the framework of risk management, the soil bioassay shows potential interests:

- determining soil infestation of a field plot in a given year,
- achieving virulence profiles present in the field plot, even in the absence of susceptible sunflower,
- following the evolution of pathogen population in quantitative and qualitative terms in comparison with the means of disease control,
- finally, adapting the means of genetics and chemical control according to the risk in the field plot.

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REFERENCES

Délos M., N. Eychenne, I. Birba, C. Duprat, and D. Tourvieille de Labrouhe. 2000. Mise au point d'une méthode de contamination artificielle en vue de l'expérimentation de fongicides sur *Plasmopara halstedii*. p. G1-G10. In: Proc. 15th Int. Sunfl. Conf., Toulouse, France.

Goossen, P.G., and W.E. Sackston. 1968. Transmission and biology of sunflower downy mildew. Can. J. Bot. 46:5-10.

Gulya, T.J., D. Tourvieille de Labrouhe, S. Masirevic, A. Penaud, K. Rashid, and F. Viranyi. 1998. Proposal for standardized nomenclature and identification of races of *Plasmopara halstedii* (sunflower downy mildew). p. 130-136. In: Proc. ISA Symposium III, Fargo, ND, USA. Int. Sunfl. Assoc., Paris, France.

Moinard, J., E. Mestries, A. Penaud, X. Pinochet, D. Tourvieille de Labrouhe, F. Vear, M.-C. Tardin, I. Pauchet, and N. Eychenne. 2006. An overview of sunflower downy mildew. Phytoma – La Défense des Végétaux 589:34-38.

Sackston, W.E. 1981. Downy mildew of sunflower. p. 545-575. In D.M. Spencer (ed.), The Downy Mildews. Academic Press, London, UK.

Tourvieille de Labrouhe, D. and P. Walser. 2005. Un test simple pour déceler la présence du mildiou sur une parcelle. Oléoscope 83:12.

Tourvieille de Labrouhe, D., E. Pilorgé, P. Nicolas, and F. Vear. 2000. Le Mildiou du Tournesol. Versailles, France: CETIOM-INRA, 70 p.

Vear, F., F. Serre, S. Roche, P. Walser, and D. Tourvieille de Labrouhe. 2007. Recent research on downy mildew resistance useful for breeding industrial use sunflowers. Helia 30(46):45-54.

Large scale field evaluations for Sclerotinia stalk rot resistance in cultivated sunflower

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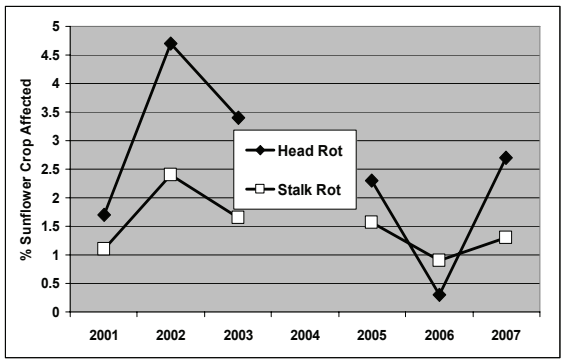
ABSTRACT

An artificial inoculation procedure to incite Sclerotinia basal stalk rot of sunflower was developed that is appropriate for large scale field evaluations. The procedure employs a dried, millet-based mycelial inoculum, without sclerotia. A measured amount of inoculum is deposited in a continuous furrow alongside each row, with ~ 60 g used per 7 m row. Thus, for each 1000 rows in a nursery we use ~ 60 to 70 kg of inoculum, allowing for spillage and inoculum deposited between rows. Preliminary results demonstrated that mycelium produced on oats and millet are equally infective, but the spherical shape of millet seeds facilitates the use of mechanized inoculation equipment. For large scale field inoculations, we modified a granular chemical applicator mounted on a tractor-driven cultivator to deposit uniform amounts of inoculum. The application needs to be made when the sunflower plants are at the V-6 stage or earlier, when the plants are shorter than the tool bar upon which the applicator is mounted. Since the inoculum is deposited in a furrow 20 to 25 cm away from the young plants, the initial symptoms of infection do not appear until 4 to 5 weeks after inoculation, at which time their root systems have contacted the inoculum. Based upon four years of field trials with commercial hybrids, this method has proven capable of producing sufficient and uniform levels of stalk rot, allowing statistical identification of the most resistant hybrids.

Key words: disease testing – *Helianthus* – inoculation methods – *Sclerotinia sclerotiorum* – sunflower.

INTRODUCTION

Basal stalk rot and wilt of sunflower caused by *Sclerotinia sclerotiorum* continues to be one of the major diseases affecting sunflower in North America, along with Sclerotinia head rot (Berglund, 2007; Gulya, 2003, 2004a; Lamey et al., 2002). During the period from 2001 to 2007, the incidence of stalk rot affected fields has ranged from 16 to 35% while head rot in the same period has ranged from 9% to 51% fields affected (Fig. 1). The severity, or percentage of the crop affected, during the same period has ranged from 0.9 to 2.4% for stalk rot and from 0.3 to 4.7% for head rot (Fig. 2). In an effort to develop resistant germplasm and to assess hybrid resistance to both Sclerotinia diseases, we have made an effort to develop artificial inoculation procedures to generate consistent and statistically sound data. While many papers have been published on inoculation procedures for head rot, there is a dearth of information on stalk rot inoculation techniques. We began a study in 2002 to develop a field inoculation method which would produce a reliable level of stalk rot with which to identify sunflower germplasm with improved levels of resistance. This initial study demonstrated the superiority of mycelial inoculum compared to sclerotia (Gulya, 2004b). The next objectives were (1) to adapt this to large scale evaluations encompassing thousands of rows at multiple locations, and (2) verify the method with commercial hybrids tested over several years.



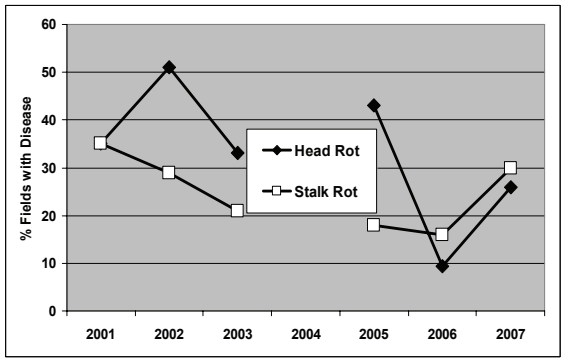


Fig. 1 and 2. Changes in disease severity (% of crop affected, left graph) and disease incidence (% of fields with disease, right graph) for Sclerotinia stalk rot and head rot in North Dakota fields surveyed between 2001 and 2007, using data obtained from the National Sunflower Association annual survey. The survey was not conducted in 2004.

MATERIALS AND METHODS

The inoculation procedure we developed is a version of that initially developed by Mancl and Shein (1982). *Sclerotinia sclerotiorum* inoculum was produced by growing the fungus on autoclaved white proso millet for 7 to 9 days (before any sclerotia developed), drying the inoculum to 10% moisture, and storing it at 4ºC until needed. Using a granular chemical applicator (Gulya et. al, 2005), the inoculum was placed in a furrow ~ 25 cm from each row, about 8 to 10 cm deep. Each 7 m row received ~ 50 to 60 g of inoculum. For each 1000 rows, we used approximately 60 kg. or 135 pounds of millet-based inoculum. Each location was inoculated 5 to 6 wk after planting when the plants were approximately at the V-6 stage, or no more than 45 cm tall. This permitted the use of a tractor-drawn inoculator with minimal damage to the plants. Plots were evaluated for disease incidence at least twice, with the first evaluation in late August (12 to 14 wk after planting and 7 to 9 wk after inoculation), and the second evaluation two weeks later. A plant showing wilt and/or a basal stalk rot lesion was recorded as diseased, and the percent of diseased plants was calculated. Statistical analysis was done using SAS software.

Each year, starting in 2004, U.S. seed companies were asked to submit experimental or commercial hybrids for inclusion in both a stalk rot trial and a head rot trial, the latter which was conducted by personnel from North Dakota State University. Since both diseases are of major concern to U.S. producers, it was felt that information on a hybrid's performance against both diseases was essential. The stalk rot trials have been planted at five locations in eastern North Dakota and northwestern Minnesota each year. Four replications of single row plots, each 7 m long and on 75 cm centers, were planted, starting in late May, with the last location usually planted within three weeks of the earliest planting. A widely grown oilseed hybrid, Cargill 270, was chosen as the long-term susceptible check variety, while the resistant check was a hybrid produced using two USDA inbreds specifically developed for stalk rot resistance (HA 412 x RHA 409). There were six to eight rows of both the resistant and susceptible varieties per replication.

RESULTS

The use of a granular chemical applicator, driven by an electric motor, and mounted on a tractor driven cultivator, allowed us to uniformly deposit *Sclerotinia* mycelial inoculum (grown on millet) beside rows of young sunflowers. Initial symptoms of Sclerotinia wilt do not appear until 5 to 6 wk following inoculation, by which time the roots of the plants had grown and reached the inoculum. By having clear plastic tubes attached to the granular chemical applicator, a person could observe if the millet inoculum was flowing freely and thus minimize the possibility of rows not receiving a uniform dosage.

Each year from 2004 to 2007 some field trials had unforeseen problems, such as flooding, hail storms, extended drought, and downy mildew infestation, that either ruined the plot for Sclerotinia evaluations or made the results statistically insignificant. Thus, the number of stalk rot trials yielding usable information varied from two to four in any given year. In consultation with seed company researchers, a minimum of three statistically sound data sets were considered necessary for the data to be

published. In addition to presenting the information annually at the Sunflower Research Workshop (Gulya and Henson, 2006), the stalk rot and head rot ratings are submitted to North Dakota State University which annually publishes a bulletin on sunflower hybrid performance (containing agronomic data as well as disease ratings), available in hard copies and on-line (Berglund and Grady, 2006).

Starting in 2004, when 75 hybrids were tested, we observed that the gradation in disease incidence from the most resistant to the most susceptible hybrids was continuous, as would be expected from a polygenically controlled quantitative trait, which precludes categorizing the entries into discrete groupings such as "resistant" or "susceptible" (Fig. 3). In 2004, there were four hybrids with less infection than the resistant USDA check (21% infection), but there was no statistical difference between them and the check. While confection hybrids are generally considered to have less resistance to most diseases than oilseed hybrids, there were some confection hybrids with reasonably high levels of resistance, as shown by the banded bars in Fig. 3. For complete information on the performance of the hybrids tested, please consult NDSU publication A-652 (Berglund and Grady, 2006) which is revised annually with new data posted in January.

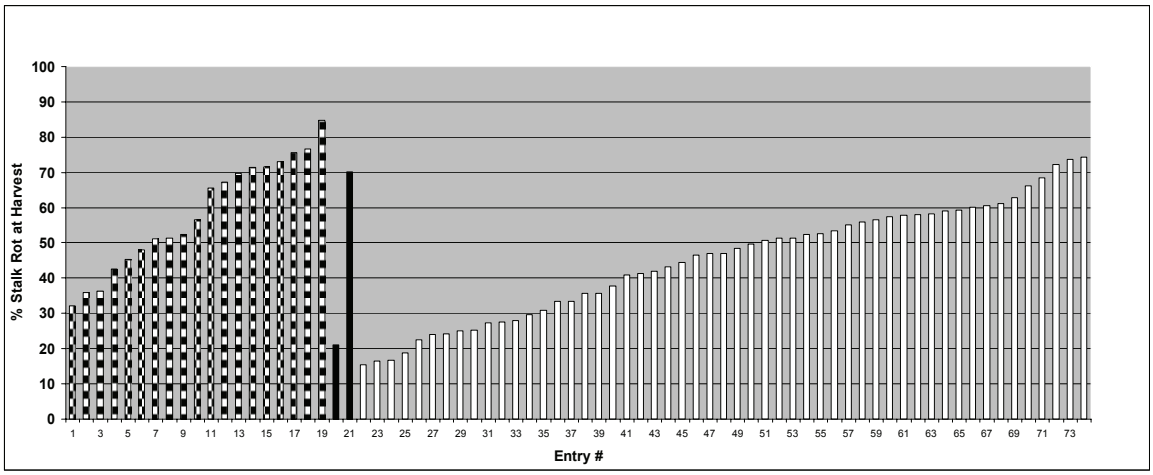


Fig. 3. Histogram of the stalk rot ratings (% diseased plants at physiological maturity) of 75 hybrid entries averaged over two locations in the 2004 tests. The banded bars on the left represent the confection hybrids while the remainder of the entries were oilseed hybrids. The black bars are the resistant check, with 21% infected plants, and the susceptible check, with 70% infected plants.

The stalk rot ratings of hybrids tested in subsequent years followed the pattern observed in the first year, with a continuous range of reaction and no discernible categories. While the range of infection varied from location to location and between years (Table 1), we were able to separate the most susceptible and the most resistant hybrids in each year. In 2007, for example, there were 18 hybrids which had stalk rot levels less than the resistant USDA check, but none of them were statistically different from each other (Fig. 4). Hybrids performing better than the resistant check were tested a second year, at up to five locations. Thus, under ideal conditions a hybrid may have been evaluated at up to 10 locations over two years.

Table 1. Summary statistics for stalk rot evaluations (% diseased plants at maturity) of commercial hybrids in inoculated field trials during 2004 to 2007.

	2004	2005	2006	2007
Number of Hybrids	75	89	97	97
Average % Stalk Rot	48	37	14	27
Minimum	15	10	2	3
Maximum	85	71	42	58
Number of Locations	2	3	4	3
Susceptible Check	70	54	23	35

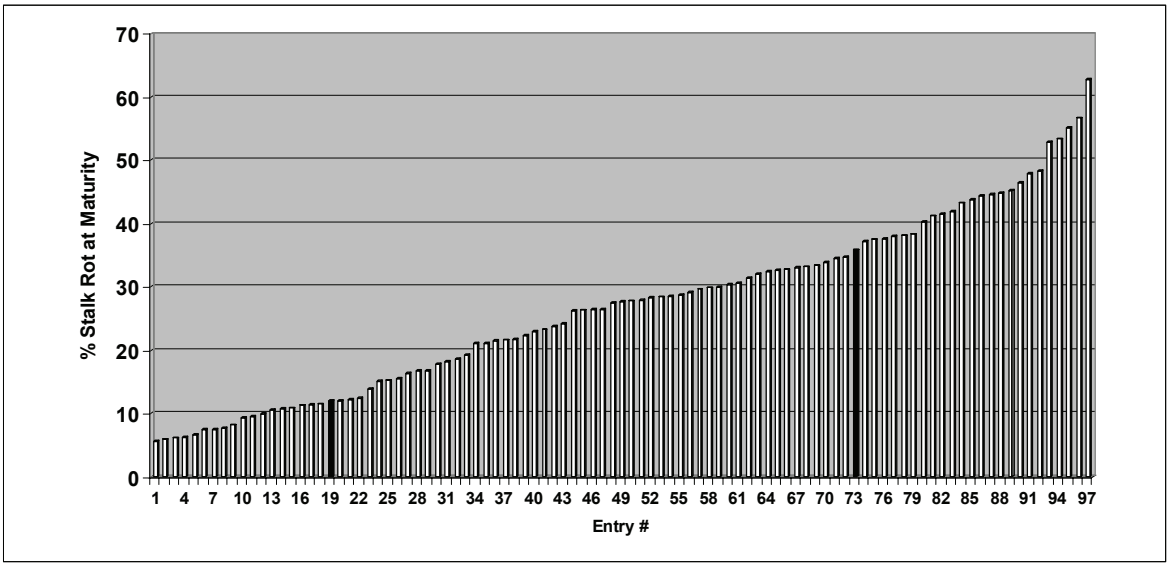


Fig. 4. Histogram of the stalk rot ratings of 97 commercial hybrids entered in the 2007 field trials, averaged over three locations. The black bar at position 19 is the resistant check, with 12% infected plants, and the black bar at position #73 is the susceptible check with 36% infected plants.

DISCUSSION

During the period 1970 to 2000, our USDA Sunflower Unit relied on fields naturally infested with *S. sclerotiorum* to screen sunflower for resistance to stalk rot. The uniformity of disease was often spotty and after repeated crops we often observed declines in disease incidence due to naturally occurring biological control. The field plots were also often located at long distances from our laboratory, all of which contributed to our decision to look for an artificial inoculation technique. Our initial method of placing a measured amount of inoculum beside each plant with a "corn jab planter" was satisfactory, but not practical with large numbers of rows requiring large numbers of people. Thus, the mechanization of the inoculation process not only produced a uniform amount of disease, but allowed us to greatly expand our efforts and test at multiple locations.

The current method of field testing for Sclerotinia stalk rot resistance is also used to evaluate USDA breeding material and other germplasm of interest. For example, the USDA Plant Introduction Collection of cultivated sunflower currently has ~ 800 accessions recently added which do not have stalk rot data listed in the USDA's Germplasm Resource Information Network (GRIN) database (http://www.ars-grin.gov/cgi-bin/npgs/html/crop.pl?7), so these are currently being evaluated, initially for stalk rot and subsequently for head rot resistance. While this method could be used for evaluating wild *Helianthus*, with the variable phenotype of each species, it may be more appropriate to either inoculate wild species by hand in the field, or in the greenhouse with a technique modeled after Grezes-Besset et al. (1994) and modified by Block et al. (2007, 2008).

We have made minor modifications to our stalk rot evaluation methods over the past four years. For example, to minimize the loss of plants due to downy mildew infection, we treat all seeds with a fungicide mix, regardless if they already have a commercial coating. We use fenamidone and zoximide (Gulya, 2002), at rates of 125 g and 250 g, respectively, which effectively protects against downy mildew with no effect on *Sclerotinia*. We have noted that field plots which do not receive any precipitation for several weeks following inoculation often develop little or no stalk rot. On small plots, this problem could be partially prevented by applying some water at the time of inoculation, or alternately, if drip or furrow irrigation were available, this also would minimize the negative impact of dry soils. We have yet to observe any decline in disease incidence at locations which are reused annually, but we do follow a three-year rotation at these locations. In an attempt to make statistical separation of entries more precise, we are continuing to study ways to improve our evaluations, including increasing the number of replications, modifying the experimental design, and increasing the amount of inoculum.

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REFERENCES

Berglund, D.R. 2007. 2006 National Sunflower Association survey: yield, cultural practices and yield limiting factors. In: Proc. 29th Sunflower Research Workshop, Fargo, ND, USA, 15 p. http://www.sunflowernsa.com/research/research-workshop/documents/Berglund_NSA_Survey_07.pdf

Berglund, D., and K.A. Grady. 2006. North Dakota and South Dakota hybrid sunflower performance testing. NDSU Extension Service Bulletin A-652 (revised). Available on-line at: http://www.ext.nodak.edu/extpubs/plantsci/rowcrops/a652.pdf

Block, C.C., T.J. Gulya, and L.F. Marek. 2007. Greenhouse evaluation of wild sunflower species for resistance to Sclerotinia wilt. Phytopathology 97:S11.

Block, C., T. Gulya, and L. Marek. 2008. Evaluation of wild sunflower species for resistance to Sclerotinia wilt. In: Proc 30th. Sunflower Research Workshop, Fargo, ND, USA. http://www.sunflowernsa.com/research/default.asp?contentID=338

Grezes-Besset, B.H., G. Tournade, O. Arnauld, R. Urs, P. George, P. Castellanet, and A. Toppan. 1994. A greenhouse method to assess sunflower resistance to Sclerotinia root rot and basal stem infection. Plant Breed. 112:215-222.

Gulya, T.J. 2002. Efficacy of single and two-way fungicide seed treatments for the control of metalaxyl-resistant strains of *Plasmopara halstedii* (sunflower downy mildew). In: The BCPC Conference - Pests & Diseases 2002. British Crop Protection Council. p. 575-580. In: Proceedings Brighton Crop Protection Conference, November 18-21, Brighton, UK.

Gulya, T.J. 2003. Sunflower crop survey – 2002. Disease assessment across eight states. In: Proc 25th Sunflower Research Workshop, Fargo, ND, USA, 10 pp http://www.sunflowernsa.com/research/research-workshop/documents/102.PDF.

Gulya, T.J. 2004a. Sunflower disease incidence and distribution in Midwestern U.S. in 2003. In: Proc. 26th Sunflower Research Workshop, Fargo. ND, USA, 9 p. http://www.sunflowernsa.com/research/research-workshop/documents/Gulya_Disease_Midwest_2003_04.PDF.

Gulya, T. J. 2004b. An inoculation method for Sclerotinia stalk rot. In: Proc. 26th Sunflower Research Workshop, Fargo, ND, USA, 6 p. http://www.sunflowernsa.com/research/research-workshop/documents/Gulya_StalkRot_04.PDF.

Gulya, T.J. 2005. A large-scale field inoculation method to induce Sclerotinia stalk rot in sunflower. In: 13th International Sclerotinia Workshop, June 12-16, Monterey, CA, USA.

Gulya, T.J., and R. Henson. 2006. Advances in managing Sclerotinia stalk rot of sunflower with resistant hybrids. In: Proc.28th Sunflower Research Workshop, Fargo, ND, USA, 9 p. http://www.sunflowernsa.com/research/research-workshop/documents/Gulya_Sclerotinia_06.pdf.

Lamey, A., M. Dietrich, and M. Draper. 2002. Sunflower crop survey in North Dakota and South Dakota. p. 1-11. In: Proc. 24th Sunflower Research Workshop, Jan 17-18. http://www.sunflowernsa.com/research/research-workshop/documents/45.pdf.

Mancl, M., and S. Shein. 1982. Field inoculation of sunflower for *Sclerotinia sclerotiorum* basal stalk rot and virulence of isolates from various hosts. p. 167-170 In: Proc. 10th. Int. Sunflower Conf., Surfers Paradise, Australia.

Study on an *in vitro* screening test for resistance to *Sclerotinia sclerotiorum* in sunflower

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ABSTRACT

An *in vitro* method that assayed callus induction on a medium amended with culture filtrate of *Sclerotinia sclerotiorum* was evaluated. Four double haploid R-lines obtained through the method of gamma-induced parthenogenesis at Dobroudja Agricultural Institute were involved (DH-R-128, DH-R-116, DH-R-7 and DH-R-2). The experiment was carried out at two levels, under field and laboratory conditions. After field infection, lines DH-R-128 and DH-R-116 demonstrated high to moderate resistance. Under laboratory conditions, *S. sclerotiorum* filtrate was added to the nutrition medium for callus induction from sunflower hypocotyl explants. Three variants of filtrate concentration in the nutrition medium were tested. The callus induction reaction of *Helianthus annuus* L. explants cultivated on a medium amended with *S. sclerotiorum* filtrate was evaluated. It was established that the higher filtrate concentrations suppressed the reaction of the explants to various degrees for the different lines. In lines DH-R-116 and DH-R-128 a better callus induction reaction was observed in comparison to the other two lines. The results showed that the test for resistance to *S. sclerotiorum* based on the callus induction allowed to identify materials with high to moderate resistance to the pathogen. The test cannot distinguish the differences between high and moderate levels in resistance and in susceptibility.

Key words: callus induction – *in vitro* test – resistance – *Sclerotinia sclerotiorum* – sunflower

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a very important oil seed crop worldwide and is a main source of vegetable oil in Bulgaria. Multiple factors determine the productivity of sunflower hybrids and varieties. In this respect, a significant effect is brought about by the causal agents of different diseases such as sclerotinia (*Sclerotinia sclerotiorum),* downy mildew (*Plasmopara helianthi)*, phoma (*Phoma macdonaldii)*, and phomopsis (*Phomopsis helianthi*). Each of these diseases can significantly decrease sunflower productivity, but *S. sclertiorum* (Lib.) de Bary is considered to be one of the most devastating pathogens distributed in almost all production regions. The losses may be up to 100% under suitable conditions (20 ^{o}C and 70% air humidity) in fields where the fungus is spread (Maširević and Gulya, 1992; Rashid, 1993). In spite of the efforts of many researchers, no chemical control on the spreading of the pathogen has been found yet (Nelson and Lamey, 2000). At this stage, the only way to reduce damage from a sclerotinia attack is by the development of forms with genetic resistance; this is a priority in a number of research projects and investigations (Ronicke et al., 2004, 2005). Simultaneously, alternative approaches have been sought for fast selection and screening of the new forms developed which have demonstrated some degree of resistance to the pathogen (Grezes-Besset et al., 1994; Verzea et al., 2004).

The aim of this investigation was to study the effect of the cultural filtrate from *S. sclertiorum* on the callus induction reaction of cultivated sunflower (*Helianthus annuus* L.) and to find out if there is a correlation between *in vitro* and *in vivo* reaction of the plant material to the pathogen.

MATERIALS AND METHODS

The investigation was carried out at two levels – under laboratory and field conditions. We worked with four doubled haploid R-lines developed by the method of gamma-induced parthenogenesis at Dobroudja Agricultural Institute, Bulgaria. The lines were of different origin: lines DH-R-116 and DH-R-128 were produced from hybrid materials with parental forms obtained as a result of interspecific hybridization; lines DH-R-2 and DH-R-7 were obtained from *H. annuus* L. hybrids.

Preparation of inoculum and inoculation of lines under field conditions

Ten plants from each of the studied lines were inoculated by the Straw-method (Encheva and Kiryakov, 2002) at stage 5-6th pair of leaves. A petiole of the fourth pair of leaves from each plant was cut, so that 3 cm of it was left on the stem. A plastic straw (30 x 6 mm) with one end closed was inserted in the place of

incision. The straw contained an agar disc from the periphery of a 3 day old culture of isolate Ss-1 on nutrient medium PDA at 22±1°C. The reaction of the plant was rated three times every 7 days according to a 6-degree scale as follows: 0 – no symptoms at the place of inoculation; 1 - a whitish spot on the petiole (high resistance); 2 – a spot at the base of the petiole reaching to the stem (resistance); 3 – a spot spreading on a part of the stem (intermediate resistance), 4 – a spot spreading on the entire stem (susceptibility); 5 – breaking of the stem (high susceptibility). The last rating of the plant's reaction determined the final evaluation of their resistance to the pathogen.

Laboratory methods

S. sclerotiorum isolate SsPh1 was cultivated on a liquid medium (PDB) for seven days at room temperature (22-25°C), then it was filtered by the method of Miklas et al. (1992) applied to bean. Following cold sterilization in a laminar box, the obtained filtrate was stored at +4° C within 7 days.

Twenty seeds from each line were sterilized in 2.5% solution of potassium hypochlorite for 20 minutes; after the seed coat was removed, the seeds were then plated on medium MS (Murashige and Skoog, 1962) for formation of young plantlets. After 7-9 days cultivation, hypocotyl explants were removed and transferred onto medium for callus induction. The medium consisted of MS + 1.5 mg/l NAA + 0.5 mg/l BAP amended with *S. sclerotiorum* cultural filtrate. Four variants were tested depending on the amount of added filtrate: variant 1 (control, without added filtrate) and variants with added filtrate in the following concentrations: 5 ml/l (variant 2); 10 ml/l (variant 3); and 20 ml/l (variant 4). The number of calli induced was evaluated after one month of cultivation.

The experiment was designed in 5 replications (5 Petri dishes per variant for each line). Cultivation of explants was done under controlled conditions: 25±1ºC, light 6000 lux and photoperiod 16/8 hr.

RESULTS AND DISCUSSION

Field evaluation

After infection under field conditions best results were observed in line DH-R-128, where the reaction of infected plants was within the range 0-3 (Table 1).

Table 1. Field evaluation of the reaction of double haploid R lines to artificial infection with *S. sclerotiorum* inoculum

Genotype	**Plant reaction to *Sclerotinia sclerotiorum***									
plant №	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***
DH-R-128	3	1	1	0	1	0	0	2	0	1
DH-R-116	1	1	2	3	3	3	1	1	2	2
DH-R-2	2	4	4	3	2	4	3	4	3	4
DH-R-7	5	5	5	5	5	5	5	5	5	5

In contrast to line DH-R-128, all plants of line DH-R-7 were completely damaged by the pathogen. A moderate reaction was observed in the other two lines, inclining towards the moderate resistance margin in line DH-R-116 and towards the susceptibility margin in line DH-R-2. Taking the origin of the lines as a starting point, no definite conclusion about their reaction to the disease can be reached; however, the expression of this reaction can be analyzed generally. Emphasis is mainly placed on the wild species of genus *Helianthus* as a main source of resistance genes to both *S. sclerotiorum* and all diseases of sunflower of economic importance (Škorić, 1992; Cerboncini et al., 2002). This, to a great extent, confirmed the result we obtained in line DH-R-128, and partially, in line DH-R-116. Parallel to this, forms having a lower susceptibility to sclerotinia attack have been found in cultivated sunflower as well (Castano et al., 1993; Degener et al., 1999 a,b). In the present study, the line DH-R-2 can be referred to as being in this category.

Callus induction reaction

All DH-R-lines had normal processes of callus induction in the control (variant 1); line DH-R-128 showed a slightly weaker reaction than the rest of the lines (Table 2).

The higher concentrations of the filtrate prevented the callus induction reaction of the plants, the degree of suppression being different for the different lines. Regardless of the differences, the general trend of the reaction of the lines is expressed as a progressive decrease in callus initiation with the increase in the *S. sclerotiorum* filtrate concentration (Fig. 1).

Table 2. Callus induction in a culture of hypocotyls explants of *Helianthus annuus* L. on a medium amended with *Sclerotinia sclerotiorum* filtrate

Genotype		Number of plated explants							Number of calli obtained						
DH-R-116	**Replicates**	**1**	**2**	**3**	**4**	**5**	**Σ**	**X mean**	**1**	**2**	**3**	**4**	**5**	**Σ**	**X mean**
	variant 1	28	32	31	30	31	**152**	**30.4**	28	32	31	30	31	**152**	**30.4**
	variant 2	30	29	20	40	34	**153**	**30.6**	30	29	20	37	30	**146**	**29.2**
	variant 3	32	31	29	29	33	**154**	**30.8**	32	31	26	18	30	**137**	**27.4**
	variant 4	32	16	43	32	41	**164**	**32.8**	22	7	9	15	11	**64**	**12.8**
DH-R-128	**Replicates**	**1**	**2**	**3**	**4**	**5**	**Σ**	**X mean**	**1**	**2**	**3**	**4**	**5**	**Σ**	**X mean**
	variant 1	25	33	29	30	29	**146**	**29.2**	25	33	29	25	21	**133**	**26.6**
	variant 2	25	37	32	29	32	**155**	**31.0**	19	33	30	19	32	**133**	**26.6**
	variant 3	33	28	23	25	25	**133**	**26.6**	12	10	17	7	12	**58**	**11.6**
	variant 4	31	28	19	27	28	**133**	**26.6**	11	0	4	17	7	**39**	**7.8**
DH-R-2	**Replicates**	**1**	**2**	**3**	**4**	**5**	**Σ**	**X mean**	**1**	**2**	**3**	**4**	**5**	**Σ**	**X mean**
	variant 1	24	28	16	20	25	**113**	**22.6**	24	26	14	20	23	**107**	**21.4**
	variant 2	20	22	25	21	24	**112**	**22.4**	2	9	11	6	7	**35**	**7.0**
	variant 3	29	21	25	30	26	**131**	**26.2**	0	0	0	0	15	**15**	**3.0**
	variant 4	30	25	22	20	33	**130**	**26.0**	0	0	0	0	0	**0**	**0**
DH-R-7	**Replicates**	**1**	**2**	**3**	**4**	**5**	**Σ**	**X mean**	**1**	**2**	**3**	**4**	**5**	**Σ**	**X mean**
	variant 1	20	25	28	22	23	**118**	**23.6**	15	25	28	22	23	**113**	**22.6**
	variant 2	23	17	17	20	22	**99**	**19.8**	11	6	3	7	5	**32**	**6.4**
	variant 3	17	21	17	23	20	**98**	**19.6**	0	3	3	4	0	**10**	**2.0**
	variant 4	24	26	22	20	22	**114**	**22.8**	1	0	0	1	0	**2**	**0.4**

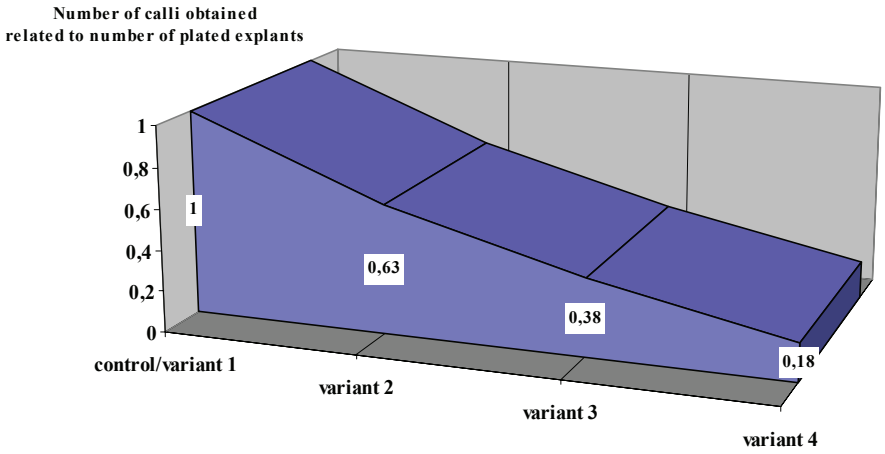

Fig. 1. General evaluation of the callus induction reaction on the investigated lines according to *S. sclerotiorum* filtrate concentration.

This trend was expressed to a higher degree in lines DH-R-2 and DH-R-7; in variants 3 and 4 their callus induction intensity was zero (Fig. 2).

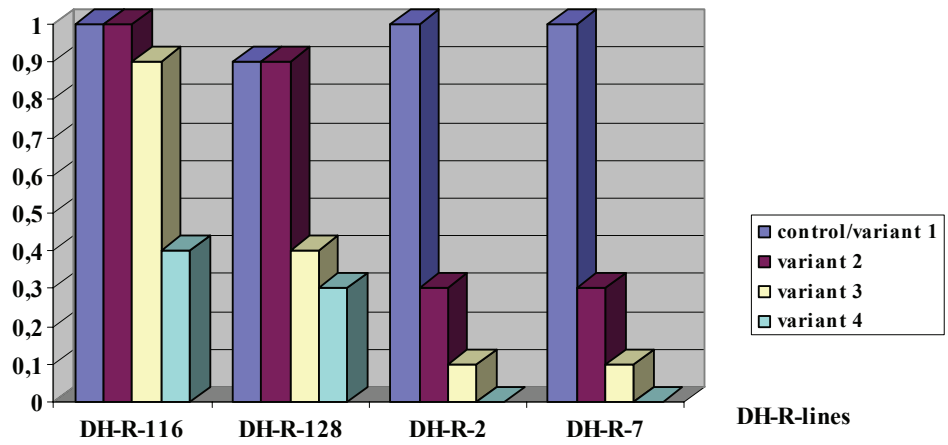

Fig. 2. Callus induction reaction of sunflower DH-R-lines according to the genotype and *S. sclerotiorum* filtrate concentration in the nutrient medium.

Differences were not observed in lines DH-R-116 and DH-R-128 between the control and variant 2. The callus induction in these lines was impeded by the higher concentrations of the filtrate in variants 3 and 4, but, in contrast to the other two investigated lines, callus initiation was not completely blocked.

The results from the *in vitro* investigation showed that line DH-R-116 had the best callus induction reaction in all four variants. Some similarity in the reactions was established for lines DH-R-116 and DH-R-128, with the exception of variant 3, where a sharp decrease in the callus genesis of line DH-R-128 was observed. This decrease was observed in variant 4 of line DH-R-116. In variant 4, the differences between the two lines were insignificant.

The comparison between the other two lines (DH-R-2 and DH-R-7) did not show any significant differences in the callus initiation of all four variants.

The comparison of the results from the field evaluation of the resistance to the *in vitro* reaction of the investigated lines revealed a certain correspondence expressed in the generalization that lines DH-R-128 and DH-R-116 showed resistance under field conditions and the best callus induction reaction, and lines DH-R-2 and DH-R-7 showed a susceptibility to *S. sclerotiorum* under field conditions and weaker in the *in vitro* reaction. By considering the results in detail, the fact comes out that although line DH-R-128 had the best evaluation in the field trial, it ranked second after line DH-R-116 under laboratory conditions. The lack of differences in the *in vitro* reaction of line DH-R-2, which had a relatively lower level of susceptibility to *S. sclerotiorum* under field conditions than line DH-R-7, also indicated that the laboratory test should most probably be more precise involving additional steps/variants between variant 3 and variant 4, so that the differences between the lines could be expressed.

CONCLUSIONS

The *in vitro* test for resistance to *S. sclerotiorum* based on the callus induction reaction is good enough to differentiate high to moderate resistant materials from materials with high to moderate susceptibility to the pathogen. The test cannot detect the differences between high and moderate levels in resistance and in susceptibility.

REFERENCES

Castano, F., F. Vear, and D.T. Labrouhe. 1993. Resistance of sunflower inbred lines to various forms of attack by *Sclerotinia sclerotiorum* and relations with some morphological characters. Euphytica 68:85-98.

Cerboncini, C., G. Beine, P.C. Binsfeld, B. Dresen, H. Peisker, A. Zerwas, and H. Schnabl. 2002. Sources of resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary in a natural *Helianthus* gene pool. Helia 25(36):167-176.

Degener, J., A.E. Melchinger, and V. Hahn. 1999a. Breeding for *Sclerotinia* resistance in sunflower: A modified screening test and assessment of genetic variation in current germplasm. Plant Breed. 117:367-372.

Degener, J., A.E. Melchinger, and V. Hahn. 1999b. Optimal allocation of resources in evaluating current sunflower inbred lines for resistance to *Sclerotinia*. Plant Breed. 118:157-160.

Encheva, V., and I. Kiryakov. 2000. A method for evaluation of sunflower resistance to *Diaporthe*/*Phomopsis helianthi* Munt. - Cvet et al. Bulgarian J. Agric. Sci. 8:219-222.

Grezes-Besset, B., G. Tournade, O. Arnauld, R. Urs, P. George, P. Castellanet, and A. Toppan. 1994. A greenhouse method to assess sunflower resistance to *Sclerotinia* root and basal stem infections Plant Breed. 112:215-222.

Maširević, S., and T.J. Gulya. 1992. *Sclerotinia* and *Phomopsis* - two devastating sunflower pathogens. Field Crops Res. 30:271-300.

Miklas, P.N., K. Grafton, G.A. Secor, and P.E. McClean. 1992. Use of pathogene filtrate to differentiate physiological resistance of dry bean to white mold desease. Crop Sci. 32:310-312.

Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Nelson, B., and A. Lamey. 2000. *Sclerotinia* diseases of sunflower. NDSU Extension Service.

Rashid, K. 1993. *Sclerotinia* head rot and mid-stem infection of sunflower in Manitoba. In: Proc. 5th Sunflower Res. Workshop, Fargo, ND, 14-15 Jan., Natl. Sunflower Assoc., Bismarck, ND, USA.

Ronicke, S., V. Hahn, and W. Friedt. 2005. Resistance to Sclerotinia sclerotiorum of 'high oleic' sunflower inbred lines. Plant Breed. 124:376–381.

Ronicke, S., V. Hahn, R. Horn, I. Grone, L. Brahm, H. Schnabl, and W. Friedt. 2004. Interspecific hybrids of sunflower as a source of *Sclerotinia* resistance. Plant Breed. 123:152-157.

Škorić, D. 1992. Results obtained and future directions of wild species use in sunflower breeding. p. 1317-1348. In: Proc. 13th Int. Sunflower Conf., Pisa, Italy.

Verzea, M., F. Raducanu, M. Baldini, S. Raranciuc, and I. Moraru. 2004. Evaluation of genetic variability of some sunflower genotypes to the *Sclerotinia sclerotiorum* by different resistance tests and AFLP analyses. Cercetari de Genetica Vegetala si Animala,Vol. 8.

EST-derived markers highlight genetic relationships among *Plasmopara halstedii* French races

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ABSTRACT

Twelve EST-derived markers were isolated from *Plasmopara halstedii* (Oomycetes), the causal agent of sunflower downy mildew. A total of 25 single nucleotide polymorphisms (SNPs) and five indels were detected by single-strand conformation polymorphism (SSCP) analysis and developed for high-throughput genotyping of 24 isolates. These markers reveal a good level of genetic diversity and were useful in detecting genotypic variability of French populations. A deficit in heterozygotes indicated that *P. halstedii* is a selfing species. For the first time, these markers allowed to reveal genetic relatedness among 14 races characterized over a 19-year period of *P. halstedii* monitoring in France.

Keywords: evolution – pathotype – SNP – sunflower downy mildew – virulence profile.

INTRODUCTION

Sunflower downy mildew due to *Plasmopara halstedii* (Berlese & de Toni) is potentially one of the most damaging diseases in sunflower. *P. halstedii* is a homothallic oomycete that alternates one sexual generation with several asexual generations. It is an obligate endoparasite that cannot be cultivated independently from its plant host. *P. halstedii* develops gene-for-gene interactions with its host *Helianthus annuus* and presents several physiological races known as pathotypes. Genetic resistance in cultivated sunflower varieties is the most efficient control method against the disease but the efficiency of major resistance genes is regularly challenged. To date, at least 20 different pathotypes have been described in different parts of the world (Tourvieille de Labrouhe et al., 2000).

Our understanding of the recurrent breakdown of sunflower major resistance genes can be improved based on new findings concerning the key processes governing the evolution of *P. halstedii* populations. Indeed, knowledge of the evolutionary potential of plant pathogen species has improved the management of resistance genes and maximized their durability in space and time (McDonald and Linde, 2002). The population genetics approach can be used to evaluate the major forces driving pathogen evolution, i.e. selection, mutation, recombination, genetic drift and gene flow. Previous genetic studies of *P. halstedii* in Europe have reported low levels of genetic and genotypic diversity in this species, with no clear genetic structure revealed with RAPD markers (Komjati et al., 2004; Roeckel-Drevet et al., 1997, 2003), ISSR (Intelmann and Spring, 2002) or ITS sequences (Spring et al., 2006). This precluded any reliable conclusions on the mode of reproduction, genetic structuring or the extent to which pathotypes are related in this species. Single nucleotide polymorphisms (SNPs) are promising molecular markers for population genetics as they are widespread throughout the genome, co-dominant, specific and have a high resolving power. The development of new methods for screening for DNA polymorphism has rendered possible the extensive development of such makers for plant pathogen species. With a total of 174 nucleotide sequences available in the international nucleotide sequence database, *P. halstedii* is a typical example of a non-model organism for which genomic resources are very scarce. We used the 145 cDNA sequences available to design a set of EST-derived markers that may be used for future population genetic studies. Here we report the characterization of 12 polymorphic markers based on SNPs and size variations (insertion-deletion) in Expressed Sequence Tags (ESTs) of *P. halstedii* and the development of high-throughput genotyping methods for 10 of these markers. We used these 12 EST-derived markers to perform a genetic analysis of the "reference races" of *P. halstedii* characterized over a 19-year period of monitoring in France (1988-2006).

MATERIALS AND METHODS

Sampling. We analyzed 24 isolates of *P. halstedii* collected in France between 1966 and 2006. Fourteen of these isolates are "references isolates", corresponding to the first description of the pathotype concerned in France (Table 1). The other 10 isolates (Table 1) were obtained from the French Plant Protection Service monitoring program (Moinard et al., 2006).

Virulence profile determination. Downy mildew pathotypes are defined on the basis of virulence profiles on a set of differential hosts carrying different *Pl* resistance genes. Resistance tests were performed as described by Cohen and Sakston (1974), with the modifications proposed by Mouzeyar et al. (1993): 15 days after inoculation, plants were incubated for 48 h in a saturated atmosphere. Plants were scored as susceptible if sporulation was observed in cotyledons and leaves, and as resistant if no sporulation or only light sporulation was seen on cotyledons. Pathotypes were named according to the international nomenclature of *P. halstedii* pathotypes proposed by Gulya et al. (1998) (Table 2).

Table 1. Race (pathotype), collection site and date of isolation in France for the 24 isolates of *Plasmopara halstedii*. The star (*) indicates isolates corresponding to the “reference pathotype”.

Race	Collection site ("*département*")	Year of collection
100*	Unknown	1966
100	Charente-Maritime	1993
100	Cher	1993
710*	Indre	1988
710	Cher	1993
710	Unknown	2000
710	Maine-et-Loire	2004
710	Deux-Sèvres	2006
703*	Lot-et-Garonne	1989
703	Tarn	1993
703	Lot-et-Garonne	2001
703	Haute-Garonne	2004
703	Gers	2006
300*	Aude	1995
700*	Haute-Garonne	1995
304*	Aude	2000
314*	Manche	2001
307*	Haute-Garonne	2002
704*	Deux-Sèvres	2002
714*	Gers	2002
334*	Charente	2004
707*	Lot-et-Garonne	2004
717*	Gers	2004
730*	Tarn	2005

Table 2. Name of race (pathotype), date of first isolation in France and virulence profiles for the 14 French reference isolates of *Plasmopara halstedii*.

Race name[1]	Isolation year	Virulence profiles according to differential hosts[2]								
		D1	D2	D3	D4	D5	D6	D7	D8	D9
100	1966	S	R	R	R	R	R	R	R	R
710	1988	S	S	S	S	R	R	R	R	R
703	1989	S	S	S	R	R	R	S	S	R
300	1995	S	S	R	R	R	R	R	R	R
700	1995	S	S	S	R	R	R	R	R	R
304	2000	S	S	R	R	R	R	R	R	S
314	2001	S	S	R	S	R	R	R	R	S
307	2002	S	S	R	R	R	R	S	S	S
704	2002	S	S	S	R	R	R	R	R	S
714	2002	S	S	S	S	R	R	R	R	S
334	2004	S	S	R	S	S	R	R	R	S
707	2004	S	S	S	R	R	R	S	S	S
717	2004	S	S	S	S	R	R	S	S	S
730	2005	S	S	S	S	S	R	R	R	R

[1] according to Gulya et al. (1998)
[2] S: susceptible (compatible interaction); R: resistant (incompatible interaction)

Molecular markers. A total of 124 ESTs of *P. halstedii* were screened for their polymorphism by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). DNA extraction was performed on infected plant tissue as previously described for *Plasmopara viticola* by Delmotte et al. (2006). Marker amplification reactions were carried out in a final volume of 25 µl containing 10 ng of genomic DNA, 2 mM of MgCl2, 150 µM of each dNTP, 4 pmol of each primer and 0.2 U *Taq* Silverstar DNA polymerase (Eurogentec) in reaction buffer. Reactions were performed with the following program: an initial denaturation step of 4 min at 96°C, followed by 38 cycles of 40 s denaturation at 96°C, 50 s annealing at 57°C, 90 s elongation at 72°C, and a final elongation step of 10 min at 72°C. Sequence polymorphism was revealed on a 6% non-denaturing polyacrylamide gel with migration at 4°C at 10 W overnight. The gel was silver-stained. For each of the different profiles of polymorphic EST markers, five alleles were sequenced in order to determine the mutations responsible for the polymorphism.

Statistical analyses. Genepop version 3.2a was used to calculate expected and observed heterozygosities, unbiased estimates of F_{IS} and F_{ST} and to perform exact tests for departure from Hardy-Weinberg equilibrium. The phylogenetic relationships between French pathotypes were investigated by building a neighbor-joining (NJ) tree based on allele shared distance (D_{AS}), using Populations 1.2.28 software. Bootstrap support for the nodes was calculated over 10,000 replications, using the same program. To describe the genetic structure of *P. halstedii* pathotypes, we also applied a Bayesian approach of genetic mixture analysis using the software Structure v2.2.

RESULTS

Molecular markers. Among the 124 ESTs tested by PCR-SSCP, only 12 were found to be polymorphic (9.6%). A total of five indels and 25 SNPs were revealed, one locus (Pha79) presenting 18 SNPs among the 25 (Table 3). The frequency of SNPs in coding regions was 0.52 SNP per kb and was 0.15 when the most polymorphic locus Pha79 was excluded. Five markers presented size polymorphism, with the

number of inserted or deleted nucleotides varying in a range from 2 to 63. For the marker Pha42, the deletion and SNP were linked so there were only two alleles at this locus.

Five SNPs were transformed into Cleaved Amplified Polymorphism Sequence (CAPS) markers. Four indel polymorphisms were automated on a Beckman Coulter Ceq8000 capillary sequencer using the manufacturer's recommendations and one was directly visualized on agarose gel. Finally, two markers were screened by PCR-SSCP since no enzyme discriminating the alleles could be found. The following protocol was used for CAPS markers: 1 µl of PCR product digested by 0.1 U restriction enzyme in 10X enzyme buffer for 1 hour at the appropriate temperature.

Table 3. Characterization and description of 12 EST-derived markers for *Plasmopara halstedii*: locus name, Genbank accession number, homology of sequences, primer sequences, polymorphism type, annealing temperature of primers and genotyping method used are shown.

Locus name	Genbank acc. no.	Homology	Primer sequences (5'-3')	Polymorphism	T_a (°C)	Genotyping method
Pha6	CB174585	Transportin	F: GTCGCTGATTTTATGTTTATGTGC R: TACTACCTCAGTCACATCATCACC	SNP	57	CAPS (T*sp*45I)
Pha39	CB174648	Hypothetical protein	F: GATTGGGTTCCTTGTTTGGA R: ATCTTCGCTGCCAGCTTCT	Indel	57	Sequencer
Pha42	CB174650	Hypothetical protein	F: GGATGTTGCTCGTCAAGTAGC R: ACGCATCCTACGCATTCAAC	indel	57	Sequencer
Pha43	CB174680	Hypothetical protein	F: ACTCAGGACTGGGCAACAAT R: CGACATCCTTGTGAGCTTGT	indel	57	Sequencer
Pha54	CB174708	Hypothetical protein	F: ATTTGGCAACGTCTCAGAGC R: CCATCGTAATAACATTCTTTAAAGTCC	SNP	57	CAPS (F*au*I)
Pha56	CB174714	40S ribosomal protein S2	F: GCGGTACTGGTCTATGTGCTG R: TTCAAGAAGTTTGATTTTTCATGC	SNP	57	CAPS (O*li*I)
Pha74	CB174642	Hsp 90	F: ACCTCGCATGGTTGCTTTAC R: TTGCTATTTCGGCCTACTGG	indel	57	Agarose
Pha79	CB174692	Hypothetical protein	F: GACGCCCCACTTAGCTTTC R: TTCGGGAGTAAGTGATTGAGC	SNP	57	SSCP
Pha82	CB174573	MMSDH[1]	F: ACTCGATCCATGCAGTAAGTAAG R: AGGAGGCTTTGCAGATTGAA	SNP	57	CAPS (B*sp*MI)
Pha99	CB174703	Hypothetical protein	F: CTCGCATTCAAACGGAAAAT R: CAAGCCAACTGTGCATGAAT	SNP	57	CAPS (B*sr*DI)
Pha106	CB174676	Hypothetical protein	F: TTGACGTTTATGCGAAGTGC R: CAAAGGAAGTTGTGATGGTGAG	Indel	57	Sequencer
Pha120	CB174660	Hypothetical protein	F: CTATTTAAAGGGGCCCGAAC R: CGGGTTTCCTCCATTAATCC	SNP	57	SSCP

[1]MMSDH: methylmalonic semialdehyde dehydrogenase.

Genotypic structure. Based on combinations of the 12 genomic markers, we identified 11 different multilocus genotypes among the 24 isolates analyzed. Three multilocus genotypes were found in multiple copies. A combination of eleven EST markers was sufficient to discriminate all the multilocus genotypes in the dataset, demonstrating the high resolving power of the markers (Fig. 1). Three pathotypes were represented by more than one isolate (race 100 represented by 3 isolates, races 703 and 710 each represented by 5 isolates). Isolates of the same pathotype presented an identical multilocus genotype, indicating that the three pathotypes may correspond to three clonal lineages. Conversely, two multilocus genotypes included more than one pathotype: the first multilocus genotype comprised pathotypes 100, 300 and 304 and the second comprised pathotypes 307 and 703.

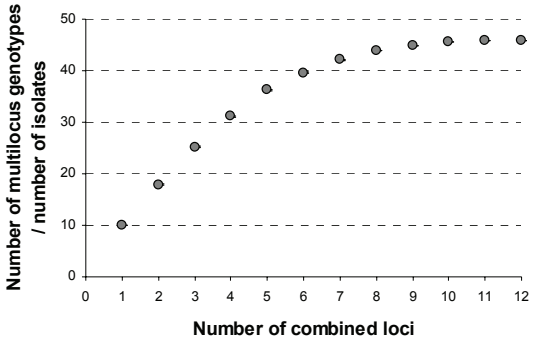

Fig. 1. Number of genotypes discriminated (number of multilocus genotypes/total number of isolates) as a function of the number of loci combined to discriminate isolates of *Plasmopara halstedii.*

Population genetic structure. The expected heterozygosity level was consistent with high levels of genetic diversity across loci ($0.349<H_E<0.677$). However, observed heterozygosity levels were much lower, with a mean H_O of 0.026. Only three of the 11 distinct multilocus genotypes were heterozygous: two at two loci and one at one locus. Tests of deviation from Hardy-Weinberg equilibrium revealed significant strong heterozygote deficits with respect to expectations under the assumption of random mating. All loci presented significant and positive F_{IS} values, with an overall F_{IS} of 0.948.

Bayesian assignment analyses showed three genetic groups of isolates: the first cluster was constituted by a single multilocus genotype including 3 pathotypes: 100, 300 and 304. The second cluster included 4 multilocus genotypes and 5 pathotypes: 703, 307, 700, 730, 707. The third cluster included 6 pathotypes with different multilocus genotypes: 710, 334, 314, 714, 717 and 704. Pathotypes 334, 707 and 730 were clearly 'mixed' genotypes that presented alleles belonging to two different clusters (Fig. 2).

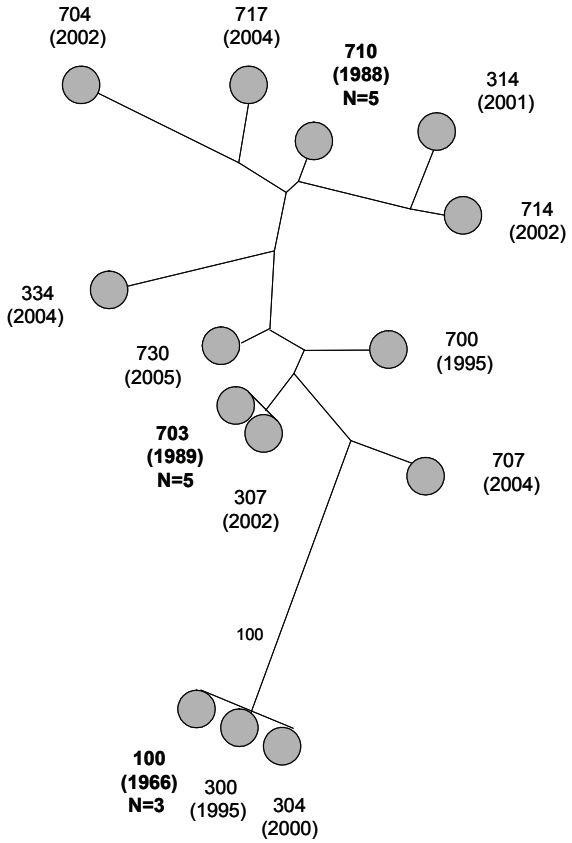

Fig. 2. NJ clustering from the allele shared distance matrix calculated among the 14 pathotypes of *Plasmopara halstedii* based on 12 EST-derived markers. Date of the first description in France of each pathotype is indicated in brackets. Pathotypes 100, 703 and 710 were represented by three to five isolates (N) and isolates belonging to one same pathotype presented an identical multiclocus genotype. Numbers above branches of the tree are bootstrap percentages after 10,000 re-samplings.

DISCUSSION

The EST-derived markers used in this study displayed high levels of intraspecific polymorphism which made it possible to infer the reproduction mode of *P. halstedii* and to assess the relationships among French pathotypes. These markers are specific and could therefore be used for the high-throughput genotyping of isolates directly from sporulating lesions collected from host leaves, avoiding the need for labor-intensive isolate subculture. *P. halstedii* is a homothallic species, and is therefore able both to

outcross and to self. However, our results suggest that *P. halstedii* is mainly a selfing species, with only limited outcrossing.

The finding that *P. halstedii* pathotypes cluster into three genetically differentiated groups, each including one of the first races described in France (i.e. 100, 703, 710), sheds new light on sunflower downy mildew evolution. Races 100, 703 and 710 correspond to three clonal lineages that not only present a strong genetic differentiation but also have very distinct virulence profiles. Given the strong geographic structuration of race distribution in France, we hypothesize that these results reflect (at least) three different introductions of this pathogen in France: the first introduction corresponds to race 100 (before 1966) that is now widely distributed in France, the second to race 710 in the North of France (before 1988) and the third to race 703 in South-West of France (before 1989). From then on, the triple introduction of *P. hasltedii* might have provided the raw genetic materials for more complex evolutionary processes of race emergence such as recombination between pathotypes or accumulation of mutation in clonal lineages (further referred as clonal evolution). These three introductions of *P. halstedii* could have provided the raw genetic materials for more complex evolutionary processes, such as recombination between pathotypes or the accumulation of mutations in clonal lineages (clonal evolution), in the emergence of new races.

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REFERENCES

Cohen, Y., and W. E. Sackston. 1974. Seed infection and latent infection of sunflower by *Plasmopara halstedii*. Can. J. Bot. 52:231-238.

Delmotte, F., W. J. Chen, S. Richard-Cervera, C. Greif, D. Papura, X. Giresse, G. Mondor-Genson, and M. F. Corio-Costet. 2006. Microsatellite DNA markers for *Plasmopara viticola*, the causal agent of downy mildew of grapes. Mol. Ecol. Notes 6:379-381.

Gulya, T., D. Tourvieille de Labrouhe, S. Masirevic, A. Penaud, K. Rashid, and F. Viranyi. 1998. III Symposium Sunflower Downy Mildew, Fargo, ND, USA.

Intelmann, F., and O. Spring. 2002. Analysis of total DNA by minisatellite and simple-sequence repeat primers for the use of population studies in *Plasmopara halstedii*. Can. J. Microbiol. 48:555-559.

Komjati, H., C. Fekete, and F. Viranyi. 2004. Genetic and molecular characterization of *Plasmopara halstedii* isolates from Hungary. p. 193-201. In: P. Spencer-Philips and M. Jeger (eds), Advances in downy mildew research, vol. 2. Kluwer Academic Publisher, London, UK.

McDonald, B. A., and C. Linde. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Ann. Rev. Phytopathol. 40:349-379.

Moinard, J., E. Mestries, A. Penaud, X. Pinochet, D. Tourvieille de Labrouhe, F. Vear, M.-C. Tardin, I. Pauchet, and N. Eychenne. 2006. Le mildiou du tournesol: panorama du pathogène. Phytoma 589:34-38.

Mouzeyar, S., D. T. d. Labrouhe, and F. Vear. 1993. Histopathological studies of resistance of sunflower (*Helianthus annuus* L.) to downy mildew (*Plasmopara halstedii*). J. Phytopathol. 139:289-297.

Roeckel-Drevet, P., V. Coelho, J. Tourvieille, P. Nicolas, and D. Tourvieille de Labrouhe. 1997. Lack of genetic variability in French identified races of *Plasmopara halstedii*, the cause of downy mildew in sunflower *Helianthus annuus.* Can. J. Microbiol. 43:260-263.

Roeckel-Drevet, P., J. Tourvieille, T. J. Gulya, G. Charmet, P. Nicolas, and D. Tourvieille de Labrouhe. 2003. Molecular variability of sunflower downy mildew, *Plasmopara halstedii*, from different continents. Can. J. Microbiol. 49:492-502.

Spring, O., M. Bachofer, M. Thines, A. Riethmuller, M. Goker, and F. Oberwinkler. 2006. Intraspecific relationship of *Plasmopara halstedii* isolates differing in pathogenicity and geographic origin based on ITS sequence data. Eur. J. Plant Pathol. 114:309-315.

Tourvieille de Labrouhe, D., E. Pilorgé, P. Nicolas, and F. Vear. 2000. Le mildiou du tournesol. INRA and CETIOM, Paris, France.

Effect of sowing date and initial inoculum of *Alternaria helianthi* on sunflower in the south region of Brazil

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ABSTRACT

Three field experiments were carried out in Londrina, PR, south region of Brazil, in 1997/1998, 1998/1999 and 1999/2000 growing seasons, to investigate Alternaria leaf spot severity in sunflower sown at different dates and the effect of inoculum of the first sowing dates on subsequent ones. Four sowing dates (October, November, December and January) and two sowing types (contiguous and isolated) were used to simulate different levels of initial inoculum. Disease severity, under natural conditions in the field, was evaluated weekly, with reference to a diagrammatic scale of this disease, used to calculate the value of the area under disease progress curve (*AUDPC*). Marked plants were harvested individually, for evaluation of grain yield. Disease severity was highest when plants were sown in December regardless of the year. Sowing sunflower in October resulted in high yield and low disease severity. Sanitation measures to reduce initial inoculum concentration delayed the onset of the disease by 11 days.

Key words: *Alternaria* leaf spot – epidemiology – *Helianthus annuus* – primary inoculum.

INTRODUCTION

The potential for increasing sunflower (*Helianthus annuus* L.) cultivated area in Brazil can be limited by leaf blight and stem spot diseases, caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara. The disease is reported to affect leaves, stem and sunflower heads, causing necrotic brown to black lesions, with a round or angular shape. Due to the frequent occurrence of climate conditions favorable for disease epidemics, i.e. high relative humidity and temperatures between 25°C and 30°C, Alternaria leaf blight is the most important sunflower disease in Brazil, occurring in all regions and sowing dates (Leite, 2005).

For many foliar diseases, once an epidemic has been initiated, infectious lesions within the crop are the predominant source of initial inoculum for newly planted tissues (Vanderplank, 1963; Jeger, 1982). Two main factors can influence disease severity: sowing date and presence of fungal inoculum in the production area. Preliminary studies about the development of Alternaria leaf spot during one growing season showed that lowest disease severities were observed in sunflower sown in October and November in the south region of Brazil and the fungal inoculum from the first sowing plots was important for pathogen dissemination to the last sowing plants (Carvalho et al., 1995).

Methods to reduce initial inoculum, i.e. Vanderplank's sanitation measures, can be used to decrease disease severity. Vanderplank (1963) used simple exponential equations to describe how sanitation measures could delay the onset of the disease, by reducing the amount of initial inoculum from which an epidemic starts. He argued that, assuming that the disease progress rate (r) is not affected by sanitation, if initial inoculum was reduced by sanitation from x_0 to x_{0s}, then the epidemic would be delayed by the time taken for disease to increase from x_{0s} to x_0. The relationship between the inoculum ratio (x_0 / x_{0s}) and the time delay in the epidemic (Δt) could be described by $\Delta t = \ln (x_0 / x_{0s}) / r$.

The main objective of this study was to investigate Alternaria leaf spot severity in sunflower sown on different dates, in the south region of Brazil, and the effect of inoculum from the first sowing plots on the last ones.

MATERIALS AND METHODS

Field experiments

Three field experiments were carried out in Londrina, State of Paraná, in the south region of Brazil (latitude 23°11'50" south; altitude 585 m), in three growing seasons: 1997/1998, 1998/1999 and 1999/2000. The experimental sunflower hybrid SE02, developed by the Embrapa Soja genetic breeding program, was used. All experiments followed the randomised complete block design, with four sowing

dates, two sowing types and four replications. Each plot consisted of four 5m rows, with between-row spacing of 0.90 m; three sunflower plants were left per linear meter. The trials received the conventional cultural practices of commercial fields, including sowing and top-dressing fertilisation, spraying against insects, and sprinkle irrigation, when necessary (Castro et al., 1996). The trials were planted in an area intensively used for sunflower experimentation.

To establish several levels of disease severity, sowing was carried out in the months of October, November, December and January of each year, in two sowing types: contiguous and isolated plots. The first type had all the plots of the different sowing dates located side by side, while, in the other type, plots of the different sowing dates were separated by six rows of corn, a non-host barrier to the fungus. No artificial inoculation of *A. helianthi* was performed; disease occurred by natural infection of the plants. The pathogen was identified by isolation in laboratory and plant inoculation in glasshouse.

Assessments of disease severity, area under disease progress curve and yield

The evaluation of disease severity and yield was made on the two central rows of each plot, disregarding 0.5 m at each row end. The single plant approach was adopted (Kranz and Jörg, 1989), in which 10 or 8 plants of each plot were marked, thus a total of 320 plants for the first trial and 256 for the second and third trials were obtained. Plants were marked after the appearance of the fourth true leaf (V4 growth stage) (Schneiter and Miller, 1981), and an attempt was made to select individuals of the same development stage, height and vigour.

The leaf areas (*LA*) (cm^2) of all leaves of each marked plant were estimated weekly, starting from the appearance of the fourth true leaf (V4 growth stage). For this, the maximum width (cm) of each leaf (*L*) was measured with a ruler. Leaf area was calculated using the equation $LA = -155.86 + 22.40\ L$ (R^2=0.90) (Leite and Amorim, 2002). Assessment of the severity of Alternaria leaf spot was simultaneous with the evaluation of leaf area, with the aid of a diagrammatic scale, which was previously elaborated and validated (Leite and Amorim, 2002). Marked plants affected by other diseases or showing any problems in their development were discarded.

The area under disease progress curve (*AUDPC*) for each plant was calculated by trapezoidal integration using the formula (Bergamin Filho et al., 1997):

$$AUDPC = \sum_{i=1}^{n-1}((S_i + S_{i+1})/2)(t_{i+1} - t_i)$$

where S_i=$S(t_i)$, n was the number of assessments, S was disease severity (in percentage) and $(t_{i+1} - t_i)$ was the interval (days) between two consecutive assessments.

Marked plants were harvested individually, after physiological maturity (R9) (Schneiter and Miller, 1981), for evaluation of grain yield (kg ha^{-1}).

Data analysis

Data of *AUDPC* and grain yield were submitted to ANOVA, using the factorial design (sowing dates x sowing types), with four replications. Duncan's multiple range test (p=0.05) was performed to detect the significant differences for *AUDPC* and yield means among sowing dates and sowing types, using SAS software (SAS Institute, USA).

Data were also analysed by non-linear regression, using the software STATISTICA 5.0 (Statsoft, Tulsa, USA). Data were fitted individually by negative exponential model, $Y=B_1 \exp(-B_2 X)$, where Y represents the yield component, X represents *AUDPC*, B_1 represents the intercept and B_2 represents the slope.

To calculate the initial inoculum, Alternaria leaf spot severity data for each sowing date and sowing type were individually fitted by logistic model (Berger, 1981), $Y=1/(1+B_1 \exp(-B_2 X))$, where Y is the disease severity, X is the number of days after sowing, B_1 is the parameter related to initial inoculum and B_2 is the disease progress rate, for the three consecutive years (1997/1998, 1998/1999 and 1999/2000).

The time delay in the epidemic was calculated based on the theory of Vanderplank (1963), which assumes that disease progress rate (r) is not affected by any measure of reducing initial inoculum. In the present work, the sanitation measure used for reducing initial inoculum was the isolated sowing type, where the different sowing dates were separated by six rows of corn, compared with the contiguous sowing type, where the different sowing-date plots were located side by side.

Using the logistic model, a constant disease progress rate (r) for each sowing date was calculated with the disease severity means of contiguous and isolated plots, for the three consecutive years. The initial inoculum for both contiguous (x_{0c}) and isolated (x_{0i}) sowing types was calculated keeping the rate

constant. The time delay in the epidemic (Δt) (days) as a function of reducing initial inoculum by the sanitation method was calculated by:

$$\Delta t = \ln (x_{0c} / x_{0i}) / r$$

where: x_{0c} was the initial inoculum present in contiguous sowing plots, x_{0i} was the initial inoculum present in isolated sowing plot and r was the constant disease progress rate for each sowing date.

RESULTS AND DISCUSSION

Sowing sunflower in four different months each year proved to be effective for obtaining a wide range of Alternaria leaf spot severity. A significant higher *AUDPC* mean was observed in plants sown in December of the three years, for both sowing types (Table 1). This variable decreased for plants sown in January, since many leaves were senescent due to the disease and were not considered for disease severity assessment. Healthy plants (*AUDPC*=0) were only observed in plants sown in October and November 1999; for the first month, considering both sowing types (contiguous and isolated plots), all plants remained disease-free. Comparing sowing types, *AUDPC* was significantly lower in isolated plots sown in January 1998 and in December 1999 (Table 1). Sunflower plants sown in January 1999 did not produce grains, as well as plants sown in contiguous plots in December 1998 (Table 1).

Table 1. Area under disease progress curve (*AUDPC*) of Alternaria leaf spot and yield of sunflower, sown on four months and two sowing types, in three consecutive years

Sowing date	*AUDPC*[1]				Yield (kg/ha)[1]			
	Sowing type				Sowing type			
	Contiguous		Isolated		Contiguous		Isolated	
1997/1998								
Oct	336.75	dB	504.19	cA	2450.54	aA	1395.04	aB
Nov	572.94	CA	485.13	cA	649.34	bA	622.3	bA
Dec	907.35	aA	867.59	aA	5.14	cA	137.76	cA
Jan	778.25	bA	630.68	bB	100.11	cA	348.65	cA
Mean	625.44				713.61			
CV (%)	10.41				24.83			
1998/1999								
Oct	163.67	dA	285.51	cA	2898.44	aA	2182.82	aB
Nov	305.26	cA	308.01	cA	920.41	bB	1214.78	bA
Dec	844.32	aA	811.82	aA	0	cB	304.82	cA
Jan	505.93	bB	667.67	bA	0	cA	0	dA
Mean	485.52				940.15			
CV (%)	17.47				15.22			
1999/2000								
Oct	0	cA	0	bA	2062.23	aA	2428.96	aA
Nov	45.92	cA	71.69	bA	1900.83	aB	2462.43	aA
Dec	771.99	aA	452.05	aB	673	bB	1886.73	bA
Jan	481.42	bA	409.77	aA	892.22	bB	1459.84	cA
Mean	279.10				1673.60			
CV (%)	19.38				19.28			

[1]For each variable and growing season, means followed by the same letter (capital letters in columns and minuscule letters in line) are not different by Duncan´s multiple range test (5%).

Data of yield and disease severity observed in this work indicate that sowing sunflower in October resulted in high grain yields and low or no disease severity. This corroborates the fact that the recommended sowing date for sunflower in the State of Paraná is from August to October (Castro et al., 1996). Silveira et al. (1993), studying sowing dates for sunflower in this State, also observed higher yields for sunflower sown in August and lower yields for sunflower sown in December.

Vanderplank´s sanitation ratio theory was used to account for the time delay in the epidemic (Δt) as a function of reducing initial inoculum by a sanitation measure. This theory was also used by Young et al. (2003), for predicting epidemics of yellow rust (*Puccinia striiformis*) on the upper canopy of wheat,

compared to disease severity on lower leaves. Vanderplank (1963) considered that the effectiveness of sanitation should be linearly related to the delay in reaching any given level of the disease. This delay (Δt) is the additional time required to reach a given severity in a crop with sanitation measures, as compared to the crop without sanitation measures (Plaut and Berger, 1981).

In this study, the time delay in the epidemic in terms of reducing the initial inoculum by the sanitation method varied from 0.75 day, on November 1998 sowing date, to 11.56 days, on December 1998 sowing date (Table 2). This confirms that sowing sunflower separated by rows of corn was enough to decrease the primary inoculum, compared with the contiguous sowing, and cause a delay in disease epidemics. The delay of 11 days on the onset of the disease is important considering the early crop cycle of 100 days. As disease is delayed, the damage to sunflower yield becomes lower.

Vanderplank (1963) considered some factors that could limit plant disease development when he first discussed the sanitation theory. He was cautious in recommending sanitation measures as a disease control strategy, particularly for diseases with high infection rates and for epidemics of a long duration. Management of Alternaria leaf spot of sunflower should not be seen as an isolated measure. The reduction in initial inoculum, which was used to simulate the effect of sanitation, may not be identical to benefits derived from actual sanitation measures (Plaut and Berger, 1981). Farmers of the same region should concentrate sunflower sowing on the same date, in order to decrease pathogen dissemination from one area to another. Low initial disease was apparently compensated for by accelerated rates of disease increase. Thus, sanitation measures in the management of compound interest diseases may be less effective than previously theorized (Plaut and Berger, 1981).

We concluded that sowing sunflower in October resulted in a high yield and low Alternaria leaf spot severity in the State of Paraná, Brazil. Sanitation measures to reduce initial inoculum concentration delayed the onset of the disease by 11 days.

Table 2. Time delay in the epidemic (Δt) and parameters of logistic function, $Y=1/(1+(1/x_0)-1)\exp(-rX)$), where Y is disease severity, X is days after sowing, x_0 is initial inoculum and r is constant disease progress rate for each sowing date, for Alternaria leaf spot of sunflower, in three consecutive years. Plants sown on four different dates and two sowing types (contiguous and isolated) were used for regression analysis.

Sowing date	r	Sowing type		Δt (days)
		Contiguous	Isolated	
		x_0	x_0	
1997/1998				
Oct	0.0601	0.0019	0.0025	-
Nov	0.0797	0.0010	0.0009	1.14
Dec	0.0620	0.0077	0.0061	3.77
Jan	0.0492	0.0105	0.0080	5.58
1998/1999				
Oct	0.1467	5.39E-07	1.02E-06	-
Nov	0.1128	5.36E-05	5.83E-05	0.75
Dec	0.0756	0.0061	0.0026	11.56
Jan	0.0563	0.0037	0.0030	3.73
1999/2000				
Oct	-	-	-	-
Nov	0.2474	9.9E-12	1.61E-11	1.97
Dec	0.0555	0.0036	0.0022	9.32
Jan	0.0226	0.0214	0.0175	8.78

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REFERENCES

Bergamin Filho, A., S.M.T.P.G. Carneiro, C.V. Godoy, L. Amorim, R.D. Berger, and B. Hau. 1997. Angular leaf spot of *Phaseolus* beans: relationships between disease, healthy leaf area, and yield. Phytopathology 87:506-515.

Berger, R.D. 1981. Comparison of the gompertz and logistic equations to describe plant disease progress. Phytopathology 71:717-719.

Carvalho V.P., A. Bergamin Filho, L. Amorim, A.M.R. Almeida, J.R.B. Farias, C. de Castro, V.B.R. Castiglioni, and R.M.V.B.C. Leite. 1995. Desenvolvimento da mancha de alternária em genótipos de girassol em diferentes épocas de semeadura. p. 77. In: Resumos 11th Reunião Nacional de Pesquisa de Girassol, Goiânia, Brazil. EMBRAPA-CNPAF, Goiânia, Brazil.

Castro, C. de, V.B.R. Castiglioni, A. Balla, R.M.V.B.C. Leite, D. Karam, H.C. Mello, L.C.A. Guedes, and J.R.B. Farias. 1996. A cultura do girassol. EMBRAPA-CNPSo, Londrina, Brazil.

Jeger, M.J. 1982. The relation between total, infectious, and postinfectious diseased plant tissue. Phytopathology 72:1185-1189.

Kranz, J., and E. Jörg. 1989. The synecological approach in plant disease epidemiology. Rev. Tropical Plant Pathol. 6:27-38.

Leite, R.M.V.B.C. 2005. Manejo de doenças do girassol. p. 501-546. In: R.M.V.B.C. Leite, A.M. Brighenti, and C. de Castro (eds), Girassol no Brasil. Embrapa Soja, Londrina, Brazil.

Leite, R.M.V.B.C., and L. Amorim. 2002. Elaboração e validação de escala diagramática para mancha de Alternaria em girassol. Summa Phytopathologica 28:14-19.

Plaut, J.L., and R.D. Berger. 1981. Infection rates in three pathosystem epidemics initiated with reduced disease severities. Phytopathology 71:917-921.

Schneiter, A.A., and J.F. Miller. 1981. Description of sunflower growth stages. Crop Sci. 21:901-903.

Silveira, J.M., J.M. da Costa, I.M. Carraro, A. Balla, and V.B.R. Castiglioni. 1993. Estudo de épocas de semeadura em girassol para o Estado do Paraná. p.19-20. In: Resumos 10th Reunião Nacional de Pesquisa de Girassol, Goiânia, Brazil. EMBRAPA-CNPSo, Londrina, Brazil.

Vanderplank, J.E. 1963. Plant diseases: epidemics and control. Academic Press, New York, USA.

Young, C.S., N.D. Paveley, T.B. Vaughan, J.M. Thomas, and K.D. Lockley. 2003. Predicting epidemics of yellow rust (*Puccinia striiformis*) on the upper canopy of wheat from disease observations on lower leaves. Plant Pathology 52:338-349.

Effects of nitrogen and water on premature ripening caused by *Phoma macdonaldii*, a fungal pathogen of sunflower

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ABSTRACT

Premature ripening (PR) caused by *Phoma macdonaldii* results in yield damage for sunflower, mainly in the South-West of France, a major production area. The aim of the study was to characterize and identify the effect of crop management systems on PR incidence and severity, in 2006 and 2007. This field study used artificial and natural inoculation to investigate the role of host resistance, N-fertilization and water regime in the Phoma epidemics and aimed to reveal the most critical factors responsible for the disease progress and plant injury. On both years, the susceptibility of the cultivar appeared as a main factor influencing PR. However, the most severe attacks were observed in conditions of high nitrogen nutrition, especially when it was associated with water stress after flowering.

Key words: crop management – disease assessment – *Phoma macdonaldii* – premature ripening – sunflower.

INTRODUCTION

Premature ripening (PR), induced by *Phoma macdonaldii* Boerema, is one of the most severe sunflower (*Helianthus annuus* L.) diseases. The disease increased in the early 1990s and the entire French sunflower cropping area is now affected (Penaud and Pérès, 1995). The term "premature ripening" was first used for sunflower by Sackston (1949) to describe wilt and stalk rot. Evidence suggests that collar girdling canker caused by *P. macdonaldii* is the primary cause of PR. Sunflower premature death is most often characterized by loss of plant vigor during mid- to late summer followed by senescence and death of the plant a few weeks before normal maturity (Gulya et al., 1984). Generally, PR plants have small heads, reduced seed yield, low seed weight, and low oil content (Donald et al., 1987).

The Phoma symptoms generally appear on the petiole, the stem and the collar of the plant (Maric and Schneider, 1979; Gulya et al., 1997). The spot may girdle the stem or the collar and the black to brown lesions may only affect the epidermal layer or penetrate into the pith of the plant. If *P. macdonaldii* is not organ specific throughout the stages of sunflower development (Penaud and Pérés, 1995), sunflower resistance depends on the organ infected and the aggressiveness of the pathogen isolates. Research carried out by CETIOM, INRA and ENSAT since 1998 has highlighted that collar infection is the best way to reproduce prematurely dead plants in the field at flowering stage (Pérès and Poisson, 2000).

Recent investigations revealed an impact of crop management on PR, as N-fertilization and water regime. However, few studies have reported on the effects of crop on the incidence of the disease. The aims of this study are to confirm the role of *P. macdonaldii* in causing PR of sunflower by artificial collar inoculation and evaluate the effects of sunflower crop management on the severity of fungus attacks at collar levels. Both of the factors under investigation in thus study may be of importance in explaining the irregular occurrence of this disease, with a special emphasis on the difference of sensitivity of two cultivars, N fertilization and water regime on PR induced by *P. macdonaldii*.

MATERIALS AND METHODS

Climate and soil: Two field experiments were carried out in Auzeville, near Toulouse (Haute-Garonne, South-West of France) over two years (2006-2007) on the experimental INRA Station. The soil was deep, silty-clay to clay with a pH of 7.8 to 8.2. From the inoculation time to the end of the experiment, the mean relative humidity was 63% and 67% and the temperature between min. 9.7-10 °C and max. 38-36°C in 2006 and 2007, respectively. Seasonal precipitation was 115 mm in 2006 and 307 mm in 2007.

Experiment design and crop management systems: The experimentation was done in a split-plot design with inoculation either artificial (AI) or natural (NI) as the main plot (800 m^2 each). Each main plot was subdivided into 2 water regimes (no irrigation vs irrigated), then 2 levels of nitrogen (0 vs 150 kg /ha) and finally 2 cultivars (cv. Heliasol RM (Semences de France) vs cv. Melody (NK Semences)). This resulted in 24 subplots of 22 m^2 in 2006 and 36 in 2007. The plant population was similar (6.7 plants/m^2). In 2006, because of a dry season, 6 irrigations were applied up to 220 mm while only 2 irrigations were applied in 2007 (80 mm). N-fertilization was applied at sowing and at early flower bud stage. The two cultivars differed by their susceptibility to Phoma black stem, cv. Heliasol being the one most affected by premature ripening.

Phoma isolates and plant inoculation: Single pycnidiospore isolates of *P. macdonaldii* derived from sunflower fragments with severe black collar lesions were used in the experiment. The isolation and conservation of *P. macdonaldii* monopycnidiospore strains (MP6 and MPH2) was done following the method described by Roustaee et al. (2000). Artificial inoculation in the field was carried out using mycelium of the fungus (vegetative part). To allow mycelium growth, single pycnidiospore isolates were transferred to Petri dishes containing potato dextrose agar (Difco) (39 g/l, pH 6) and incubated for 10 days at 25°C in the dark. The inoculation on the AI plot was carried out at star bud stage on 25 homogenous plants on the 2 central rows of 6-row plots. A disc of mycelium (6 mm diameter) was placed for 5 days at the plant collar using MP6 as single pycnidiospore in 2006 and MPH2 in 2007. Previous tests had suggested that the latter was more aggressive. Desiccation of the disc was avoided by applying a damp cotton wool plug and aluminum around the collar.

A*ssessments of Phoma macdonaldii incidence and severity:* A disease assessment method was used to evaluate *Phoma macdonaldii* incidence (proportion of necrotic areas of infected collars) and final severity (proportion of early ripened plants). The first evaluation was performed 5 days after inoculation. Severity ratings were assessed weekly on the 25 tagged plants for all treatments from 59 DAE (days after emergence) and 52 DAE until harvest in 2006 and 2007, respectively, at least 15 assessments were performed during the experiment. The disease rating scale used was proposed by Cetiom: (0) healthy plant, (1) black collar in less than ¾ of the stem diameter (2) coalescent spots on collar, (3) all the leaves are wilted but the stem is still green, (4) the plant is totally dry.

The weekly monitoring of disease progress was used to calculate the area by a disease progress curve (AUDPC) and the value was standardized by dividing it by the total number of epidemic days. The AUDPC was calculated according to the equation of Campbell and Madden (1990):

$$AUDPC = \sum_{i}^{n-1} (yi + yi+1)/2 * (t\,i+1 - ti)$$

where n is the number of evaluations, y the severity or incidence of the disease and t the thermal time of each evaluation. $(t,y) = (441, 0)$ is included as the first evaluation date, approximately 1 week before AI. Daily thermal time (DTT) was calculated using daily mean air temperature. from emergence to 120 DAE (equivalent to 1930 DTT) and 126 DAE (1776 DTT) in 2006 and 2007, respectively, the last observation date where disease rating scale might be attributed to PR and not to natural senescence. The threshold temperature was taken at 4.8 °C (Granier and Tardieu, 1998).

Statistical analysis: Final severity ratings and AUDPC values were subjected to one-way and multifactor analysis of variance (ANOVA) both in 2006 and 2007. ANOVA was performed by comparing the impact of water regime*N-fertilisation*cultivar for each inoculation treatment (AI, NI) on AUDPC values. Where significant differences were found at $P \leq 0.05$, means were compared using Fisher's protected least significant difference test (95% LSD). Analysis was performed using Statgraphics Plus 5.1 statistical software (Rockville, MA, USA).

RESULTS

Disease incidence measured by AUDPC values between the two experiments (2006 and 2007) did not differed significantly on AI plots ($P = 0.08$), whereas AUDPC on NI was significantly higher in 2007 (P=0.00*). According to the mode of inoculation (AI and NI) for each year, disease severity was measured by the final fraction of PR plants and the AUDPC did not vary significantly, except in 2006 when it was higher in AI than in NI plots (Table 1). This lack of significant effects on AUDPC and PR suggests that AI reproduces NI correctly.

Table 1. AUDPC values and percentage of premature ripened sunflower plants for artificial (AI) and natural (NI) inoculation with *Phoma macdonaldii* in 2006 and 2007 as a function of the cultivar, N-fertilisation and water regime.

	AUDPC[1]				PR plants (%)			
	2006		2007		2006		2007	
Treatment	AI	NI	AI	NI	AI	NI	AI	NI
	3860 a	2177 b	3755 a	3792 a	38 a	34 a	49 a	40 a
Heliasol	4141 a	2325 a	3843 a	3927 a	69 a	43 a	60 a	48 a
Melody	3787 b	2028 b	3665 b	3657 b	39 b	26 b	37 b	33 b
0-N	3812 b	2101 b	3627 b	3617 b	34 b	23 b	15 b	11 b
150-N	4116 a	2253 a	3919 a	3882 a	74 a	45 a	83 a	69 a
Unirrigated	4125 a	2184 a	3800 a	3968 a	83 a	52 a	55 a	50 a
Irrigated	3804 b	2169 a	3708 b	3616 b	25 b	17 b	42 b	31 b

[1]Calculated according to Campbell and Madden (1990).
Means were calculated using Fisher's protected LSD test a $P \leq 0.05$. The effect of cultivar, nitrogen and water regime was tested separately for AI and NI. Letters to the right of each value refer to differences between values. Means with the same letter do not differ significantly.

In AI and NI plots, sunflower crop management through cultivar, N amount and water regime increased the proportion of plants infected by *P. macdonaldii* ($P < 0.01$). All the tagged plants had a score of 2 (black coalescent spots on collar), but the evolution into PR plants (score 4) was only induced by the crop management. Cv. Heliasol was systematically more susceptible than cv. Melody to Phoma attacks whatever the season and the mode of inoculation ($P < 0.01$).

AUDPC values and PR (%) differed significantly between 150-N and 0-N and between rainfed and irrigated management for AI and NI ($P< 0.01$ for N and water) both in 2006 and 2007. High N-fertilisation and water stress (resulting from rainfed management) increased the proportion of plants infected by *Phoma macdonaldii* (at stem and collar level) and especially the percent of PR plants.

Two examples of disease progress curves for AI in 2007 are shown in Fig. 1.

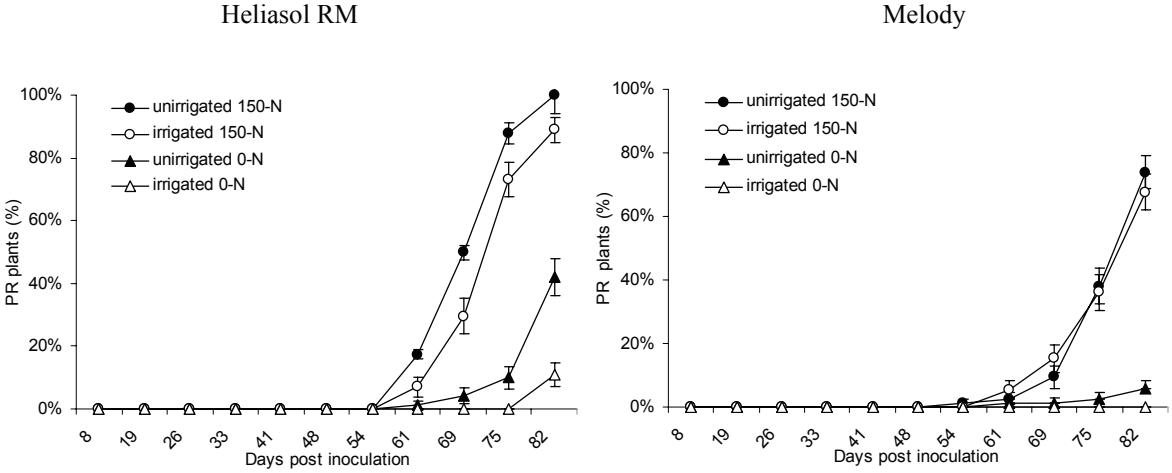

Fig. 1. Disease progress curves of premature ripening plants after artificial inoculation in 2007 for cv. Heliasol (susceptible) and cv. Melody (tolerant) for contrasted crop management systems combining nitrogen and water applications.

The susceptibility of the cultivar was the main factor influencing the final rate of PR plants, cv. Heliasol being more attacked than cv. Melody. Nitrogen fertilisation appeared as the second factor affecting disease progress for both cultivars. Water stress seems to amplify nitrogen effect and may be considered as the third factor stimulating the disease progress. High N fertilization combined with rainfed management resulted in the highest plant injury: up to 100% of PR plants were observed for cv.Heliasol (in AI and NI treatments) on 150-N and no irrigation. Effects of cultivar x nitrogen interaction were observed in 2006 for NI and for AI in 2007 ($P<0.01$).

DISCUSSION

This study intended to point out the most influencing factors of sunflower premature ripening attributed to *Phoma macdonaldii*. A preliminary investigation into the possible role of a different fitness among the fungus isolates did not clearly reveal differences in disease severity. In 2006 and 2007, no significant differences in AUDPC were found in AI plots with two different monopycnidiospores. This suggests that differences in disease severity observed in the field could not be only attributed to isolate aggressiveness but more to environment and crop management.

As expected, the proportion of PR plants was higher in 2007 than in 2006. Higher precipitation and air relative humidity in 2007 resulted in a favourable environment for natural Phoma infection. Weeraratne and Priyantha (2003) observed that HR > 80%, temperature ranging from 25 to 30°C and cloudy weather favour the development of the disease under tropical conditions. Stem injury resulting from *Phoma macdonaldii* infections at collar level probably increased because the microclimate in the lower part of the sunflower stand was more favourable to fungus development and activity. Similar conclusions were drawn by Debaeke and Pérès (2003) on stem and collar attacks. However, if disease injury is influenced by the climatic and microclimatic environment, the PR syndrome relies strongly on cropping system management.

The susceptibility of the cultivar was shown throughout both experiments in AI and NI plots. The response of cv. Heliasol and cv. Melody differed significantly towards the progression of the disease and the final incidence of the inoculation by *P. macdonaldii*. The significantly lower AUDPC values and percent of PR plants for cv. Melody indicate possible differential genotypic susceptibility to premature ripening. Such differences were already noted by Penaud (1994) and Dechamp-Guillaume et al. (2000) on stem attacks. If the susceptibility of the cultivar is one of the main factors inducing PR, host nutrition was responsible for different patterns of epidemics in the experimentation.

As suggested in the literature, mineral nutrition can exert a profound effect on disease development, with fertiliser application increasing development of the disease. The mechanisms leading to these nutrient-induced changes in disease development are complex and multifarious. These mechanisms include the effects of the mineral nutrients directly on the pathogen, on plant growth and development, and on resistance mechanisms. According to Gulya et al. (1997), high N fertilisation increased the proportion of collars infected by *P. macdonaldii*. Conversely, nitrogen deficiency did not predispose plants to PR. The role of N-fertilisation clearly demonstrated in this study should be better explained at a process level. Our investigations did not reveal if the nitrogen had an impact on the sunflower culture that could favour pathogen development or was trophic for the pathogen. Large nitrogen supplies influence the size of the canopy. Dense canopy, observed in the experimentation for a given stand density, especially in 2007, induced a microclimate which might promote inoculum production and create conditions conducive to successful infections. Spore germination of *P. macdonaldii* might be sensitive to this high relative humidity microclimate, constituting a major climatic parameter in disease epidemiology and field infection (Roustaee et al., 2000). The other approaches to the effect of N-fertilisation on the percent of PR plants may concern the possible N sources available by plant pathogenic fungi. Assimilation of N sources, depending on the tissue being colonised, would include nitrate, ammonium, amino acids, amine and protein (Snoeijers et al., 2000). In addition, available N sources may also depend on the mode of nutrition of the pathogen. *P. macdonaldii,* which is a necrotrophic fungus, could have access to a wide range of N sources. The present study did not allow us to determine clearly the role of N-fertilisation. Both nitrogen effects may act on the disease progression, and one probably more than the other. Further investigations will be set up to better understand the N-fertilisation impact on the microclimate by varying crop density of sunflower. The difficulty is that in most plant-pathogen interactions, very little is known about the N content and subsequent colonisation by fungi. This is an area that requires further investigation (Walters and Bingham, 2007).

Previous data showed that infested plants which did not get irrigation presented a higher AUDPC and final percent of PR plants compared to those which were irrigated. If its effect seems to be less influential compared to the susceptibility of the cultivar and high nitrogen supply, rainfed plots receive a significant impact from the severity of the disease. This impact is not clearly demonstrated but this effect might be more linked to the physiological status of the plants. A predisposition to disease is often observed in host plants during water deficiencies. According to the literature, there are no studies in which the biochemical and biophysical causes of predisposition to disease during water deficiencies are known with any certainty. When the plant is infected by the pathogen, changes in host plants may alter their interactions with other organisms and suggest possible mechanisms of susceptibility. Boyer (1995) proposed two mechanisms to explain how water stress increases the susceptibility of plants to attacks from pathogens: (1) reduced photosynthate production induced by drought eliminates the plants' ability to defend

themselves against pathogens and/or (2) plant growth is reduced without reducing the pathogen's ability to reproduce, thus allowing further progression and increased symptom severity in the host. These two mechanisms may be an approach to a better understanding of the water deficiency effect on disease progression in the plant leading to PR as a nitrogen effect.

This study has attempted to identify the most crucial elements of PR induced by P. macdonaldii infestation. Our data revealed that sunflower epidemiology efficiency is mainly influence by Phoma and crop management. The susceptibility of the cultivar, high N-fertilization and rainfed management have a strong impact on the disease. Nitrogen input and water stress enhance PR. The interaction of both may act to favour pathogen infection in the plant and reduce expression in host defence mechanisms. Deployment of resistant lines in combination with an integrated disease management framework is suggested as suitable tools for reducing inoculum pressure and PR plants induced by *P. macdonaldii.*

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REFERENCES

Boyer, J.S. 1995. Biochemical and biophysical aspects of water deficits and the predisposition to disease. Ann. Rev. Phytopathol. 33:251-274.

Campbell, C.L., and L.V. Madden. 1990. Introduction to Plant Disease Epidemiology. Wiley, New York.

Debaeke, P., and A. Pérès. 2003. Influence of sunflower (*Helianthus annuus* L.) crop management on Phoma black stem (*Phoma macdonaldii* Boerema). Crop. Prot. 22:741-752.

Dechamp-Guillaume, G., Roustaee, A., Larfeil, C., Barrault, G. and A. Pérès. 2000. Phoma du tournesol: bientôt un test variétal à la disposition des sélectionneurs. Oléoscope 58: 25–28.

Donald, P.A., Venette, J.P. and T.J. Gulya. 1987. Relationship between *Phoma macdoldii* and premature death of sunflower in North Dakota. Plant Dis. 71:466-468.

Granier, C., and F. Tardieu, 1998. Is thermal time adequate for expressing the effects of temperature on sunflower leaf development ? Plant Cell. Env. 21: 695–703.

Gulya, T., Charlet, L., Donald, P., and J. Venette. 1984. What do we know about premature ripening? The Sunflower, Dec. 1984, p 20-21.

Gulya, T., Rashid, K.Y, and S.M. Masirevic. 1997. Sunflower diseases: Phoma black stem. p. 319–322. In: Schneiter, A.A. (Ed.), Sunflower Technology and Production. Agronomy Monograph no. 35. ASA, CSSA, SSSA, Madison, WI, USA.

Maric, A., and R. Schneider. 1979. Black spot of sunflowers in Yugoslavia caused by *Phoma macdonaldii* Boerema. Phytopathol. Z. 94:226-233.

Penaud, A., 1994. Phoma du tournesol: la lutte s'organise. Phytoma 460:38-40.

Penaud, A., and A. Pérès. 1995. Oléoscope, Numéro spécial: Phoma du tournesol, n°15, CETIOM. ISBN: 2-908645-54-8. 37 p

Pérès, A., and B. Poisson. 2000. Le phénomène «Pieds secs» du tournesol: implication du phoma et étude du mécanisme de la contamination. p. 17-21 (vol. 2). In: Proc. 15th Int. Sunflower Conf., Toulouse, France.

Roustaee, A., Costes, D. Dechamp-Guillaume, G. and G. Barrault, 2000. Phenotypic variability of *Leptosphaeria lindquistiii* (*Phoma macdonaldii*) a fungal pathogen of sunflower. Plant Pathology 49:227-234.

Sackston,W.E. Sunflower diseases in Manitoba in 1949. p 35. In: Annu. Rep. Can. Plant Dis. Surv. 29th I.L. Conner and D.B.O. Savile, compilers. Can. Dep. Agric. Sci. Serv. Div. Bot. Plant Pathol.

Snoeijers, S.S., A. Perez-Garcia, M.H.A.J. Joosten, and P.J.G.M De Wit. 2000. The effect of nitrogen on disease development and gene expression in bacterial and fungal plant pathogens. Eur. J. Plant Pathol. 106:493-506.

Walters, D.R., and I.J.Bingham. 2007. Influence of nutrition on disease development caused by fungal pathogens: implications for plant disease control. Ann. Appl. Biol. 151: 307-324.

Weeraratne, W., and M. Priyantha. 2003. First report of Phoma black stem of sunflower in Sri Lanka and its management. Annals of the Sri Lanka Department of Agriculture, 5.

Pathological and morphological evaluation of sunflower isohybrids carrying or not the *Rcm-1* gene for *Sunflower chlorotic mottle virus* resistance

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ABSTRACT

Sunflower (*Helianthus annuus L.*) crops are affected by *Sunflower chlorotic mottle virus* (SuCMoV) which reduces yield parameters in commercial hybrids infected at early ontogenic stages. Sunflower isohybrids (between two near isogenic males differing in the *Rcm-1* resistance gene and three different females) were mechanically inoculated with SuCMoV under field conditions in two locations (Balcarce and Venado Tuerto) and evaluated for symptom expression and agronomic characteristics. Symptoms were scarce chlorotic pinpoint in all resistant hybrid combinations and severe chlorotic mottling in all susceptible hybrids, independently of the female parents. Nevertheless, higher symptoms intensity was detected in Venado Tuerto. Morphological parameters were more affected in Venado Tuerto than in Balcarce and differed among hybrid combinations.

Key words: *Helianthus annuus* – isohybrids – *sunflower chlorotic mottle virus* – virus.

INTRODUCTION

Sunflower chlorotic mottle virus (SuCMoV) is a potyvirus, which seemed to be restricted to the Americas. In Argentina, it has been associated with chlorotic mottling and plant stunting symptoms and it has been reported in several provinces including; Entre Ríos, Santa Fé, Buenos Aires and Córdoba. The virus is a member of the *Potyvirus* genus within the Potyviridae family (Dujovny et al., 1998, 2000) and it has been classified as a strain of *Potato virus Y* (Berger, 2005).

Recently, a sunflower line tolerant to SuCMoV infection has been reported (L33) and the resistance gene *Rcm-1*gene has been mapped (Lenardon et al., 2005). Breeding for virus resistance is one of the best ways to manage virus epidemics since no additional agricultural practices are required to reduce disease incidence and severity. Using molecular marker-assisted selection, the *Rcm-1*gene was incorporated to a susceptible L37S male in order to obtain a near-isogenic resistant version L37R. The objective of this work was to study the level of resistance obtained by the presence of this gene in different hybrid combinations, both in terms of symptom expression and agronomic characteristics.

MATERIALS AND METHODS

Plant Material and Experimental design

Crosses between a pair of near-isogenic male lines with (R) and without (S) the *Rcmo-1* gene and 3 susceptible female lines were performed in order to obtain 3 pairs of isohybrids. Hybrids obtained from the cross between the three females and the resistant donor L33 (source of *Rcm-1* gene) (Advanta Semillas S.A.I.C) were employed as controls.

Split plot design experiments with three replications were sown in two locations: Venado Tuerto (Santa Fe Province, 33º45´S, 61º 58´ W, November 20, 2006) and Balcarce (Buenos Aires Province, 37° 45' S, 58° 18' W, November 27, 2007). Each replication consisted of the hybrids as the main plot, and two treatments (SuCMoV inoculated versus non-inoculated) as the subplot. Each subplot was represented by three rows (20 plants per row). The middle row was used for treatment application and evaluation.

Plant inoculations
A SuCMoV isolate maintained on sunflower commercial hybrid CF 7 under greenhouse conditions was used as inoculum source for the whole experiment. Sunflower plants were mechanically inoculated at vegetative stage V 12 (Venado Tuerto) and at R1 (Balcarce) (Schneiter and Miller, 1981) with a high pressure airbrush apparatus, using a slurry prepared from infected leaves ground with phosphate buffer and abrasive (Lenardon et al., 2005).

Evaluation
Inoculation in Venado Tuerto and Balcarce were performed on December 20, 2006 and on January 15, 2007 respectively. . Symptom expression was evaluated 25 days after the inoculation and agronomic parameters (flowering date, plant height, capitulum diameter, length and width of a totally expanded leaf from middle portion of the stem) at proper times.

Statistical analysis
Results were analyzed by ANOVA. Orthogonal contrasts were planned in order to test the effects on the morphological characters of: a) the treatment (inoculated vs non-inoculated) for each hybrid and 2) the effect of the incorporated gene (R vs S) within each treatment (inoculated or non- inoculated).

RESULTS AND DISCUSSION

Inoculation was successful and all inoculated plants expressed virus symptoms. A heavy storm occurred in Venado Tuerto and some plants suffered mechanical stress and lodging before flowering so one pair of isohybrids was eliminated from the analysis.

Qualitative differences between symptoms in all isohybrid pairs were detected as expected. In all resistant hybrids symptoms were scarce chlorotic pinpoints, similar to those observed in the controls when the resistant L33 donor was employed. Nevertheless, the intensity of the symptoms was higher in Venado Tuerto than in Balcarce even in the crosses where L33 was a parent. In the first location, the chlorotic pinpoint was intense and also chlorotic ringpots were detected. In Balcarce, typical scarce chlorotic pinpoint symptoms (SCP) were observed on resistant genotypes. The susceptible counterpart of the isohybrids exhibited severe chlorotic mottling (SCM) symptoms independently of hybrid combination (Table 1).

Table 1. Symptom expression in isohybrid pairs differing in *Rcm-1* gene artificially inoculated in two locations[1].

Pedigree	Venado Tuerto	Balcarce
L16xL37S	SCM	SCM
L16xL37R	ICP + CR	SCP
L16xL33	ACP + CR	ACP + CR
L348xL37S	SCM	SCM
L348xL37R	ICP + CR	SCP
L348xL33	ACP + CR	SCP
L351xL37S	NR	SCM
L351xL37R	NR	SCP
L351xL33	NR	SCP

[1]SCM: severe chlorotic mottle; SCP: scarce chlorotic pinpoints; ACP: intense chlorotic pinpoint; ICP: intermediate chlorotic pinpoint; CR: chlorotic ringspots; NR: no results

One-two day differences in flowering date between treatments were observed according to the isohybrid (data not shown).

ANOVA analysis for both locations detected significant differences between hybrids and treatments and an interaction between hybrid x treatment ($P<0.05$) for the morphological characters with exception of head diameter in Balcarce. The means of morphological traits are presented in Table 2.

Table 2. Morphological traits in isohybrid pairs differing in the *Rcm-1* gene inoculated and non-inoculated with SuCMoV in two locations. Hybrids with L33 (resistant source) are used as controls.

Venado Tuerto

Pedigree	Plant height, cm		Head diameter, cm		Leaf length, cm		Leaf width, cm	
	Inoc.	Non inoc.	Inoc.	Non inoc.	Inoc.	Non inoc.	Inoc.	Non inoc.
L16xL37S	171	193	9	21	17	23	17	23
L16xL37R	196	203	11	18	21	24	21	24
L16xL33	177	181	13	17	23	25	21	23
L348xL37S	179	216	8	18	17	23	16	22
L348xL37R	217	222	15	19	22	23	22	23
L348xL33	195	200	12	18	19	24	17	22

Balcarce

Pedigree	Plant height, cm		Head diameter, cm		Leaf length, cm		Leaf width, cm	
	Inoc.	Non inoc.	Inoc.	Non inoc.	Inoc.	Non inoc.	Inoc.	Non inoc.
L16xL37S	173	186	12	11	20	25	19	23
L16xL37R	185	190	12	13	25	24	25	22
L16xL33	156	152	17	16	30	31	30	31
L348xL37S	180	187	12	14	17	25	15	24
L348xL37R	191	193	13	13	22	24	20	23
L348xL33	154	160	19	20	26	28	27	28
L351xL37S	171	172	17	16	20	25	19	23
L351xL37R	172	176	17	16	26	27	25	27
L351xL33	152	152	22	20	27	26	28	26

Table 3. Orthogonal contrast for morphological traits between inoculated and non-inoculated hybrids, which differ in *Rcm-1* gene in two locations.

Venado Tuerto

Inoculated vs. non-inoc.	Plant height			**Leaf length**			**Leaf width**			**Head diameter**		
	MS	F Value	p-value	MS	F Value	p-value	MS	F Value	p-value	MS	F Value	p-value
L16 xL37S	506.25	16.99	0.0026	62.08	72.40	<0.0001	65.34	80.47	<0.0001	108.37	65.49	<0.0001
L16xL37R	73.50	2.47	0.1507	16.67	19.44	0.0009	17.68	21.78	0.0005	83.10	50.22	0.0001
L348 xL37S	2041.31	68.51	<0.0001	54.00	62.97	<0.0001	51.04	62.86	<0.0001	155.14	93.75	<0.0001
L348xL37R.	48.17	1.62	0.2354	0.74	0.86	0.3728	2.04	2.51	0.1388	28.17	17.02	0.0026

Balcarce

Inoculated vs. non-inoc.	Plant height			**Leaf length**			**Leaf width**		
	MS	F Value	p-value	MS	F Value	p-value	MS	F	p-value
L16 xL37S	261.36	20.25	0.0003	36.51	9.79	0.0058	20.91	3.94	0.0626
L16 xL37R	44.61	3.46	0.0794	5.61	1.50	0.2359	9.63	1.81	0.1946
L348 xL37S	84.68	6.56	0.0196	96.0	25.75	0.0001	105.84	19.95	0.0003
L348 xL37R	10.32	0.80	0.3829	6.83	1.83	0.1927	8.64	1.63	0.2181
L351 xL37S	7.28	0.56	0.4623	26.46	7.10	0.0158	25.63	4.83	0.0413
L351 xL37R	2.04	0.16	0.6955	1.50	0.40	0.5339	5.61	1.06	0.3175

As previously described, symptoms intensity was higher in Venado Tuerto than in Balcarce. Thus, the inoculated plants in Venado Tuerto showed a significant reduction in all parameters in the S hybrids (without resistant gene) and in the R hybrids (with resistant gene) except plant height of R hybrids and leaf size (when L348 female was crossed with L37 R) (Table 3). On the contrary, in Balcarce, inoculation showed a significant reduction in all parameters in the S hybrids but it did not affect R hybrids (Table 3).

The presence of the incorporated gene did not modify the morphological characteristic of non-inoculated isohybrids (S vs R non-inoculated) (Table 4).

Table 4. Orthogonal contrast for morphological traits, between hybrids differing in *Rcm-1* gene inoculated and non-inoculated with SuCMoV in two locations.

Venado Tuerto

Pedigree	Plant height			**Leaf length**			**Leaf width**			**Head diameter**		
	MS	F Value	p-value	MS	F Value	p-value	MS	F Value	p-value	MS	F Value	p-value
L16 xL37S vs L16xL37R.inoc	684.7	23.0	0.001	24.8	28.9	0.0002	22.8	28.1	0.0002	0.2	0.1	0.7553
L16 xL37S vs L16xL37R.non inoc	170.7	5.7	0.040	1.4	1.6	0.225	0.8	1.0	0.339	9.8	5.9	0.0379
L348 xL37S vs L348xL37R. inoc	2140.6	71.8	<0.0001	40.0	46.7	<0.0001	49.3	60.7	<0.0001	61.3	37.0	0.0002
L348 xL37S vs L348xL37R. non inoc	64.4	2.2	0.176	0.03	0.03	0.863	1.7	2.1	0.173	0.5	0.3	0.6111

Balcarce

Pedigree	**Plant height**			**Leaf length**			**Leaf width**		
	MS	F Value	p-value	MS	F Value	p-value	MS	F Value	p-value
L16 xL37S vs L16xL37R.inoc	234	18.1	0.0005	36.5	9.8	0.006	39.5	7.5	0.0137
L16 xL37S vs L16xL37R.non inoc	33.8	2.6	0.1232	5.6	1.5	0.236	1.9	0.4	0.5542
L348 xL37S vs L348xL37R. inoc	200.3	15.5	0.0010	29.0	7.8	0.012	36.5	6.9	0.0172
L348 xL37S vs L348xL37R. non inoc	66.7	5.6	0.0355	3.2	0.9	0.365	1.7	0.3	0.5776
L351 xL37S vs L351xL37R. inoc	2.1	0.2	0.6889	55.2	14.9	0.001	43.7	8.3	0.0101
L351 xL37S vs L351xL37R. non inoc.	0.04	0.003	0.9580	12.3	3.3	0.086	15.4	2.9	0.1060

Complete resistance to SuCMoV has not been detected up to now. The *Rcm-1* gene produced a qualitative modification of symptom expression, which could be affected by the environment and the specific hybrid combination. Slight differences in the inoculation time could be excluded as the cause of the intensity differences between locations, because the phenotypic data used for gene mapping was obtained under field inoculation in Balcarce at younger stages and the symptoms of resistant plants were equal to those obtained for this location in the present study (Lenardon et al., 2005).

The use of this resistance gene could attenuate the effect of SuCMoV on morphological traits such as leaf area, and therefore, on some of the yield components.

REFERENCES

Berger, P.H., M.J. Adams, A.A. Brunt, J.H. Hill, J. Hammond, R.L. Jordan, R.J. Morales, S.T. Ohki, E. Rybicki, I. Uyeda, and H.J. Vetten. 2005. Potyviridae. p. 819-841. In: C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger, and L.A.Ball (eds), Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, CA, USA.

Dujovny, G., T. Sasaya, H. Koganesawa, T. Usugi, K. Shohara, and S.L. Lenardon. 2000. Molecular characterization of a new potyvirus infecting sunflower. Arch. Virol. 145:2249-2258.

Dujovny, S.G., T. Usugi, K. Shohara, and S.L. Lenardon. 1998. Characterization of a potyvirus affecting sunflower crops in Argentina. Plant Dis. 82:470-474.

Lenardon, S.L., M.E. Bazzalo, G. Abratti, C. Cimino, M.T. Galella, M. Grondona, F. Giolitti, and A.J. León. 2005. Screening sunflower for resistance to *Sunflower chlorotic mottle virus* and mapping the *Rcmo-1* resistance gene. Crop Sci. 45:735-739.

Schneiter, A.A., and J.F. Miller. 1981. Description of sunflower growth stages. Crop Sci. 21:901-903.

Study of resistance to *Sclerotinia* head disease in sunflower genotypes

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ABSTRACT

In Hungary, the most dangerous head diseases of sunflower are white and grey rots, caused by *Sclerotinia sclerotiorum* (Libert de Bary) and *Botrytis cinerea* (Persoon) respectively. In recent years, the role of fungal species well-disposed to higher temperatures (above 30°C) has also become more important. The intensity of attack by pathogens is not the same from year to year. Nevertheless, in certain years, they cause heavy yield losses and reduced quality. In 2005, the heaviest damage in seed and commercial grain production was caused by *Sclerotinia* head rot. Protection of the sunflower head has some difficulties and, in many cases, treatments do not produce any results. One of the most important aspects of our breeding work is the development of hybrids tolerant to head diseases. Heavy epidemic in 2005 made it possible to confirm the success of selection aimed at tolerance to *Sclerotinia* head rot. Genetic differences in susceptibility to *Sclerotinia* head rot were assessed in performance trials at 4 locations with 17 experimental hybrids and 3 check varieties of MGSZH (Central Agricultural Office, Directorate of Plant Production and Horticulture). Impact of sowing date on degree of infection was studied in two experiments with an earlier (15th April) and a later (2nd May) sowing time. Differences in the level of infection were scored in the plots of nearly isogenic lines and with their hybrids flowering at different dates. Efficiency of chemical treatment was evaluated on the basis of data obtained in treated and untreated plots. Temperature, humidity and rainfall were systematically recorded. Results of trials reflected differences in tolerance between hybrids. Planting date had an indirect influence because the level of damage was highly dependent on the average temperature and quantity of rainfall during bloom. Differences in percentage of infection in nearly isogenic lines and their hybrids flowering at different dates, as well as those in performance trials of hybrids sown on two dates, showed a close significant correlation r=0.84*** with the quantity of precipitation from the beginning to the end of the flowering period. Combining ability of 5 CMS female lines and 5 male restorer lines was studied by coupling model of Comstock and Robinson and analysis of variances was used for the evaluation of the experiment.

Key words: head rot – flowering – *Sclerotinia sclerotiorum* – sunflower – susceptibility – weather.

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is one of the most important pathogens in sunflower widely spread all over the world. Korf and Dumont (1972) assigned the species to *Whetzelinia,* a new genus, but objections to this disposition were raised by Dennis (1974). Not only *S. sclerotiorum*, but also *Sclerotinia minor* and *Sclerotinia trifoliorum* Fuckel, are pathogenic to sunflower (Cormack, 1946). After ontogenetic studies of *Sclerotinia* (Willets and Wong, 1971) and electrophoretic investigations (Wong and Willetts, 1973), it was concluded that *S. scelorotiorum, S. trifoliorum* and *S. minor* are three different species.

Originally identified on sunflower in 1861, the fungus has been reported from all sunflower-growing regions of the world (Gulya et al., 1997). Depending on the environmental conditions, it attacks the seedling, root, petiole, stem, and inflorescence. Since there is no 100% efficient chemical protection, hybrids should have good field resistance against this pathogen as well. As chemical control of *S. sclerotiorum* is difficult and uneconomical (Mestries et al., 1998), genetic control appears to be of great value. In the literature, there are no articles showing total resistance to *Sclerotinia sclerotiorum* in cultivated sunflower. However, reports on the identification of sunflower genotypes with low susceptibility or partial resistance are common worldwide. Some wild species include important *Sclerotinia* resistance genes (Seiler and Rieseberg, 1997; Köhler and Friedt, 1999; Degener et al., 1999). Resistance is a polygenic trait (Castaño et al., 1993.) There are two types of resistance in sunflower: (i) resistance to penetration, and (ii) resistance to mycelial extension in the tissues.

Breeders have a better opportunity to assess genetic resistance. A first approach is to make use of the natural infection, but the intensity of pathogen attacks is not the same from year to year. A second approach is to produce artifical infection (Tourvieille and Vear, 1984; Rodríguez et al., 2004). These

authors used the following procedure: Ascospores were suspended in sterile distilled water with Tween 80 (0.05%) to a concentration of 5×10^3 spores/ml (10 ml per inflorescence). Plants were checked regularly for flowering. Sunflower heads were sprayed with inoculum when the anthesis of the two outer rows was completed, and so inoculation was produced at anthesis. Control plants were sprayed with water and drops of detergent.

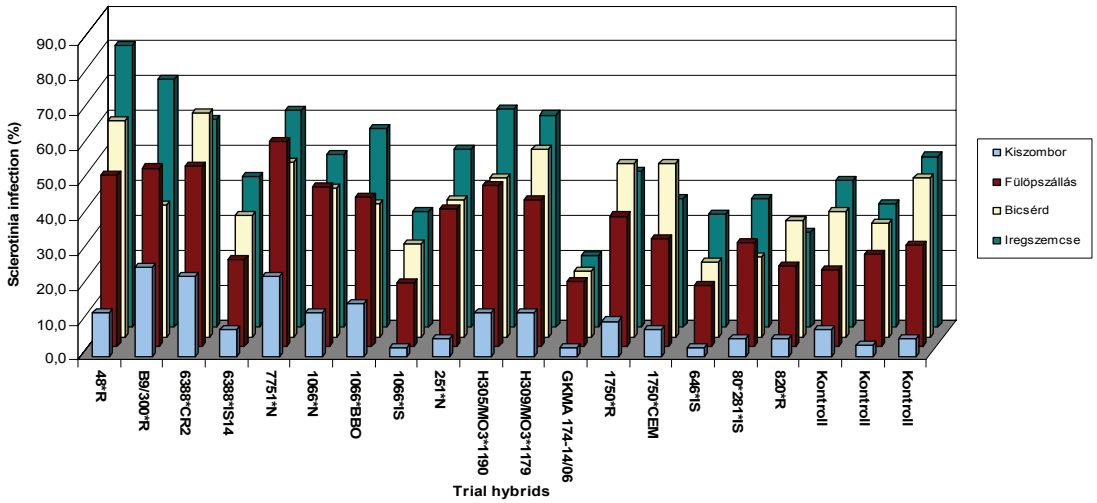
The aim of this study was to analyse genetic differences in susceptibility to *Sclerotinia* head rot at different locations.

MATERIALS AND METHODS

Genetic differences in susceptibility to *Sclerotinia* head rot were assessed in performance trials at 4 locations with 17 experimental hybrids and 3 check varieties of MGSZH (Central Agricultural Office, Directorate of Plant Production and Horticulture). Impact of sowing date on degree of infection was studied in two experiments with an earlier (15th April) and a later (2nd May) sowing time. Differences in the level of infection were scored in the plots of nearly isogenic lines and their hybrids flowering at different dates. Efficiency of chemical treatment was evaluated on the basis of data obtained in treated (6 pair of leaves, initial reproductive stage, and full flowering) and untreated plots. Temperature, humidity and rainfall were systematically. Combining ability of 5 CMS female lines and 5 male restorer lines was studied by coupling model of Comstock and Robinson (1948) and analysis of variances was used for the evaluation of the experiment.

RESULTS AND DISCUSSION

The four locations showed different levels of head infection. The highest and lowest average of attack were 47.7% and 10.2%, respectively. Degree of infection in the most susceptible genotype at the location with the heaviest infection pressure attained 80%, whereas that of the most tolerant hybrid was only 15.1% (Fig. 1.)



LSD 5%=9.3

Fig. 1. *Sclerotinia* infection of trial-hybrids at four locations, 2005

The *Sclerotinia* head rot resistance of hybrids was studied as a function of sowing time and chemical treatment. In the experiment, the mean infection was 14.3%, the highest was 30% and the lowest was 1.5% (Fig. 2). There was no significant difference between chemically treated and untreated plots.

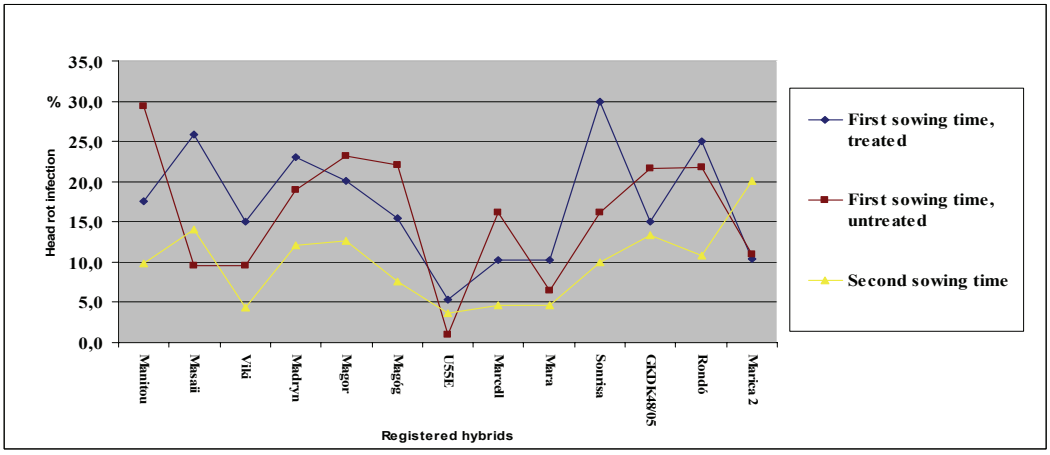


Fig. 2. The *Sclerotinia* head rot resistance of registered hybrids as a function of sowing time and chemical treatment.

Differences for disease incidence between the two sowing dates was analysed with the data of temperature and rainfall at the beginning of flowering, 50% flowering, and full flowering Significant difference was found between the percentages of infection at the two sowing times, the lower infection being on the second sowing date (Fig. 2).

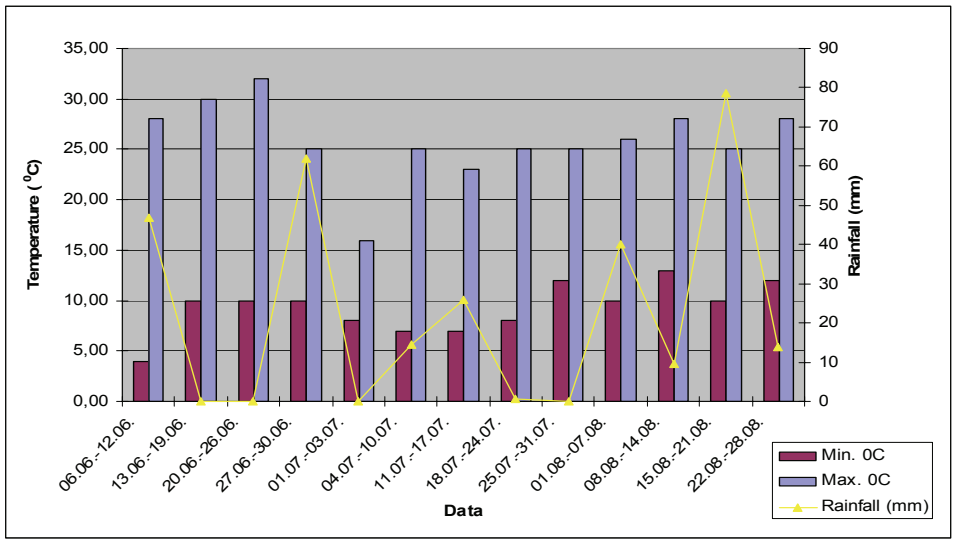


Fig. 3. The ecological conditions during flowering time.

The results of the experiments of the registered hybrids were assessed compared to the results of nearly isogenic lines and their hybrids. The hybrids sown on 28th April were flowering between 10-20 July and their average infection was 17.2%. The hybrids sown on 14th May were flowering between 18-27 July and the average infection was 9%. The ecological conditions during flowering time were examined on the basis of the data (Fig. 3).

The weather during the flowering time of the genotypes sown on 1st April was hot (30 °C), there was no rain, and the humidity was under 40%. After flowering, there was 62 mm rainfall but it did not affect the extent of infection.

The head rot infection was significantly higher in the case of the genotypes sown on 30th April, the temperature during the flowering time of these genotypes was lower, the relative humidity was more then 60% all day and the rainfall was 40 mm, compared to the plots flowering before and afterwards. In the experimental area the rainfall was 0.5 mm and there were no hot days. The degree of head rot infection of the genotypes flowering this time was less than that of the genotypes flowering till mid July.

The observations have shown that the weather during flowering time had a huge influence on the head rot infection. Besides genotype sensitivity, the degree of infection was determined by favourable weather conditions for the germination of the ascospores. The failure of the artifical infection can be attributed to the high temperature during the period after infection. Many researchers claim that the optimal temperature for spores germination and infection is 16-25°C. In the experiments at more locations, the considerable differences in the genotype susceptibility were determined by the ecological conditions duing the flowering of a given genotype. Differences in infection percentage in nearly isogenic lines and their hybrids flowering at different dates, as well as those in performance trials of hybrids sown at two dates, showed a close significant correlation (r=0.84***) with the quantity of precipitation recorded from the beginning to the end of the flowering period.

In relation to combining ability, there was a significant difference with respect to general combining ability (GCA) in the mother lines and specific combining ability (SCA) between parental lines. There was one restorer line which had a good GCA to the head rot infection, and this line transmitted the resistance to its hybrids.

REFERENCES

Castaño, F., F. Vear, and D. Tourvieille de Labrouhe. 1993. Resistance of sunflower inbred lines to various forms of attack by *Sclerotinia sclerotiorum* and relations with some morphological characters. Euphytica 68:85-98.

Comstock, R.E., and H.F. Robinson. 1948. The components of genetic variance in populations of biparental progenies and their use in estimating the average degree of dominance. Biometrics 4:254-266.

Cormack, M.W. 1946. *Sclerotinia sativa*, and related species as root parasites of alfalfa and sweet clover in Alberta. Sci. Agric. 26:448-459.

Degener, J., A.E. Melchinger, and V. Hahn. 1999. Interspecific hybrids as source of resistance to *Sclerotinia* and *Phomopsis* in sunflower breeding. Helia 22:49-60.

Dennis, R.W.G. 1974. *Whetzelinia* Korf & Dumont, a superfluous name. Kew Bull. 29:89-91.

Gulya, T.M., K.Y. Rashid, and S.M. Masirevic. 1997. Sunflower diseases. p. 263–379. In: A.A. Schneiter (ed.), Sunflower Technology and Production. Agronomy Monograph 35. ASA, CSSA, and SSSA, Madison, WI, USA.

Köhler, H., and W. Friedt. 1999. Genetic variability as identified by AP-PCR and reaction to mid-stem infection of *Sclerotinia sclerotiorum* among interspecific sunflower (*Helianthus annuus* L.) hybrid progenies. Crop Sci. 39:1456-1463.

Korf, R.P., and K.P. Dumont. *Whetzelinia*, a new generic name for *Sclerotinia sclerotiorum* and *S. tuberose*. Mycologia 64:248-251.

Mestries, E., L. Gentzbittel, D. Tourvieille de Labrouhe, P. Nicolas, and F. Vear. 1998. Analysis of quantitative trait loci associated with resistance to *Sclerotinia sclerotiorum* in sunflowers (*Helianthus annuus* L.) using molecular markers. Mol. Breed. 4:215-226.

Rodríguez, M.A., N. Venedikian, M.E. Bazzalo, and A. Godeas. 2004. Histopathology of *Sclerotinia sclerotiorum* attack on flower parts of *Helianthus annuus* heads in tolerant and susceptible varieties Mycopathologia 157:291–302.

Seiler, G.J., and L.H. Rieseberg. 1997. Systematics, origin, and germplasm resources of the wild and domesticated sunflower. p. 21-65. In: A.A. Schneiter (ed.), Sunflower Technology and Production. Agronomy Monograph 35. ASA, CSSA, and SSSA, Madison, WI, USA.

Tourvieille, D., and F. Vear. 1984. Comparaison de méthod d'estimation de la resistance du tournesol au *Sclerotinia sclerotiorum* (Lib) de Bary. Agronomie 4:517-525.

Willetts, H.J., and A.L. Wong. 1971. Ontogenetic diversity of sclerotia of *Sclerotinia sclerotiorum* and related species. Mycol. Soc. 57:515-524.

Wong, A.L., and H.J. Willetts. 1973. Electrophoretic studies of soluble proteins and enzymes of *Sclerotinia* species. Mycol. Soc. 61:167-178.

Puccinia helianthi Schw., infecciones en híbridos comerciales en Argentina y su evolución durante dos décadas

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RESUMEN

La roya negra del girasol, causada por *Puccinia helianthi* Schw., puede reducir el rendimiento y calidad de híbridos de girasol. En la República Argentina se la reconoce como endémica a la región girasolera Norte (Chaco y Santa Fe). Sin embargo, en la campaña 2006/7 ocurrió una epifitia sin precedentes en la región girasolera Centro (sur de Córdoba, La Pampa y Norte de Buenos Aires) y Sur (sur de Buenos Aires), de temprana aparición y elevada intensidad. El objetivo del presente trabajo es caracterizar la interacción entre genotipos de girasol (cultivares comerciales) y ambientes de la región centro y sur, donde se presentó roya negra. De la caracterización de esta interacción se aportará valiosa información sobre la posible existencia de razas de *P. helianthi* en la República Argentina y su directa implicancia en los planes de mejoramiento y manejo de esta enfermedad. Los genotipos resistentes identificados fueron estables a través de los ambientes; cinco genotipos presentaron comportamiento variable entre ensayos. La falta de interacción cruzada entre GxA para la manifestación de roya negra sugiere la presencia en la región Centro y Sur de girasol de una única raza de *Puccinia helianthi*.

Palabras clave: *Helianthus annuus* enfermedades - híbridos - *Puccinia helianthi* - roya negra girasol.

ABSTRACT

Sunflower rust caused by *Puccinia helianthi* is an important disease of sunflower in Argentina with a potential for causing significant yield losses in susceptible hybrids. In Argentina it is most frequently encountered affecting sunflower in the northern area (Chaco and Santa Fe states), but during the 2006/07 growing season early and severe rust outbreaks occurred in the central states of Cordoba, La Pampa and Buenos Aires. The objective of this study was to characterize the interaction between commercial cultivars of sunflower in diverse localities where the epidemic was severe. It is important to know the existence of pathotypes of *P. helianthi* in a given area to improve the effective lifespan of sunflower commercial cultivars. Results indicated that the resistant sunflower genotypes identified were stable at the different localities. Lack of cross interaction between Genotypes x Environment for the rust sunflower disease revealed the presence of only one rust pathotype in the Central and South sunflower growing areas.

Key words: diseases - *Helianthus annuus* – hybrids – *Puccinia helianthi* – rust – sunflower.

INTRODUCCIÓN

La roya negra del girasol (RN), causada por *Puccinia helianthi* Schw., puede reducir el rendimiento y calidad de híbridos de girasol (Gulya et al., 1997). En la República Argentina (RA) se la reconoce como endémica a la región girasolera Norte (Chaco y Santa Fe). Sin embargo, en la campaña 2006/7 ocurrió una epifitia sin precedentes en la región girasolera Centro (sur de Córdoba, La Pampa y Norte de Buenos Aires) y Sur (sur de Buenos Aires), de temprana aparición y elevada intensidad (Huguet et al., 2007). El manejo de la enfermedad se basa en la utilización de genotipos con resistencia genética. Las fuentes de resistencia utilizadas son las provenientes de cruzamientos y selecciones de la "Mezcla Precoz" (Bertero de Romano y Norberto Vázquez, 2003) que dio origen a la variedad "Charata INTA" también ha dado origen a líneas importantes para el mejoramiento del girasol tales como: Pergamino 71/538 (INTA Pergamino), HA-R1 y HA-R4 (USDA, Fargo, ND, USA) y MP555 y MP557 (INTA Castelar). HA-R1 se utiliza como diferencial internacional para las razas de *Puccinia helianthi*, y HA-R4 para las razas de

Puccinia helianthi y *Plasmopara halstedii* (Gulya and Masirevic, 1995; Miller and Gulya, 1995). En la RA las infecciones de roya negra han variado considerablemente año a año, la evolución en las últimas dos décadas se observa en la Fig. 1.

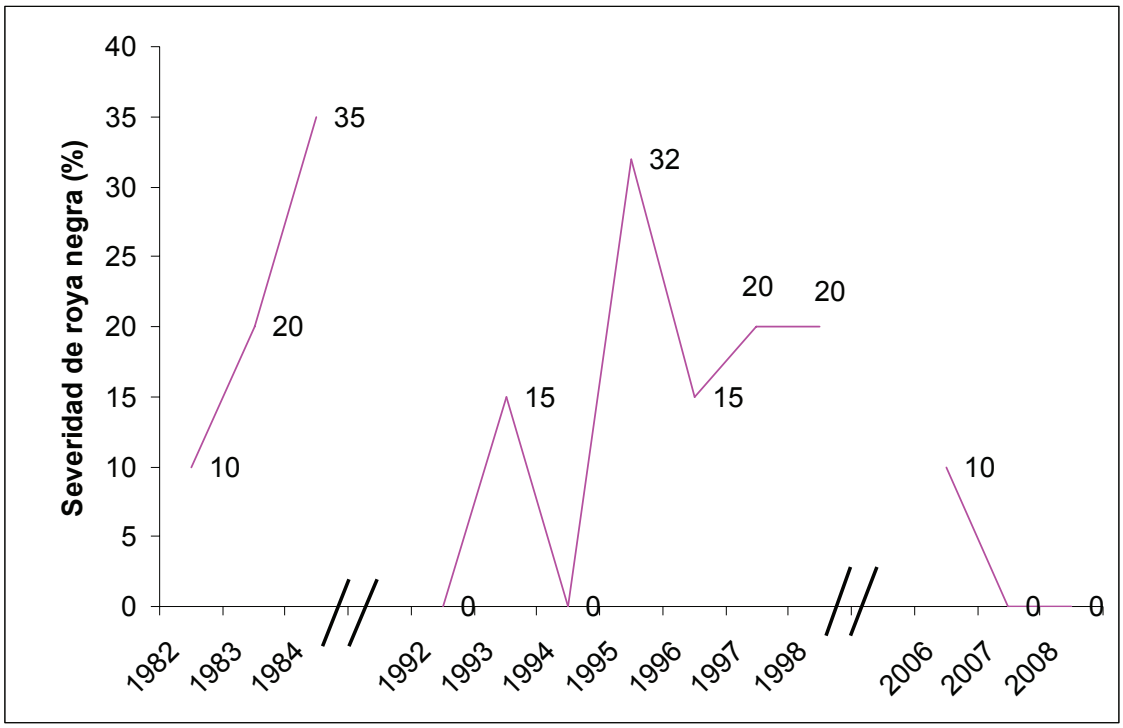

Fig. 1. Evolución de la severidad de Roya Negra en la República Argentina entre los años 1982 y 2008. Evaluaciones realizadas sobre híbridos comerciales que participaron en los Ensayos Comparativos de Rendimientos de la Red Nacional de Girasol en las campañas 1982-1984 y 2006-08 y Ensayos de NUZEA-INTA Campañas 1992-98.

Entre los principales objetivos del la Red Nacional de Cultivares Comerciales de Girasol de INTA se destacan la caracterización del comportamiento sanitario de los genotipos. A partir de varios ensayos ubicados en las zona centro y sur se identificó a un grupo acotado de cultivares comerciales resistentes a la Roya negra (18 de 85 cultivares evaluados), como así también no fueron identificados cultivares con niveles altos de susceptibilidad (Huguet et al., 2007). Aproximadamente el 10% del total de los cultivares evaluados no fueron caracterizados por su comportamiento debido a la variabilidad entre ensayos. El objetivo del presente trabajo es caracterizar la interacción entre genotipos de girasol (cultivares comerciales) y ambientes de la región Centro y Sur, donde se presentó Roya negra. De la caracterización de esta interacción se aportará valiosa información sobre la posible existencia de razas de *P. helianthi* en la RA y su directa implicancia en los planes de mejoramiento y manejo de esta enfermedad.

MATERIALES Y MÉTODOS

Cincuenta y dos híbridos comerciales de girasol fueron evaluados en cinco ensayos con presencia de la enfermedad, ubicados en la zona sur y centro de producción de la RA. El diseño utilizado fue de tres bloques completos aleatorizados. En el estadio de fin de floración se evaluó la severidad de Roya negra (ASAGIR, 2002). Se realizó análisis de la varianza donde se estimó el efecto del genotipo (G), el ambiente (A) y la interacción GxA, todos estos factores fueron considerados como de efectos aleatorios (Procedimiento GLM, SAS, Institute, Cary, NC, USA). Se estimó los componentes de la varianza mediante el procedimiento RELM (SAS, Institute, Cary, NC, USA). Para la identificación de genotipos de comportamiento variable se realizó el análisis de interacción GxA adaptado por Massiero y Castellano (1991).

RESULTADOS

Se identificó el efecto de la interacción genotipo-ambiente (*P*=0,0001), del ambiente (*P*=0,0001) y el genotipo (*P*=0,001). Del total de la variabilidad fenotípica, la interacción representó el 12%, el genotipo el 82% y el ambiente el 6%. Los cultivares Paraíso 33 (Nidera sa, Junin, Argentina), Paihuén (El Cencerro, Coronel Suárez, Argentina), CF31 (Advanta semillas sa, Venado Tuerto, Argentina), PAN7031 y PAN7047 (PANNAR Argentina sa, Venado Tuerto, Argentina) presentaron comportamiento variable entre ensayos. Ninguno de estos híbridos presentó comportamiento cruzado a través de los ambientes, de las interacciones detectadas solo existió cambio de magnitud en niveles de Roya Negra. Los genotipos resistentes identificados fueron estables a través de los ambientes.

CONCLUSIONES

Las fuentes de resistencia de Roya Negra incluida en los híbridos comerciales de girasol de la RA son estables en ambientes del la región Centro y Sur de girasol.

La falta de interacción cruzada GxA para la manifestación de Roya Negra sugiere la presencia en la región Centro y Sur de girasol de una única raza de *Puccinia helianthi*. Son necesarios posteriores trabajos para confirmar y actualizar las mismas.

REFERENCIAS

ASAGIR, 2002. Primer taller de fitopatología. Carlos Casares. www.asagir.org.ar/talleres.asp

Bertero de Romano, A., and A. Norberto Vázquez. 2003 Origen de las Variedades Argentinas de Girasol. Nidera S.A., Baigorrita, Argentina. ASAGIR.

Gulya, T., and S. Masirevic. 1995. Proposed methodologies for inoculation of sunflower with *Puccinia helianthi* and for disease assessment. p. 3-47. In: Studies on Common Methodologies of Artificial Inoculation and Population Dynamics of Sunflower Pathogens. The FAO European Research Network on Sunflower, Rome, Italy.

Gulya, T., K.Y. Rashid, and S.M. Masirevic. 1997. Sunflower diseases. In: Sunflower Technology and Productions, Agronomy Monograph Nº 35. American Society of Agronomy, Madison, WI, USA.

Huguet, N, J. Pérez Fernández, V. Quillehauquy, A. Escande, and F. Quiroz. 2007. Epifitia de roya negra por *Puccinia helianthi* en las principales regiones de producción de girasol. 4º Congreso Argentino de Girasol, ASAGIR.

Masiero, B., and S. Castellano. 1991. Programa para análisis de la interacción genotipo-ambiente - Primer Congreso Latinoamericano de Sociedades de Estadistica , Valparaiso, Chile.

Miller, J.F., and T.J. Gulya. 1995. Registration of three rust resistant sunflower germplasm populations 2001. Crop Sci. 41:601.

Results regarding the influence of *in vitro* stress induced by the *Phomopsis helianthi* filtrate on some physiological indices and on sunflower oil quantity and quality

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ABSTRACT

During 1997-2005 at ARDI Fundulea, many experiments for *in vitro* testing and selection of some Romanian sunflower genotypes with tolerance to *Phomopsis helianthi* have been performed. Fourteen out of the 30 tested genotypes were selected for their good response to the *in vitro* culture. Following the treatment applied on MS culture medium supplemented with 150ml/l filtrate, and, on the basis of the results obtained regarding the leaf index, chlorophyll content, 1000-kernel weight, seed oil percentage and its composition, genotypes with increased resistance to this pathogen have been selected. The determinations were performed by the Minolta Chlorophyll meter (SPAD units) for chlorophyll contents, nuclear magnetic resonance for oil content, and gas-chromatography for fatty acid composition of the seed oil.

Key words: chlorophyll content – *in vitro* culture – *in vitro* testing and selection – *Phomopsis helianthi*.

INTRODUCTION

Phomopsis helianthi (Diaporthe helianthi), causal agent of stem canker, is one of the most important pathogens of sunflower in Europe. It can cause significant losses in yield (10±50%) and in oil content (10±15%) when the environmental conditions are favorable for disease development. Stem canker was noticed for the first time in Yugoslavia in 1980 and in Romania in 1981 (Vrânceanu et al., 1992; Vrânceanu, 2000). In 1994, the inocula of *Phomopsis* were present in all the areas where sunflower is grown (Vear et al., 1997).

Using *in vitro* screening, the goals of this study were to contribute to the knowledge regarding the influence of stress induced by *Phomopsis helianthi* filtrate on some Romanian inbred lines and to the identification of inbred lines with a high level of tolerance to the pathogen (Raducanu et al., 1997a, 1997b; Raducanu, 1998; Hagima and Raducanu, 1998; Raducanu et al., 2002; Raducanu and Moraru, 2003; Raducanu et al., 2005).

MATERIALS AND METHODS

For *in vitro* testing to *Phomopsis helianthi* pathogen, a total of 14 Romanian inbred lines were used. As explants, immature embryos collected 10 days after pollination were inoculated on an MS medium, supplemented with 150ml/l *Phomopsis helianthi* filtrate and incubated for 21 days at 27ºC, 12/12 light/dark. After this period, phenotypically normal plants were transplanted into pots with a mixture of heavy soil and sand in 1:1 proportion and they were grown under controlled conditions until maturity.

On these plants, under different stages of vegetation, the following data were recorded: leaf index, chlorophyll content, TKW (thousand kernel weight), seed oil content and its composition.

The determinations were performed by the Minolta chlorophyll meter (SPAD units) for chlorophyll content, nuclear magnetic resonance (NMR) for oil content, and gas-chromatography (Shimadzu-GC-14B) for seed oil fatty acid composition.

The fatty acids were analyzed according to the conventional method (Schulte and Weber, 1989). The transesterification of triglycerides to fatty acid methyl esters was performed with trimethylsulfoniumhydroxid (TMSH). A capillary column (25 MX 0.32 MM ID) of 25m length on a Shimadzu gas chromatograph with flame ionization detector (FID) was used. Injector and detector were kept at 270 and 280 ºC, respectively. The carrier gas was nitrogen, with a flow rate of 20 ml/min. To calculate the total area of the peaks, an electronic integrator was used. The area of each fatty acid peak was expressed as a percentage of the total area.

The leaf index was calculated by the following formula:

L x l x 0.66 (L=length; l=width; 0.66=correction coefficient).

RESULTS

The ANOVA analyses and effects of *Phomopsis helianthi* filtrate on leaf index, chlorophyll content, 1000-kernel weight, seed oil percentage and its composition are presented in Tables 1 to 9.

Table 1. ANOVA of the leaf area of some Romanian sunflower genotypes after *Phomopsis helianthi* treatment

Source of variation	SS	DF	MS	F value
Genotypes (A)	34198.51	13	2630.65	60.049***
A error	1139.02	26	43.808	-
Treatment (B)	2584.12	1	2584.12	67.034***
A x B	4020.47	13	309.267	8.022***
B error	1079.37	28	38.549	

Table 2. The effects of *Phomopsis helianthi* filtrate on leaf area in some Romanian sunflower genotypes

NO.	**Genotypes**	Average leaf area (cm^2 / genotype)			
		Control		treatment	
		Average	Difference from average[1]	Average	Difference from control[2]
1	LC 4001	17.366	-16.125^^	22.800	-10.691
2	LC 4002	12.933	-20.558^^^	162.000	-17.291**
3	LC 4005	27.566	-5.925	25.466	-8.025
4	LC 4006	41.800	8.308	26.000	-6.891
5	LC 4007	23.866	9.625	14.100	19.391***
6	LC 4010	62.166	28.675^^^	44.000	10.508
7	LC 4011	36.166	2.675	34.833	1.341
8	LC 4016	14.600	-18.891^^	15.166	-18.325**
9	LC 4018	37.433	3.914	16.100	-17.391**
10	LC 4019	55.633	22.141^^^	31.833	-1.658
11	LC 4020	110.666	77.175^^^	62.566	29.075***
12	LC 4022	16.266	-17.225^^	11.533	-21.958***
13	LC 4024	68.300	34.802^^^	49.866	16.375***
14	LC 4025	21.766	-11.725^	20.166	-13.325*
	Average	39.037		35.216	

[1]^, ^^, ^^^ Significantly different from average for P<0.05; P<0.01; P<0.001, respectively.
[2]*, **, *** Significantly different from control for P<0.05; P<0.01; P<0.001, respectively.

Table 3. ANOVA of the chlorophyll content in some Romanian sunflower genotypes after *Phomopsis helianthi* filtrate treatment

Source of variation	SS	DF	MS	F value
Genotypes (A)	3418.58	13	262.968	19.575***
A error	349.271	26	13.433	-
Treatment (B)	460.615	1	460.615	43.124***
A x B	593.004	13	45.615	4.271***
B error	299.075	28	10.681	

Table 4. The effects of *Phomopsis helianthi* filtrate on chlorophyll content in some Romanian sunflower genotypes

NO.	**Genotypes**	Average chlorophyll content (SPAD/units)			
		Control		Treatment	
		Average	Difference from average[1]	Average	Difference from control[2]
1	LC 4001	24.500	-5.508	28.500	-1.508
2	LC 4002	22.433	-7.575	17.600	-12.408**
3	LC 4005	26.633	-3.375^^	20.766	-9.241**
4	LC 4006	30.233	0.225	23.866	-6.141**
5	LC 4007	30.400	0.391^^^	25.400	-4.608
6	LC 4010	35.800	5.791	36.000	-6.141**
7	LC 4011	35.800	5.791	39.866	9.858**
8	LC 4016	38.800	8.917^^	31.933	4.925
9	LC 4018	42.733	12.725	31.566	1.558
10	LC 4019	42.866	12.725	36.800	6.792**
11	LC 4020	42.733	12.725	31.266	-1.258
12	LC 4022	26.166	-3.841^^^	24.266	-5.741
13	LC 4024	38.500	8.492^^^	20.800	-9.408
14	LC 4025	22.866	-7.142^	18.900	-11.108***
	Average	32.890		27.680	

[1]^, ^^, ^^^ Significantly different from average for P<0.05; P<0.01; P<0.001, respectively.
[2]*, **, *** Significantly different from control for P<0.05; P<0.01; P<0.001, respectively.

Table 5. ANOVA of the TKW in some Romanian sunflower genotypes after *Phomopsis helianthi* filtrate treatment.

Source of variation	SS	DF	MS	F value
Genotypes (A)	819.046	13	63.003	21.632***
A error	75.726	26	2.912	-
Treatment (B)	150.934	1	150.934	117.678***
A x B	77.645	13	5.972	4.657***
B error	35.912	28	12.826	

Table 6. The effects of *Phomopsis helianthi* filtrates on TKW in some Romanian sunflower genotypes

NO.	**Genotypes**	Average TKW(g)			
		Control		Treatment	
		Average	Difference from average[1]	Average	Difference from control[2]
1	LC 4001	13.766	-0.408	13.533	-0.640
2	LC 4002	18.733	4.599^^^	18.366	4.192**
3	LC 4005	20.966	6.792^^^	18.900	4.726***
4	LC 4006	21.223	7.059^^^	15.600	1.426
5	LC 4007	12.300	-1.874	10.366	-3.807**
6	LC 4010	16.800	2.626^	10.600	-3.574**
7	LC 4011	17.566	3.392^^	15.466	1.292
8	LC 4016	14.666	0.492	13.033	-1.106
9	LC 4018	14.000	-0.174	10.933	-3.240**
10	LC 4019	14.033	-0.140^^^	10.466	-3.707
11	LC 4020	18.933	4.759^^^	12.933	-1.240
12	LC 4022	12.700	-1.474	11.100	-3.074**
13	LC 4024	11.700	-2.474^	9.400	-4.744***
14	LC 4025	9.700	-4.474^^^	8.966	-5.207***
	Average	14.459		12.833	

[1]^, ^^, ^^^ Significant different from average for P<0.05; P<0.01; P<0.001, respectively.
[2]*, **, *** Significantly different from control for P<0.05; P<0.01; P<0.001, respectively.

Table 7. ANOVA of the oil content in some Romanian sunflower genotypes after *Phomopsis helianthi* filtrate treatment

Source of variation	SS	DF	MS	F value
Genotypes (A)	1302. 19	13	100.169	48.209***
A error	54.022	26	20. 778	-
Treatment (B)	516.031	1	512.031	160.344***
A x B	195.958	13	15.073	4.684***
B error	90.111	28	3.218	

Table 8. The effects of *Phomopsis helianthi* filtrate on the oil content in some Romanian sunflower genotypes

NO.	**Genotypes**	Average oil content (%)			
		Control		Treatment	
		Average	Difference from average[1]	Average	Difference from control[2]
1	LC 4001	29.333	- 4.972^^^	27.200	- 7.104***
2	LC 4002	37.700	3.395^	27.600	6.671***
3	LC 4005	39.266	4.961^^^	33.633	0.671
4	LC 4006	39.200	4.895^^^	36.333	2.028
5	LC 4007	27.200	- 7.104^^^	29.033	- 5.271***
6	LC 4010	41.900	7.595^^^	36.000	1.695
7	LC 4011	29.933	- 4.371^^^	27.366	-6.938***
8	LC 4016	36.233	1.928	28.433	-5.871***
9	LC 4018	38.033	3.728^^^	33.333	1.005
10	LC 4019	41.100	7.795^^^	38.500	4.195*
11	LC 4020	43.866	9.561^^^	35.400	1.095
12	LC 4022	38.866	4.561^^^	30.000	- 4.305*
13	LC 4024	33.700	- 0.538	30.133	-4.171*
14	LC 4025	37.566	3.261^	34.033	-2.272
	Average	36.706		31.928	

[1]^, ^^, ^^^ Significantly different from average for P<0.05; P<0.01; P<0.001, respectively.
[2]*, **, *** Significantly different from control for P<0.05; P<0.01; P<0.001, respectively.

Table 9. Retention time for fatty acids from the standard solution

No. peak	Retention time (min.)	Fatty acid (formula)	The fatty acid
1	12.55	C 16:0	Palmitic acid
	17.48	C 18:0	Stearic acid
2	17.48	C 18:1	Oleic acid
3	20.36	C 18:2	Linoleic acid
4	22.65	C 18:3	Linolenic acid

DISCUSSION

The results obtained by gas chromatography underlined the fact that of the five fatty acids from sunflower oil, oleic acid decreases after treatment in all genotypes, excepting the LC 4010 line. At the same time, the linoleic acid percentage increases after treatment in nine out of the tested lines. We positively noticed the fact that the linolenic acid, which reduces oil stability, was detected only in three genotypes but in very small quantities. ANOVA for the leaf index emphasized a very different behavior of the tested lines, with significant positive or negative differences between genotypes, depending on both tolerance degree to disease and response to the *in vitro* culture. Eight genotypes in which the leaf area was not diminished by the treatment as compared with the control have been identified.

As regards the chlorophyll content, it was ascertained that for all tested genotypes, at the treatment variant, the average/variant was diminished with 5.2 SPAD units vs. the control.

In variants treated with the filtrate, TKW was drastically diminished in seven out of the 14 genotypes. The oleic acid content showed a higher decrease in comparison with the control in all lines excepting the LC 4010 line.

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REFERENCES

Hagima, I., and F. Raducanu. 1998. Some relationships between *Helianthine* and resistance to *Phomopsis* in some sunflower genotypes. European Society for New Methods in Agricultural Research. Brno, Czech Republic, 125 p.

Raducanu, F. 1998. The using of cells and tissues culture in sunflower breeding for *Sclerotinia sclerotiorum* and *Phomopsis helianthi* resistance. Sunflower Yearbook. p. 83-84.

Raducanu, F., and I. Moraru. 2003. The reaction of some Romania genotypes at in vitro stress induced by *Phomopsis helianthi* filtrate. Proceeding of European ESSA, Environmental Stress and Sustanable Agriculture, p. 407.

Raducanu, F., I. Moraru, and G. Soare. 1997a. Actualitati si perspective in biotehnologiile vegetale. p. 191-197. ISBN 973-98095-1-0.

Raducanu, F., I. Moraru, and G. Soare. 1997b. Testarea *in vitro* pentru rezistenta la *Diaporthe/Phomopsis helianthi Munt. Cvet*. a unor linii consangvinizate de floarea soarelui. Anale INCDA. p.150-155.

Raducanu, F., I. Moraru, and E. Petcu. 2002. Genetic variability of some Romanian sunflower genotypes under *in vitro* stress induced by *Phomopsis helianthi* filtrate. Romanian Agricultural Reserach 17/18:. 9-15.

Raducanu, F., E. Petcu, and I. Moraru. 2005. Probleme actuale ale geneticii, Biotehnologiei si Ameliorarii. Efectul filtratului de *Phomopsis helianthi* asupra unor caractere fiziologice si biochimice la unele genotipuri de floarea-soarelui.

Schulte, E., and K. Weber. 1989. Sculte Herstellung der Fettsauremethylesteraus Fetten mit Trimethylsulfoniumhydroxid oder Natriummethylat. Fat Sci. Technol. 91:181-183.

Vear, F., M. Garreyn, and D. Tourvieille de Labrouhe. 1997. Inheritance of *Phomopsis (Diaporthe helianthi)* in sunflower. Plant Breed. 116:277-281.

Vrânceanu, A.V., F. Stoenescu, N. Pârvu, and N. Csep. 1992. Genetic variability of sunflower reaction to the attack of *Phomopsis helianthi* Munt. Cvet. Helia 23-25.

Vrânceanu, A.V. 2000. Floarea-soarelui hibrida. Ceres, Bucharest, Romania.

The impact of the new races of broomrape (*Orobanche cumana* Wallr.) parasite in sunflower crop in Romania

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ABSTRACT

The pathogenic composition of broomrape populations has changed over the years, slowly at first, then rapidly in Eastern Europe, Turkey and Spain. In Romania there are three important areas infested with broomrape (*Orobanche cumana* Wallr.), which are different in their infestation degree and presence of different virulence groups. A new highly virulent population of broomrape has attacked sunflower in Romania in 2005. Many commercial hybrids from different companies lost their resistance to this parasite. In the sunflower germplasm collection of Fundulea Institute has been identified a restorer line, AO-548, fully resistant to this new broomrape population. Since this line could be used directly as a parent to produce commercial hybrids, as well as a source of resistance to broomrape in sunflower breeding programs, the inheritance of resistance to the new population of *Orobanche cumana* was studied. Using the cytoplasmic male sterile inbred line AD-66, very susceptible to this population of the parasite, as a female parent, progenies of the cross with AO-548: F_1, F_2 and BC_1 to both parents, as well as the parental lines, were analysed for their reaction to this broomrape population. The results of the observed resistant/sensitive plants vs expected ratio (15:1 and 3:1) indicated that the inheritance of resistance to broomrape in the line AO-548 is conferred by two independent dominant genes.

Key words: broomrape – inheritance – resistant – sensitive – sunflower – virulence.

INTRODUCTION

Broomrape (*Orobanche cumana* Wallr./*Orobanche cernua* Loefl.) attacks sunflower crop in parts of Europe, the Near East and China (Vrânceanu, 2000). In the last few years the parasite migrated to Western Europe (in 2007 this parasite attacked sunflower crops in France).

Sunflower breeding for resistance to this parasite was started by Pustovoit in 1912 at VNIIMK Institute in Krasnodar, Russia (Sackston, 1992). Using the method of growing all available sunflower material in heavily infested plots, Pustovoit had, by 1927, selected strains with up to 99% of resistant plants (Pustovoit, 1967). By that year, however, previously resistant sunflowers were succumbing to what turned out to be a complex of new races of broomrape. Repeated selection produced lines resistant to the new race complex. Pustovoit did not try to determine the nature of genes controlling this resistance.

Some early Soviet sunflower breeders did study genetic ratios. Meister (1936) reported that resistance to broomrape was inherited as a dominant character, and referred to simple segregation ratios. Later, scientists found that resistance to broomrape races A and B derived from the perennial *H. tuberosus* L. was controlled by a single simply inherited dominant gene (Burlov and Kostyuk, 1976; Pogorletsky and Geshele, 1976).

The virulence of the parasite populations has changed over the years. Vrânceanu et al. (1980) reported on five virulence groups (races or groups of races) of broomrape encountered in Romania, and five types of effective resistance against the respective groups. These investigators set up a series of differentials permitting identification of the five virulence groups, although not the individual races of the pathogen, as each resistance type was effective against a specific race group. The results of complex crossing studies demonstrated a gene-for-gene relationship between virulence groups in the broomrape and resistance in sunflower. They successfully introduced gene *Or5,* which gives resistance to all five race groups, into inbred lines with high combining ability that were the parents of existing or prospective hybrids, and released resistant hybrids.

The different reactions of resistance in varieties of different pathogen sensitivities in sunflower have been reported in recent years. Ciriaev (1987) reported the oligogenic resistance controlled by two genes. Domínguez (1996) has identified the line R-41 having resistance to broomrape controlled by two independent dominant genes. Melero-Vara and Fernández-Martínez (2004) have reported two independent recessive genes for resistance to broomrape.

In Romania, the race F was identified in 1997, as well as the gene (one dominant gene) conferring resistance to this race (Pacureanu-Joita et al., 1998)

Melero-Vara et al. (1989), and other authors quoted works indicating the chemical control of broomrape, but agreed that genetic resistance is the most important method for controlling the parasite. IMI resistance sunflower hybrids may be another way to control it.

This paper presents the results obtained in identifying a new race of *O. cumana* in sunflower crop in Romania, as well as a source of resistance and its inheritance.

MATERIALS AND METHODS

Different sunflower hybrids were tested in fields naturally infested with broomrape, in two important areas in Romania. The investigators set up a series of differentials permitting identification of different virulence groups of the parasite. The different sunflower genotypes (lines and populations) were tested for resistance to the broomrape attack, with a view to identifying new sources of resistance to the most virulent populations of the parasite. Crosses between sensitive lines and new resistant ones were performed in order to establish the inheritance of the genetic resistance. The testing was performed under artificial inoculation using broomrape seeds from two infested areas in Romania. The Panchenko (1975) method was used for testing the artificial infestation conditions.

RESULTS

In Romania, more than 55% of the sunflower cultivated area is infested with broomrape. There are three important areas with a high infestation degree and the presence of different virulence groups (Fig. 1). The high infestation degree in the first area, situated near the Black Sea, is given by race F, and race G has also been identified in this area. In the second area, situated in Ialomita-Braila, race F is well represented.

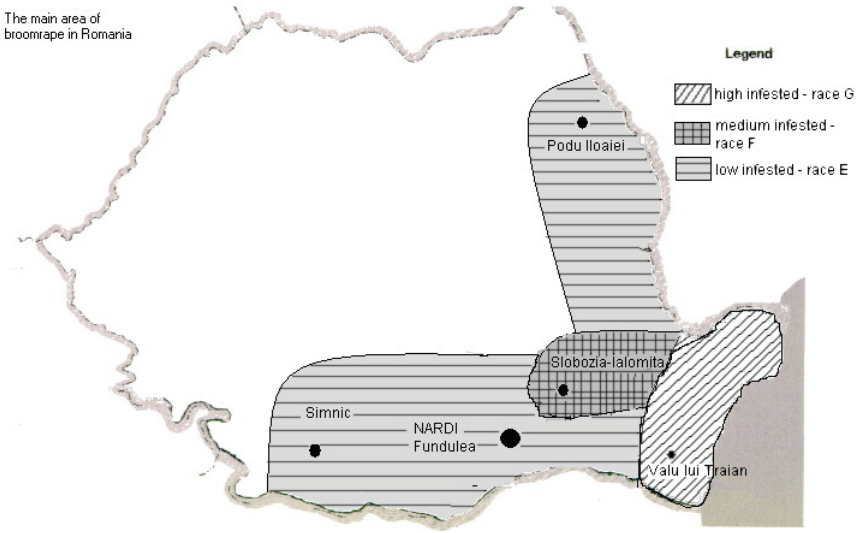

Fig. 1. The main areas of broomrape infestation in sunflower crop in Romania**.**

In recent years, the parasite *Orobanche cumana* has developed new races in a short time in sunflower crops in Romania, compared to the first period (Fig. 2). So, if 15 years have passed since the identification of races A and B until race E appeared, as well as from race E to race F, the races G and, may be, H, have appeared in a shorter time and spread quickly over a large area.

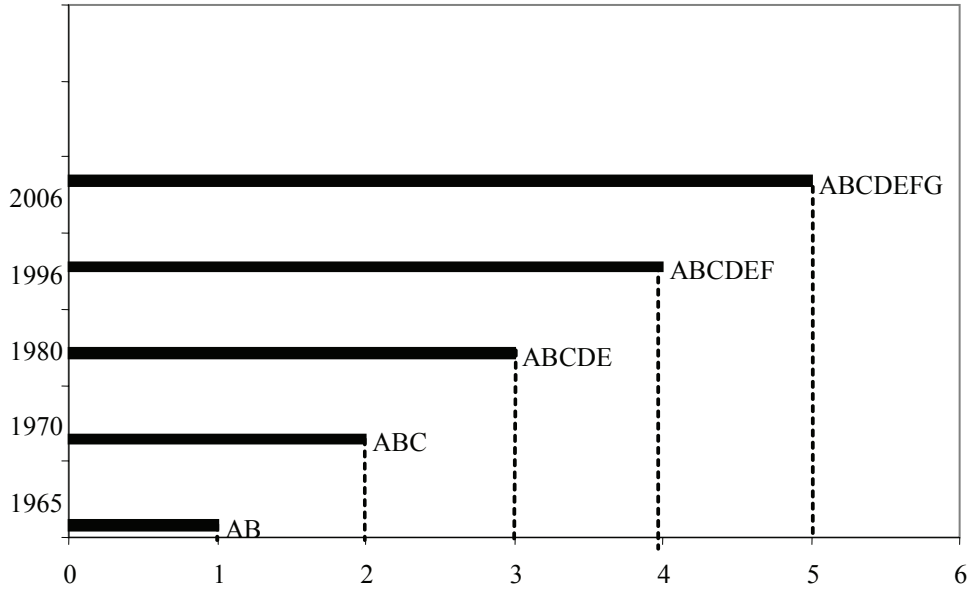

Fig. 2. The evolution of the broomrape races in sunflower crop in Romania.

In 2006, in a sunflower crop cultivated in Tulcea area, near the Black Sea, some of the hybrids resistant to the race F lost their resistance, being infested at a high percentage (Fig. 3). The hybrids having resistance to the races G or H , were fully resistant.

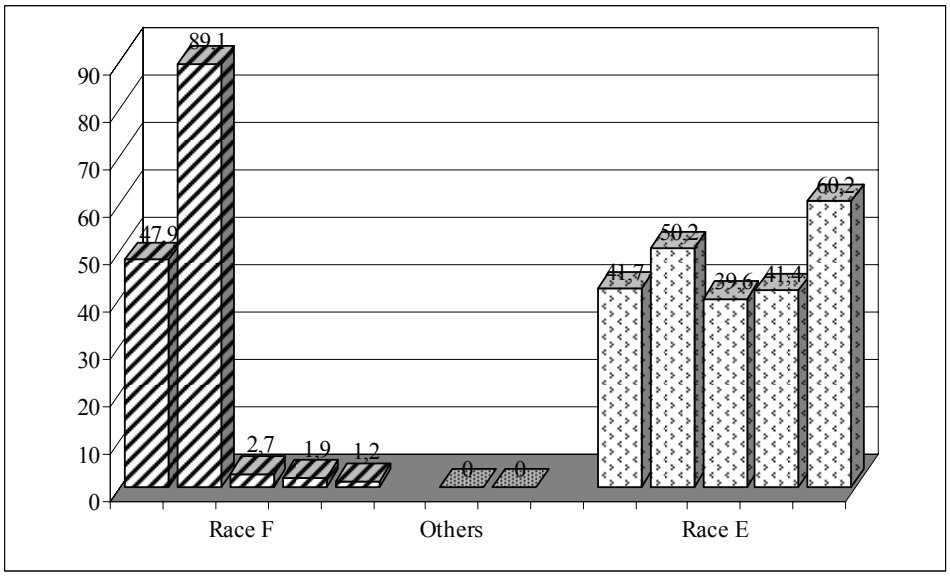

Fig. 3. The behaviour of some sunflower cultivated hybrids in an infested area with broomrape, Romania, 2006

Using broomrape seeds collected from this area, different inbred lines used as differentials for the races E, F and G have been tested, as well as some resistant hybrids, under artificial infestation conditions in the greenhouse. At the same time, the same genotypes were tested, using the broomrape seeds collected from Ialomita-Braila area. The results (Table 1) showed that the differential for the race F, the inbred line LC 1093, lost its resistance in Tulcea area, having full resistance in the Braila-Ialomita area. The inbred line AO-548 is resistant in both cases. The same behaviour was shown by the hybrids having as parents these two lines (Favorit and Daniel).

Table 1. The reaction to the broomrape attack in different sunflower genotypes (Fundulea, 2006-2007)

Sunflower genotype	Reaction to the broomrape races	Source of broomrape			
		Ialomita -Braila		Tulcea - Constanta	
		Number of infested sunfl. plants	Infestation degree (%)	Number of infested sunfl. plants	Infestation degree (%)
P-1380-2	E - A	10/10	41.7	10/10	77.4
LC-1093	F - A	0/10	0.0	3/10	1.9
Kd-3-2	F - A	0/10	0.0	2/10	1.2
AO-548	G - A	0/10	0.0	0/10	0.0
Od-832-2b	F - A	0/10	0.0	3/10	1.8
Favorit	F - A	0/10	0.0	5/10	2.3
F-225	F - A	0/10	0.0	7/10	2.9
PR64A83	(E)F - A	8/10	19.7	10/10	73.1
PR64A71	(G)H	0/10	0.0	2/10	0.9
Daniel	G - A	0/10	0.0	0/10	0.0

The test conducted under natural infestation conditions in Braila area, using some differentials for the races E, F and G, confirmed that race G was still not present in this area (Table 2).

Table 2. The reaction of the broomrape attack to sunflower under natural infestation conditions – Braila, Romania, 2007

Sunflower genotypes	Reaction to the broomrape races	Infestation degree (%)
P-1380-2	E - A	49.7
LC-1093	F - A	0.0
O-7455	E - A	8.7
Sel-10481	E - A	19.7
Kd-3-2	F - A	0.0
AO-548	G - A	0.0
AD-66	Sensitive	69.7

All tests for resistance to broomrape, which were performed in 2006 and 2007, have shown that, in all the infested areas, the restorer inbred line AO-548 was fully resistant. This line was crossed with AD-66, a CMS line, in order to establish the inheritance of resistance in this restorer line. The F_1 generation was obtained in the field, after which, the crosses and selfings to obtain BC_1 and F_2 generations were carried out under artificial infestation conditions, in pots, in the greenhouse. The plants were kept in pots until maturity, after that they were uprooted and their roots carefully washed to observe any established broomrape nodules. The plants free of nodules or stalks in the roots were considered resistant. The observed ratio of resistant and susceptible plants, in each generation, as well as the goodness of fit of observed – expected ratios are shown in Table 3. The F_2 progeny segregated at a ratio of 15:1 (resistant:susceptible), whereas the BC1 on the susceptible parent, AD-66, segregated according to 3:1

(resistant:susceptible), indicating that resistance to *Orobanche cumana* in AO-548 line is conferred by two single genes with independent action.

Table 3. Broomrape resistant and susceptible sunflower plants in the parental, F1, F2 and BC1 generations of crosses between AD-66 (cms) and AO-548, and the goodness of fit of observed (vs) expected ratios

Material (generations)	Plants		Expected ratio	P%
	Resistant	Susceptible		
AD-66 (P1)	-	15	-	
AO-548 (P2)	15	-	-	
F1	15	-	-	
F2	189	14	15:1	80-90
BC1 (AD-66)	50	15	3:1	50-70
BC1 (AO-548)	65	-	-	

DISCUSSION

The parasite *Orobanche cumana* has became more and more dangerous for the sunflower crop in Romania. In 2006, most resistant sunflower hybrids cultivated in an infested area with this parasite were attacked, some of them at a high attack degree (80%).

The behaviour of some sunflower genotypes regarding resistance to broomrape, under natural and artificial infestation conditions, has shown that the parasite virulence is increasing. The inbred line AO-548 was fully resistant.

The inheritance of resistance to broomrape in AO-548 line is conferred by two independent dominant genes. This line has a good combining ability, being used directly in the obtention of commercial hybrids.

REFERENCES

Burlov, V.V., and S.V. Kostyuk. 1976. Development of counterparts restoring pollen fertility and resistant to broomrape (*Orobanche cumana* Wallr.) and downy mildew (*Plasmopara helianthi* Novot.). p. 322-326 (vol. 1). In: Proc. 7th Int. Sunflower Conf., Krasnodar, USSR.

Ciriaev, P.V. 1987. Ustoicivosti samoopâlennîh linii podsolnocinika k zarazihe. Naucino-tehn.Biulleteni VNIIMK, Krasnodar, 11(97):3-5.

Dominguez, J. 1996. R-41 a sunflower line, carrying two genes for resistance against a highly virulent Spanish population of *Orobanche cernua*. Plant Breed. 115:203-204.

Meister, G.K. 1936. Selektia I Semenovodstvo, No.7

Melero-Vara, J.M., J. Dominguez, and J.M. Fernández-Martínez. 1989. Evaluation of differential lines and a collection of sunflower parental lines for resistance to broomrape (*Orobanche cernua*) in Spain. Plant Breed. 102:322-326.

Melero-Vara, JM., and J.M. Fernández-Martinez. 2004. Genetic resistance to broomrape in sunflower crop in Spain. Abstract COST Meeting in Bucharest, Romania, 4-6 November, 2004.

Panchenko, A.I. 1975. A screening method of evaluating the breeding materials for resistance to broomrape. Vestnik selkohoz, nauki, No.2.

Păcureanu-Joița, M., A.V. Vrânceanu, G. Soare, A. Marinescu, and I. Sandu. 1998. The evaluation of the parasite-host interaction in the system *Helianthus annuus* L. – *Orobanche cumana* Wallr. In Romania. p. 153-158 (vol. 1). In: Proc. 2nd Balkan Symposium in Field Crops.

Pogorletsky, B.K., and E.E. Geshele. 1976. Sunflower's immunity to broomrape, downy mildew and rust. p. 238-243 (vol. 1). In: Proc. 7th Int. Sunflower Conf., Krasnodar, USSR.

Pustovoit, V.S. 1967. p. 1-35. In: Handbook of Selection and Seed Growing of Oil Plants (In Russian) Transl. 1973, Isr. Progr. Sci. Transl., Jerusalem.

Sackston, W.E. 1992. On a treadmill: Breeding sunflowers for resistance to disease. Ann. Rev. Phytopathology 30:529-551.

Vrânceanu, A.V. 2000. Floarea soarelui hybrida. Editura Ceres, B.

Vrânceanu, A.V., V.A. Tudor, F.M. Stoenescu, and N. Pârvu. 1980. Virulence groups of *Orobanche cumana* Wallr., differential hosts and resistance sources and genes in Sunflower. p. 74-82 (vol. 1). In: Proc. 9th Int. Sunflower Conf. Torremolinos, Spain.

Distribution and dissemination of sunflower broomrape (*Orobanche cumana* Wallr.) race F in Southern Spain

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ABSTRACT

Sunflower broomrape (*Orobanche cumana* Wallr) is the most important problem in the sunflower crop in Southern Spain. The dissemination and dispersion of the virulent race F of this parasite has been evaluated during the last 10 years. The sunflower crop acreage in Southern Spain was divided into 8 large areas, where the presence and intensity of broomrape race F in sunflower fields was evaluated in the years 2001, 2003, 2005 and 2007. In two of these areas, irrigation lands and Antequera, broomrape race F was not found. In one area, Jerez, the progress of infection and dispersion showed a very slow advance. In the Seville-Huelva and Villamartín areas, the presence of race F accounted for 79% and 69% respectively, although without causing serious damage to the sunflower yield. In the other three areas studied, Córdoba, Écija and Carmona, the broomrape race F infestation was very high, causing important damage to sunflower production.

Key words: broomrape race F – broomrape distribution – *Orobanche cumana.*

INTRODUCTION

Sunflower broomrape (*Orobanche cumana* Wallr.) is a major disease in southern Europe, the Black Sea Region, Ukraine and China (Sackston, 1992; Parker, 1994). This obligate holoparasitic angiosperm attaches itself to the roots of infected plants depleting them of nutrients and water and causing important yield losses. In cases of severe infections, these losses can reach up to 50% and near 100% of sunflower production. Throughout sunflower broomrape history, different races of this holoparasite have developed in infested areas where sunflower has been traditionally cultivated (Vrânceanu et al., 1980).

In Spain, the broomrape was detected for the first time in Toledo province in 1958, attacking confectionery sunflower (González Torres et al., 1982). At the beginning of the 1980's the first broomrape infections in oil sunflower were noticed in Cuenca and El Coronil (Sevilla). In the early 1990's, in the surroundings of Écija (Sevilla) and La Almarcha (Cuenca), all the available commercial hybrids at that time were affected. Race studies showed that these infections were caused by broomrape race E (Melero-Vara, 1999; Domínguez, 2004). After 1993, several sunflower hybrids resistant to this race, most of them carrying the Or5 gene as the resistance source, were developed and commercialized. These hybrids are still being grown currently in most of the areas where sunflower is sown in Spain.

In 1995, a serious broomrape attack on resistant hybrids carrying the Or5 gene was detected for the first time in Spain near Écija (Sevilla) (Alonso et al., 1996). This new broomrape pathotype was determined as race F (Alonso et al., 1996). From that moment until the end of the 1990's, several spots of race F broomrape have been detected in Sevilla, Córdoba and Cuenca provinces (Domínguez, 1999). We report here a study carried out to evaluate the dissemination and dispersion of broomrape race F in the South of Spain during the present decade.

MATERIALS AND METHODS

The sunflower crop acreage in Southern Spain was divided into 8 large areas, where the presence and intensity of the broomrape virulent race F in sunflower fields was evaluated for four years. These areas are shown in Fig. 1 and Table 1. In each selected area, several sunflower fields were chosen at random. The sampling method consisted of selecting roads and ways including each area. Then the sunflower fields close to them located at a distance of 2-3 km. between each field were visited and evaluated. The total numbers of evaluated fields were 453 in 2001, 629 in 2003, 602 in 2005 and 565 in 2007.

For each tested field the following ranks of attack were considered:

- Large plots infected with race F: these are the sunflower fields with presence of plots larger than 1 Ha, highly infected by broomrape and associated with high yield losses.

- Small plots infected with race F: these are sunflower fields with more than 30-50% of infected sunflower plants with a low number of broomrapes per plant or with presence of small plots with a high broomrape infection. The incidence in sunflower yield is low.
- Presence of race F: these are the sunflower fields with a 10-25 % of infected sunflower plants with a low number of broomrapes per plant. The incidence in sunflower yield is practically void.
- Broomrape absence: sunflower fields with an under 10% broomrape infection in sunflower plants. In 2001 and 2003, these fields were clearly associated with non broomrape-infected fields. However, with the presence from 2005 onwards of sunflower race F-resistant hybrids in some studied areas, the absence of broomrape could be associated either with a non infected field or with a race F-resistant sunflower hybrid. In 2005 and 2007 assessments, the classification of a field with broomrape absence was assigned to one category or another by two methods. The first was a direct one, by obtaining information from the farmer about the hybrid sown. The second was a non direct one, on the basis of the information supplied by commercial staff of seed companies.

RESULTS AND DISCUSSION

The evaluation of the evolution of sunflower broomrape race was carried out in Andalusia (Southern Spain). The sunflower crop in this region accounts for approximately 45% of the total sunflower production in Spain, also being the area in which the mean performances are higher. Moreover, Andalusia is the region where broomrape race F has spread most, causing large losses in sunflower production. In order to study the evolution and dispersion of race F of broomrape, the sunflower growing area was divided into 8 large areas (Fig. 1), basically considering their record of the presence of former broomrape races. Table 1 shows for each area the surface taken up by sunflower in the four years of the study.

Fig. 1. Distribution of sunflower crop in Andalusia, subdivided into 8 large areas.

The results of the evaluation showed that throughout these last years, there were only two areas (E and G) where broomrape race F was not found in any field. This may be due to different reasons. In area E, irrigated land, the sunflower is a minor crop that is sown in rotation with corn and cotton. This area is relatively close to areas with a large broomrape presence, and, therefore, the broomrape seed dissemination by short distance movement, wind, water, tillage operations or infested farm equipment (Eplee et al., 1998), could be possible. The absence of *Orobanche* race F in this area could be due to the fact that both cotton and corn are trap crops, that promote the germination of broomrape seeds but without starting any infection in these crops (Rodríguez-Ojeda et al., 2001), resulting in the reduction of broomrape inocula in the soil. The second area with the absence of broomrape was area G, Antequera. This was probably due to the fact that this area is isolated from the rest of sunflower crop areas by large olive groves.

Table 1. Sunflower areas evaluated in Andalusia, location name and number of hectares cultivated in each area during 4 years.

Area	Location name	2001	2003	2005	2007
A	Sevilla-Huelva	28,381	26,822	18,619	20,463
B	Carmona	47,818	47,494	25,125	36,834
C	Écija	47,818	47,494	25,125	36,834
D	Córdoba	50,792	50,822	23,780	36,854
E	Irrigated land	28,189	28,045	15,934	21,722
F	Villamartín	44,339	44,002	24,394	34,276
G	Antequera	19,473	17,050	10,612	10,743
H	Jerez-Arcos	34,582	34,061	26,615	27,574
Total		301,392	295,788	170,204	225,300

The six remaining studied areas can be divided into 2 groups (Fig. 2 and Fig. 3). The areas of Écija, Córdoba and Carmona, C, D and B, respectively, where at present the broomrape race F is considered to be a serious problem for the sunflower crop, and the areas including Villamartín, Jerez and Sevilla-Huelva, F, H and A, respectively, where broomrape race F has been detected later and could be a potential problem for sunflower crop within the next years.

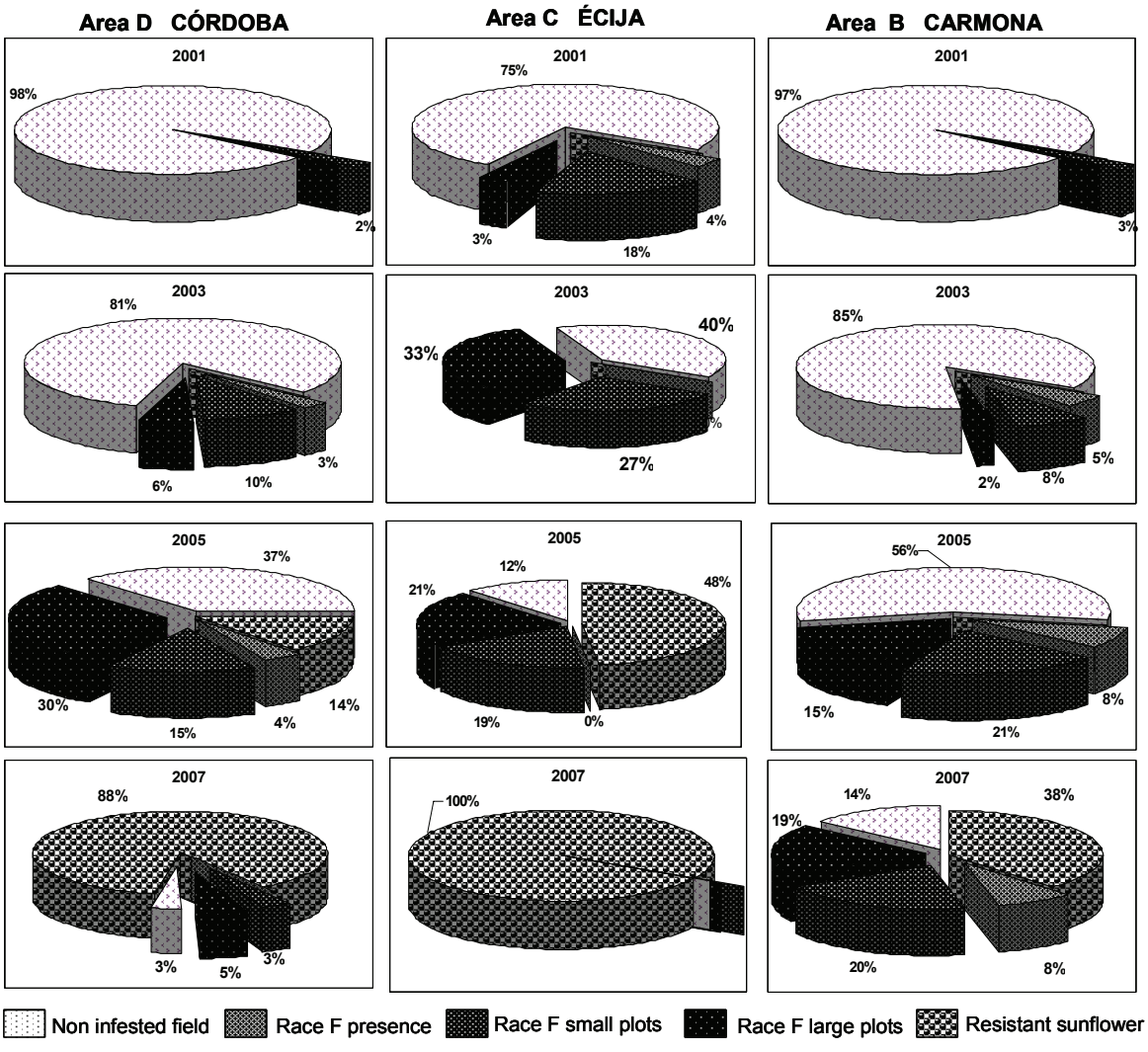

Fig. 2. Distribution of broomrape race F in Córdoba, Écija and Carmona areas during the years 2001, 2003, 2005 and 2007.

The first spots of race F were detected near Écija in the province of Sevilla in 1995 (Alonso et al., 1996). Within the following years, other small spots of this race appeared in new sunflower fields in a radius of about 20 km round Écija (Domínguez, 2004). In 2001 the fields affected by race F reached 25%, with large plots infested in 18% of the cases (Fig. 2). The spreading of race F in this area has been relatively slow, especially if compared with the broomrape race E evolution, which took place in the early 1990's, when the period from the appearance of the first fields with broomrape race E until its presence in over 80% of the fields, was only 4-5 years. From 2001, there has been a constant spread of race F in this area, from a complete absence of broomrape race F in 75% of the fields in 2001 to 40% in 2003, 12% in 2005 and, finally, with the whole sunflower cropped area sown with race F resistant hybrids in 2007 (Fig. 2).

The epidemic rate of growth in the Carmona area has followed a similar pattern to the one in Écija, although with a later appearance of the first race F infections. On the contrary, even though the presence of the first disease spots in Córdoba was simultaneous to that in Carmona, the spreading of race F towards Córdoba has been much faster than in the two previous areas (Fig. 2). One of the most important vectors in broomrape dissemination is the machinery movement among the different growing areas (Eplee et al., 1998). The higher rate of growth of race F in Córdoba may be due to the movement of combine-harvesters from Écija to the Córdoba area, where the harvest is up to 10-15 days later with regard to Écija.

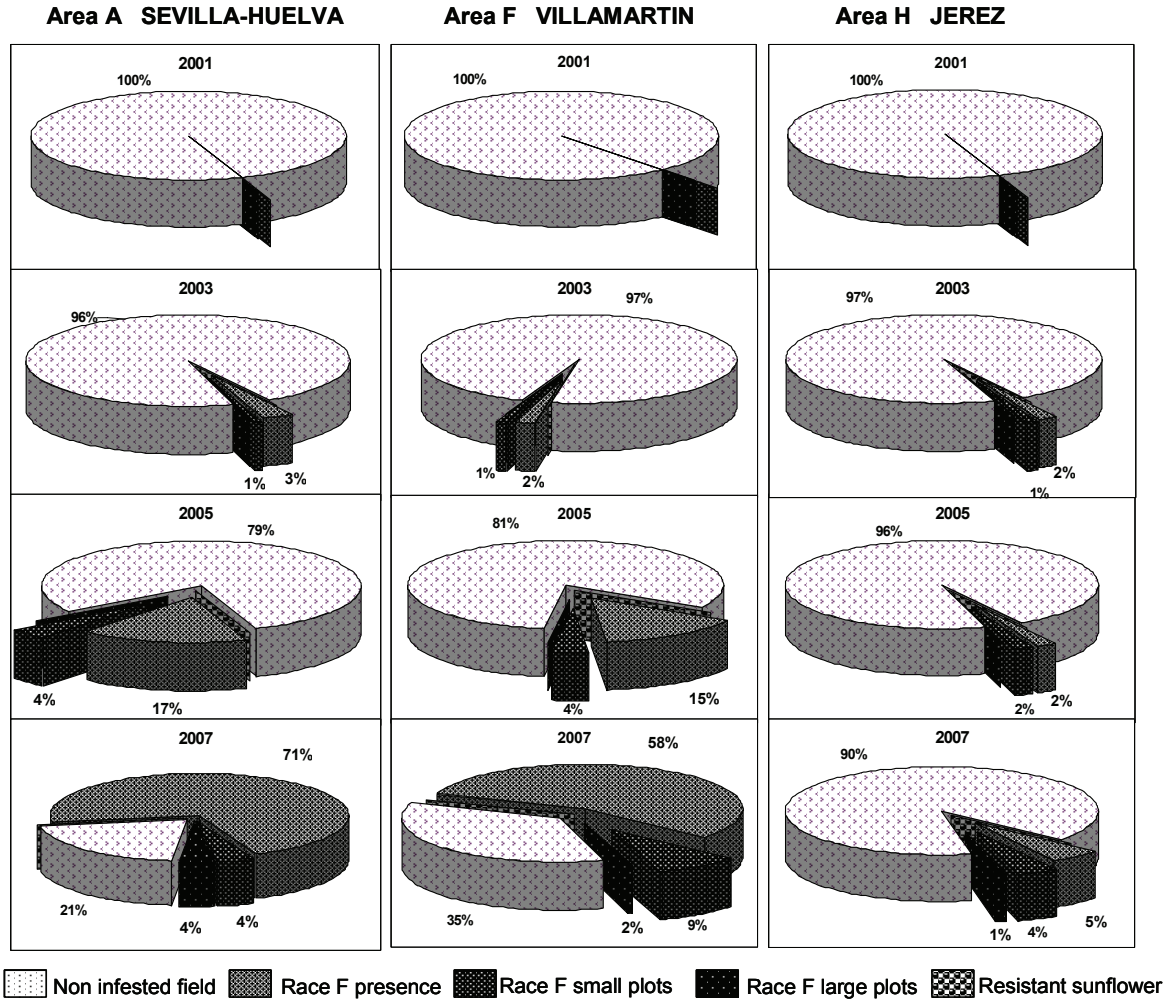

Fig. 3. Distribution of broomrape race F in Seville-Huelva, Villamartín and Jerez areas from 2001 to 2007.

The areas A and F, corresponding to Sevilla-Huelva and Villamartín, showed a similar behaviour regarding the appearance and dispersion of race F both in space and time. In both cases the first infections were detected in 2003, and four years later, the percentage of infected fields reached 79% and 69%, respectively (Fig. 3). It is worth mentioning that in these areas the infection with race F is present in many sunflower fields with 10-25 % of infected plants and a low broomrape number per plant, in contrast to B, C and D areas, where, in the first epidemic stages, the broomrape appears in small plots with a high broomrape infection. This fact may be due to multi-infestations with broomrape seed from the most infected areas (Écija and Carmona) through the combine-harvester movement. In areas A and F the sunflower harvest is carried out around 10 and 15 days later than in B and C areas, with the resulting machinery movement in this direction during harvest.

In the H area, around Jerez, the race F appearance took place in 2003 and with a similar intensity to that of A and F areas. Nevertheless, the rate of growth has been much lower and, in 2007, this race was present in only 10% of the sunflower fields. This situation is similar to that observed in the early 1990's regarding broomrape race E dispersion, when Jerez was the area in which broomrape appeared the latest in comparison to the rest of Andalucía and the spread was quite slow (unpublished results). The reason why broomrape presence and its expansion in the Jerez area is slower than in other sites, both for race E and race F, is unknown, at least by the authors.

The distribution knowledge and the broomrape F race rate of growth in a large area such as Southern Spain, with a sunflower surface of over 250000 has in recent years, may be considered as a model for designing strategies both for farmers and plant breeders. For the former, so that they can prevent broomrape seed dispersion among farms. For the latter, to be able to design alternative systems in order to fight the damage caused by broomrape in sunflower crop. This could be especially interesting in Andalusia, where the presence of a more virulent race (race G) which attacks race F resistant hybrids (Molinero-Ruiz and Melero-Vara, 2005) has already been reported.

REFERENCES

Alonso, L.C., J. Fernández-Escobar, G. López, M.I. Rodríguez-Ojeda, and F. Sallago. 1996. New highly virulent sunflower broomrape (*Orobanche cernua* Loelf.) pathotypes in Spain. p. 639-644. In: M.T. Moreno, J.I Cubero, D. Berner, D. Joel, L. Musselman, and C. Parker (eds), Advances in parasitic plant research. Proc. 6th Int. Symp. Parasitic Weeds, Córdoba, Spain. Congresos y Jornadas 36/96. Junta de Andalucía, Consejería de Agricultura y Pesca, Seville, Spain.

Domínguez, J. 1999. Inheritance of the resistance to *Orobanche cumana* Wallr. in sunflower: A review. p. 115-119. In: J.I. Cubero, M.T. Moreno, D. Rubiales, and J. Sillero (eds), Resistence to *Orobanche*: The state of the art. Congresos y Jornadas 51/99. Junta de Andalucía, Consejería de Agricultura y Pesca, Seville, Spain.

Domínguez, J. 2004. Visión actual de las infestaciones del cultivo del girasol por jopo. Vida Rural 55:12-15.

Eplee, R., R. Norris, and R. Jacobsohn. 1998. Mitigating epidemiology of *Orobanche*. p. 173-176. In: K. Wegman, L.J. Musselman, and D.M. Joel (eds), Current problem of *Orobanche* researches. Proc. 4th Int. Workshop on *Orobanche*, Albena, Bulgaria.

González-Torres, R., R. Jiménez-Díaz, and J.M. Melero-Vara. 1982. Distribution and virulence of *Orobanche cernua* in sunflower crops in Spain. Phytopath. Z. 104:78-89.

Melero-Vara, J.M. 1999. Pathogenic variability in *Orobanche cumana* Wallr. p. 149-155. In: J.I. Cubero, M.T. Moreno, D. Rubiales, and J. Sillero (eds), Resistence to *Orobanche*: The state of the art. Congresos y Jornadas 51/99. Junta de Andalucía, Consejeria de Agricultura y Pesca, Seville, Spain.

Molinero-Ruiz, M.L., and J.M. Melero-Vara. 2005. Virulence and aggressiveness of sunflower broomrape (*Orobanche cumana*) populations overcoming the *Or5* gene. p 165-169. In: G.J Seiler, (ed.), Proc. 16th Int. Sunflower Conf., Fargo, ND, USA.

Parker, C. 1994. The present state of *Orobanche* problem. p. 17-26. In: A.H. Pieterse, J.A.C. Verkleijand, and Ter Borgs (eds), Biology and management of *Orobanche* and related *Striga* research, Royal Tropical Institute, Amsterdam, The Netherlands.

Rodríguez-Ojeda, M.I., L.C. Alonso, and J. Fernández-Escobar. 2001. Effect of different crops on the germination of *Orobanche cernua* Loelf. (*O. cumana* Wallr.) seeds. p. 124. In: Proc. 7th Int. Parasitic weed symposium. Nantes, France.

Sackston, W.E. 1992. On a treatmill: breeding sunflower or resistance to disease. Annu. Rev. Phytopathol. 30:529-551.

Vrânceanu, A.V., V.A. Tudor, F.M. Stoenescu, and N. Pirvu. 1980. Virulence groups of *Orobanche cumana* Wallr., differencial host and resistance sources and genes in sunflower. p. 74-82. In: Proc. 9th Int. Sunflower Conf., Torremolinos, Spain.

Development of resistance to insect pests attacking the stem and head of cultivated sunflower in the central and northern production areas of North America

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ABSTRACT

There is a need to provide successful and economical management tools for the sunflower producer to reduce losses from the spectrum of insect pests that attack the crop in the major production regions. The use of plant resistance can be a useful strategy in a long-term integrated pest management approach for crop protection. The goal of this project was to investigate host plant resistance as a potentially valuable management resource and screen sunflower accessions, interspecific crosses, and lines for those having reduced seed damage from larval feeding by the sunflower moth, red sunflower seed weevil, and banded sunflower moth and reduced densities of sunflower stem weevil larvae in the stalks. Trials were conducted in the central and northern Plains of the U.S. to screen germplasm in the areas where the different insects have caused economic losses. The discovery of germplasm that has lower insect damage can provide the seed companies with breeding material to be incorporated into hybrids targeted to locations where specific insect problems occur. A long-term goal is to identify germplasm with resistance or tolerance to more than one insect pest. The 2005 and 2006 trials revealed that the most resistant lines had a 70-90% reduction in weevil or moth seed damage or numbers of weevil larvae in stalks compared to the most susceptible lines evaluated. After each year of testing, lines with low damage have been retested to confirm their resistance to attack. Trials were conducted again for all four insect pest species in 2007.

Key words: banded sunflower moth – germplasm – pest management – red sunflower seed weevil – sunflower moth –sunflower stem weevil.

INTRODUCTION

The major insect pests attacking cultivated sunflower include the sunflower stem weevil, *Cylindrocopturus adspersus* (LeConte), the sunflower moth, *Homoeosoma electellum* (Hulst) (Lepidoptera: Pyralidae), the red sunflower seed weevil, *Smicronyx fulvus* LeConte (Coleoptera: Curculionidae), and the banded sunflower moth, *Cochylis hospes* Walsingham (Lepidoptera: Tortricidae). (Charlet et al., 1997; Knodel and Charlet, 2007). Strategies to reduce crop losses for these pests have concentrated on insecticidal control, but host-plant resistance would provide producers with a sustainable integrated pest management approach for crop protection with lower input costs.

The sunflower stem weevil has caused yield losses in North Dakota, Colorado, Kansas, and Texas (Charlet et al., 1997; Armstrong, 1996; Charlet et al., 2002). Mature larvae overwinter in sunflower stalks and adults emerge in early summer. After mating, females deposit eggs in the stem at the base of the plant. Weevil larvae feed inside the stalk, descending to the lower portion of the stalk or root crown by late August. Larvae construct overwintering chambers by chewing cavities into the stem cortex. High larval populations in a plant can weaken the stem through pith destruction, tunneling, or overwintering chambers, causing it to break at soil level resulting in a loss of the sunflower plant prior to harvest. Stalk breakage is most severe during drought stress or when high winds occur as plants are drying prior to harvest (Charlet, 1987; Knodel and Charlet, 2002).

The sunflower moth causes yield losses to cultivated sunflower in the southern and central Plains. Larvae overwinter in the soil in Texas and adults are carried on northerly winds to the central and northern Plains. Female moths deposit eggs in blooming sunflower heads. Larvae feed and develop in the sunflower head, destroying seeds and reducing oil content. Feeding damage in the head may provide an entrance site for the Rhizopus head rot fungal pathogen. Larvae exit the seed when mature and drop into the soil to overwinter (Rogers, 1978, 1992; Charlet et al., 1997).

The banded sunflower moth has been a persistent pest of sunflower in the northern Plains and populations also are present in the central Plains. Adults emerge from the soil in mid-July and are present in the field until mid-August. Adults congregate in field margins on weeds or adjacent crops during the day and then fly into the sunflower field in the evening. Females lay eggs on the outside of the bracts of the sunflower head and larvae feed on the florets, developing seed, and mature seeds. After completing development, larvae drop from the heads and spin cocoons in the soil and overwinter as mature larvae (Charlet and Gross, 1990; Charlet et al., 1997).

The red sunflower seed weevil is a pest of cultivated sunflower in both North and South Dakota, but is also present in the central Plains (Charlet and Glogoza, 2004). Larvae overwinter in the soil, emerge in July, and after mating, females deposit eggs inside the developing sunflower seeds. Larvae feed and develop in the seeds, destroying a portion of the kernel and reducing oil content. When mature, the larvae exit the seeds and drop into the soil in late August or September to overwinter (Brewer, 1991; Rogers, 1992; Charlet et al., 1997).

Plant resistance is an important strategy in a sustainable pest management program for sunflower. Our goal was to evaluate selected sunflower accessions, interspecific crosses, and lines for reduced seed damage from larval feeding by the sunflower moth, red sunflower seed weevil, and banded sunflower moth, and lower populations of stem weevil larvae in stalks. Lines that have less insect damage can provide germplasm for incorporation into hybrids targeted to locations where specific insect problems occur. Our long-term objective is to identify germplasm with resistance or tolerance to more than one insect pest. This will increase grower confidence in the crop and facilitate maintenance and expansion of sunflower acreage in both the central and northern Plains production regions of the United States.

MATERIALS AND METHODS

Sunflower stem weevil

Plots were established at the Northwest Research Extension Center, Kansas State University, Colby, KS. Field trials in 2005 screened 14 selected sunflower hybrids, 8 accessions or Plant Introductions (PIs) obtained from the USDA, ARS, Plant Introduction Station at Ames, Iowa, 12 interspecific crosses, and hybrid '894'. In 2006 we screened 9 selected commercial sunflower hybrids, 5 retested accessions, 4 accessions that had previously shown low levels of seed damage from the banded sunflower moth, 4 accessions and an interspecific cross that previously had shown low sunflower moth damage, 5 retested interspecific crosses, 2 susceptible checks, and hybrid '894'. The lines were planted each year in single rows 7.6 m long and each was replicated three times in a randomized block design and planted on 9 and 8 May in 2005 and 2006, respectively. As a result of a phenotypic recurrent selection program that genetically combined lines with quantitatively-controlled insect tolerance factors from earlier trials, 60 S_1 line progeny rows also were subjected to insect infestation in 2006 in a separate trial planted on 8 May. The lines were planted in single 7.6 m rows in a block design with checks randomly placed within the trial. Five other hybrids, crosses, or lines (Hir 1734-1, HA 89, Str 1622-2, PI 497939, hybrid '894') were included as checks within the trial. Five stalks (~ 46 cm length plus the root crown) per row were removed in October each year and sent to the USDA, ARS, Northern Crop Science Laboratory, Fargo, ND, for evaluation. Stalks were held in the cold until evaluated. The stalks were then split and the numbers of weevil larvae in each stem determined. Because of time constraints, only one half of each stalk was evaluated and then converted to number per stalk. The degree of resistance or tolerance was measured by comparing the number of weevil larvae per stalk with the germplasm having the lowest number of insects in the trial.

Sunflower moth

Plots were planted at Colby, KS. Sunflower moth feeding damage in the 2004 trials was very low with an average of 0 to 2% in the material evaluated. Because of the reduced amount of damage, the trial was repeated in 2005. Germplasm selected for testing included retested accessions and interspecific crosses with less than 4% feeding damage in 2003, selected susceptible checks and hybrid '894'. Other

accessions were added because of low damage in sunflower stem weevil, red sunflower seed weevil, and banded sunflower moth screening trials. Seven new accessions also were added. Germplasm selected for evaluation in 2006 included retested accessions and interspecific crosses with less than 4% feeding damage in 2005. The susceptible checks Hir 1734-1 and 01-4094-1 (04-628) were included as was hybrid '894'. All accessions were obtained from the USDA Plant Introduction Station. The entries were replicated three times in a randomized block design and were planted 9 and 8 May in 2005 and 2006, respectively. In a separate trial in 2006, 58 S_1 line progeny rows also were subjected to insect infestation. Five other hybrids or lines (04-628, Cropland 378, HA 89, Str 1622-2, Hybrid '894') were included as checks within the trial. The lines were planted in single 7.6 m long rows on 8 May in a block design with checks randomly placed within the trial. Physiologically mature heads were harvested between 23 August and 12 September each year. Five heads were removed from each row and shipped to Fargo, for evaluation. The heads were dried, threshed, the seed cleaned, and subsamples of 100 seeds per head evaluated for number of seeds damaged by moth larval feeding. The degree of resistance or tolerance to the sunflower moth was measured by comparing the percentage of seeds damaged among those tested.

Red sunflower seed weevil

Plots in 2005 were established at two locations: Highmore, SD, and Prosper, ND. Field trials at each site screened the same germplasm: 2 interspecific crosses, 17 accessions obtained from the USDA Plant Introduction Station and hybrid '894'. Plots in 2006 were planted at the same locations. Field trials at each site screened the same germplasm: 2 interspecific crosses, 4 retested accessions, 5 accessions with low banded sunflower moth damage, 2 interspecific crosses and 5 accessions with low sunflower moth damage from previous trials, and hybrid '894'. The entries were planted in a randomized block design with three replications on 16 June at Highmore and on 20 May at Prosper in 2005 and 7 and 9 June at Highmore and on 18 May at Prosper in 2006. In a separate trial in 2006, 60 S_1 line progeny rows also were subjected to insect infestation at the same two locations. Four other hybrids, crosses or lines (PI 431542, Hir 828-3, HA 89, and hybrid '894') were included as checks within the trial. The lines were planted in single 7.6 m long rows on 7 and 9 June (Highmore) and on 18 May (Prosper) in a block design with checks randomly placed within the trial. At Highmore because of very dry conditions in 2005, up to ten heads from each row were harvested in October and shipped to Fargo for evaluation. At Prosper, five heads were randomly removed from each row from mid-September to early October each year and taken to Fargo for evaluation. Harvest occurred in early November at Highmore and heads were sent to Fargo for evaluation. The heads from both locations were dried, threshed, and the seed cleaned. Subsamples of 100 seeds per head from each nursery were evaluated for number of seeds damaged by seed weevil larval feeding and the percentage of damaged seeds determined. Resistance or tolerance to the banded sunflower moth was measured by comparing the percentage of damaged seeds among the germplasm evaluated in the trials.

Banded sunflower moth

In 2005, plots were established at Prosper, ND. Five interspecific crosses, one new line, 17 accessions obtained from the USDA Plant Introduction Station and hybrid '894' were screened. The entries were planted in a randomized block design with three replications on 20 May. Plots in 2006 also were planted at Prosper, ND. Field trials screened 2 interspecific crosses, a new line, 11 retested accessions, 6 new accessions, an accession with low sunflower moth damage and two accessions with low seed weevil damage from previous trials, and hybrid '894'. The treatments were planted in a randomized block design with three replications on 18 May. In a separate trial, 60 S_1 line progeny rows also were subjected to insect infestation. Five other hybrids or lines (PI 251902, Par 1673-2, HA 89, P21VRI, and hybrid '894') were included as checks within the trial. The lines were planted in single 7.6 m long rows on 18 May in a block design with checks randomly placed within the trial. Five heads were randomly removed from each row when plants were physiologically mature in mid-September both years and taken to Fargo for evaluation. The heads were dried, threshed, the seed cleaned, and subsamples of 100 seeds per head evaluated for number of seeds damaged by moth larval feeding. The degree of resistance or tolerance to the banded sunflower moth was measured by comparing the percentage of seeds damaged among those tested.

RESULTS AND DISCUSSION

Sunflower stem weevil

In the 2005 trial, the mean number of sunflower stem weevil larvae occurring in the germplasm tested ranged from 7 to 70 larvae per stalk. Among all the individual stalks evaluated, numbers ranged from 0 to a high of 166 per stalk. Among the 35 lines or hybrids tested, 13 were below 25 and five below ten weevil larvae per stalk. The line with the best performance in the trial was accession PI 431516 with a mean of only 6.6 larvae per stalk. This was the first year in which this line was tested. Three interspecific crosses Str 1622-2, Hir 828-2 and Hir 828-3 had less than 20 weevil larvae per stalk. Accession PI 497939 only had 9 larvae in 2005, 12 in 2004, and only six in 2003. The accession PI 386230 had only 9 larvae per stalk and was among the ten lowest in 2004. Hybrid '894', which had only an average of 16 larvae per stalk in 2004, had over 30 larvae per stalk in 2005. The commercial hybrid with the lowest density among those tested was Fontanelle 902NS with 21 larvae per stalk.

In 2006, the mean number of larvae occurring in the material tested ranged from 5 to 51 larvae per stalk. Among the 31 lines or hybrids tested, 21 were below 25 and three below ten weevil larvae per stalk. One of the two accessions with the best performance in the trial was accession PI 431516 with a mean of only 6.5 larvae per stalk. This was the second year in which this line was tested, and in 2005 it had the lowest number of larvae in the trial. The line with the lowest number of larvae in the trial was PI 386230 with a mean of 5 larvae per stalk; in 2005 it was among those with the lowest larval density per stalk and was among the ten lowest in 2004. The accession Ames 3454 had 9 larvae per stalk, the same as in 2005. The results from the trial evaluating the S1 lines showed high numbers of larvae occurring in some of the stalks with means from 0 to140 larvae per stalk among those tested. However, a total of 22 showed average larval densities of less than 25 per stalk. Thirty-two were selected for reevaluation in 2007.

Sunflower moth

Other than two lines which showed over 30% damage, the remaining 34 tested showed an average of less than 10% seed damage per head in the 2005 trial. Although some inconsistencies in the results were evident compared to those in previous years, a number of lines that have repeatedly had low damage also were among those tested with reduced percent seed damage again in 2005. The susceptible line 01-4094-1 was again the most damaged of those evaluated. Eleven lines with low damage in 2003 sustained an average of 2% or less damage per head in 2005. This group included hybrid '894'. Others with less than 2% damage included four that had previously shown reduced seed damage in trials for banded sunflower moth (PI 505651, PI 291403, PI 494861 and PI 494859), one in trials for sunflower stem weevil (Ames 3391), and one in trials for red sunflower seed weevil (Ames 3269). Two of the accessions that were new in the 2005 trial also had less than 2% seed damage from sunflower moth feeding (PI 170405 and PI 193775).

Insect pressure from the sunflower moth was very heavy in 2006 as shown by the amount of seed damage in the trial; the damage ranged from 1 to 81% seed damage among the selected accessions and lines evaluated. The amount of damage sustained by the accessions tested was surprising because, other than the susceptible checks, those included in the 2006 trial only had shown 4% or less damage in 2005. However, hybrid '894', which had the lowest amount of damage in the trial, also was among the lowest in 2005 with only 0.3% damage. Others in the 2006 trial with lower damage levels included PI 170385 (9.6%) and Ames 3269 (11.6%) which averaged 2.5% and 1.1% damage, respectively, in 2005. PI 170414 averaged only 10.6% damage in 2006 and had averaged 0% damage in 2005, although only 3 heads were evaluated. The results from the trial evaluating the S1 lines showed feeding damage levels from 0.2 to 70% among those tested. A total of 36 showed average percentage damage of less than 10%. The check, hybrid '894' again showed lower damage from moth feeding in this trial. The best of these lines were retested in 2007 to confirm their resistance to damage by the sunflower moth.

Red sunflower seed weevil

The damage at Highmore in 2005 indicated high levels of weevil infestation, with a range of 2 to 59% seed damage among the germplasm tested at this location. Those showing damage levels of 18% or less included the three accessions PI 431545, Ames 3269, and PI 431542; however, the results were from only a limited number of heads evaluated. Ames 3269 had been tested in both 2003 and 2004 and showed only 13% damage each year. PI 431542 had the least damage of all germplasm in 2005 as well as in 2004. Hybrid '894' averaged 43% seed damage which was higher than the 2004 trial in which it averaged 24% damage. The density of red sunflower seed weevil at the Prosper trial was much lower than the Highmore location, based on the amount of seed damage. Percentage damage ranged from a high of 4% in accession PI 431569 to 0.7% in Ames 3269. Hybrid '894', which scored near the middle of the selected germplasm

evaluated at Highmore, was near the bottom in level of seed damage at Prosper at 1.5%. The accessions 431542 and Ames 3269 scored near the bottom in percentage of damage at both locations. Some others showed inconsistent results, but the differences were likely because of the lower levels of damage that occurred at Prosper.

High levels of red sunflower seed weevil occurred in the 2006 trial with a range of 7 to 52% seed damage among the germplasm tested at Highmore. Eight lines showed damage levels of 15% or less. Ames 3269 had the lowest amount of damage in both 2003 and 2004, was one of the lowest in 2005, and showed only 13% damage in 2006. Three of the least damaged accessions had shown low damage from sunflower moth in earlier trials (PI 175728, PI 162453, and PI 193775). The results from the trial evaluating the S1 lines showed feeding damage levels from 0.3 to 40% among those tested. Of the 59 evaluated, 25 showed average percentage damage of less than 13%. The best of the lines were retested in 2007 to confirm their resistance to red sunflower seed weevil damage. The density of red sunflower seed weevil at the Prosper trial was lower compared to the Highmore location based on the amount of seed damage from the germplasm evaluated. Percentage damage ranged from a high of 0.9% to 24%. There were a number of inconsistencies at the two sites. Hybrid '894', which scored near the middle of the selected germplasm evaluated at Highmore (34%), was at the bottom in the level of seed damage at Prosper with 0.9%. The accession Ames 3269 scored at the bottom in percentage of damage at Highmore, but was the most damaged of those tested at Prosper. Some others, however, were similar in amount of damage between the two locations likely because of the lower levels of damage at Prosper. Only 47 of the S1 lines were evaluated for damage due to lodging from a wind storm. The results from the trial showed feeding damage levels from 20 to 0.6% among those tested. Of those evaluated, 31 showed average percentage damage of less than 4%. The best of the lines were retested in 2007.

Banded sunflower moth

The percentage of banded sunflower moth feeding damage ranged from 8% in hybrid '894' to 58% in accession PI 431542 in 2005. The accessions PI 251902 and PI 170391, which had less than 11% damage in 2005, had only 8% damage in 2004 and less than 5% in the previous year. PI 265503 which sustained only 11% in the 2005 trial incurred 12% damage in 2004 and less than 7% in the 2003 trial. Hybrid '894', which sustained the least amount of damage in the trial, had less than 9% damage in the 2004 trial and only 4% in the 2003 trial. All of the interspecific crosses had greater than 18% feeding damage.

In the 2006 trial, the percentage of banded sunflower moth feeding damage ranged from a low of 0.5% in accession PI 432516 to 29% in interspecific cross Par 1673-2. The majority of the tested germplasm sustained less than 10% damage from moth larval feeding. PI 162453 which had shown reduced sunflower moth damage in previous trials sustained less than 6% feeding damage from banded sunflower moth. The accessions PI 170401 and PI 505651 had less than 2% damage and were also low in 2005. Three of the new PIs tested in 2006 (PI 195573, PI 219649, PI 432516) were the lowest in the trial, showing less than 2% feeding damage. The results from the trial evaluating the S1 lines showed feeding damage levels from 0.4 to 14% among those tested. A total of 19 were lost to lodging from wind. Of the remaining 41 S1 lines, 17 showed average percentage damage of less than 3%. The best of these lines were retested in 2007 to confirm their resistance to banded sunflower moth damage.

CONCLUSIONS

Evaluation of sunflower germplasm for resistance to important sunflower seed-feeding and stem-infesting pests has been conducted in regions where these insects have caused economic losses. Nurseries for the sunflower moth and sunflower stem weevil were located in KS, for the banded sunflower moth in ND, and nurseries for the red sunflower seed weevil were placed in ND and SD. Results from both 2005 and 2006 identified promising resistance in germplasm against the four insects studied. There was a reduction in seed damage of 90% and 80% between the most susceptible and the most resistant line in the sunflower moth and banded sunflower moth trials, respectively. The red sunflower seed weevil trials in both locations had genotypes with a 70% to 80% reduction in seed damage and 90% fewer larvae per stalk in the stem weevil trials. After each year of testing, lines, accessions, or interspecific crosses with low damage are retested to confirm their resistance to attack. Trials were again conducted for all four insect pest species in 2007 and the results are currently being evaluated. The lines that are determined to be the most resistant in the 2007 trials will be random-mated to begin development of the next cycle of S_1 progeny lines.

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REFERENCES

Armstrong, J.S. 1996. Development of a degree-day based prediction model for adult sunflower stem weevil, *Cylindrocopturus adspersus*, emergence. p. 49-51. In: Proc. 18th Sunflower Research Workshop, Natl. Sunflower Assoc., Fargo, ND, 11-12 January 1996.

Brewer, G.J. 1991. Oviposition and larval bionomics of two weevils (Coleoptera: Curculionidae) on sunflower. Ann. Entomol. Soc. Am. 84:67-71.

Charlet, L.D. 1987. Seasonal dynamics of the sunflower stem weevil, *Cylindrocopturus adspersus* LeConte (Coleoptera: Curculionidae), on cultivated sunflower in the northern Great Plains. Can. Entomol. 119:1131-1137.

Charlet, L.D., and P.A. Glogoza. 2004. Insect problems in the sunflower production regions based on the 2003 sunflower crop survey and comparisons with the 2002 survey. Proc. 26th Sunflower Research Workshop, Natl. Sunflower Assoc., Fargo, ND, USA, 14-15 January 2004. http://www.sunflowernsa.com/research/research-workshop/documents/143.pdf.

Charlet, L.D., and T.A. Gross. 1990. Bionomics and seasonal abundance of the banded sunflower moth (Lepidoptera: Cochylidae) on cultivated sunflower in the northern Great Plains. J. Econ. Entomol. 83:135-141.

Charlet, L.D., G.J. Brewer, and B. Franzmann. 1997. Insect pests. p. 183-261. In: A. A. Schneiter (ed.), Sunflower Technology and Production. Agron. Ser. 35. Am. Soc. Agron., Madison, WI, USA.

Charlet, L.D., J.S. Armstrong, and G.L. Hein. 2002. Sunflower stem weevil (Coleoptera: Curculionidae) and its larval parasitoids in the Central and Northern Plains of the USA. BioControl 47:513-523.

Knodel, J.J., and L.D. Charlet. 2002. Biology and integrated pest management of the sunflower stem weevils in the Great Plains. North Dakota State Univ. Coop. Ext. Serv. Bull. E-821. 8p.

Knodel, J., and L. Charlet. 2007. Pest management - insects, p. 26-53. In: D. R. Berglund [ed.], Sunflower production. North Dakota State University Extension Service Bull., A-1331. 119 p.

Rogers, C.E. 1978. Sunflower moth: feeding behavior of the larva. Environ. Entomol. 7:763-765.

Rogers, C.E. 1992. Insect pests and strategies for their management in cultivated sunflower. Field Crops Res. 30:301-332.

Integrated pest management of the banded sunflower moth in cultivated sunflower in North Dakota

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ABSTRACT

Banded sunflower moth, *Cochylis hospes* Walsingham (Lepidoptera: Tortricidae), is a key insect pest of cultivated sunflowers in North Dakota. We investigated pest management strategies to reduce feeding injury caused by the banded sunflower moth in commercial oilseed and confection sunflower fields located in north central North Dakota during 2005-2006. Seed damage from banded sunflower moth was more concentrated on field edges than at 20 m, 40 m and 150 m in the field. As a result, edge spraying was as effective as whole field spraying in controlling banded sunflower moth when populations were low to moderate. Early planted sunflower had a higher percentage of seed damage than later planted sunflower regardless of sunflower type. There was a positive linear relationship between the percent of damaged seed and the subsequent number of banded sunflower moth larvae emerging from heads. The presence of sunflower in adjacent fields had a diluting effect on field densities of banded sunflower moth. In contrast, when sunflower was not present in adjacent fields, fields had a concentrating effect with higher densities of banded sunflower moth. Sixty-one percent of banded sunflower moth reared were parasitized by two species of parasitoids: *Glypta prognatha* Dasch (Hymenoptera: Ichneumonidae) and *Chelonus phaloniae* (Mason) (Hymenoptera: Braconidae). Parasitism rates were negatively impacted by insecticide spraying in field edges. Parasitoids were effective in searching from field edges to 40 m into the field and were not dependent on the presence of sunflower in the landscape.

Key words: banded sunflower moth – biological control – *Cochylis hospes* Walsingham – insecticide control – integrated pest management – sunflower.

INTRODUCTION

Banded sunflower moth, *Cochylis hospes* Walsingham (Lepidoptera: Tortricidae), is a major pest of sunflower in the northern Plains and populations have been increasing in recent years in North Dakota. Adults begin to emerge from the soil about mid-July and are present in the field until mid-August (Mundal et al., 2006). Adults tend to congregate in field margins on weeds or adjacent crops during the day and then move to the crop in the evening. Eggs are deposited on the outside of the bracts of the sunflower head. Larvae feed in the florets and developing seeds, and also destroy mature seeds. At maturity, larvae drop to the ground and spin cocoons in the soil to overwinter (Charlet and Gross, 1990; Charlet et al., 1997). The primary management strategy for control of banded sunflower moth has been the use of insecticides, although research has also shown that delayed planting can reduce feeding damage (Knodel and Charlet, 2007). In addition, crop management programs relying primarily on insecticide usage can be detrimental to parasitoid diversity and activity. Several parasitoid species attack banded sunflower moth (Charlet, 1999, 2001). However, in most years the control exerted by parasitoids is inadequate to maintain banded sunflower moth populations below economic injury levels. Understanding the population dynamics of the pest and its natural enemies will provide valuable information that could improve control of banded sunflower moth in cultivated sunflower. The integration of different pest management strategies has the potential to provide more effective control with reduced input costs for sunflower producers.

The goal of this project was to investigate the integration of pest management strategies to reduce input costs and overall feeding injury caused by banded sunflower moth in commercial confection and oilseed sunflower fields. The effectiveness of treating only the margins of sunflower fields was evaluated for reducing economic losses from banded sunflower moth in early and late planted fields. In addition, the impacts of landscape and parasitoid complex were determined on populations of banded sunflower moth. The discovery of the most effective combination of control tactics to manage banded sunflower moth will enable producers to reduce yield loss and save money by lowering insecticide treatment costs.

Cooperation from a certified crop consultant ensured that commercial sunflower producers could readily adapt results. This helps to validate the field research in a real-life setting.

MATERIALS AND METHODS

Sunflower fields in north central North Dakota were selected in cooperation with a certified crop consultant. During 2005, eight commercial oilseed sunflower fields in Renville and Bottineau counties of ND were either treated or untreated only around the perimeter of the field with a registered sunflower insecticide, Lorsban (chlorpyrifos, Dow AgroSciences LLC, Indianapolis, IN, USA). Lorsban was applied at 1 pt per acre and 3 GPA by air when the crop was at the 10% ray petal stage. In the past several years, many fields in this region have been treated with insecticides only on the outer 61 m of the field. The influence of landscape, including sunflowers in adjacent fields, was also studied to determine the impact on abundance and field distribution of both the pest and its parasitoids. A total of eight fields were sampled on 31 August 2005. Five randomly selected sunflower heads containing mature banded sunflower moth larvae were collected from the edge, 20 m and 40 m into the field on each side of the field (a total of 60 heads per field). The heads were bagged individually, labeled, and returned to the USDA, ARS laboratory at Fargo. Banded sunflower moth larvae were extracted from the heads and reared in the laboratory to determine pest and parasitoid density, parasitoid species richness, and parasitism rates. Each head was dried, threshed, and subsamples of 100 seeds were evaluated for seed damage.

In 2006, commercial fields were selected from both confection and oilseed sunflower that were either treated or untreated, and planted early (prior to mid-May) or late (late May to mid-June). There were three replicates of the following treatments: (1) early planted, sprayed, oilseed fields; (2) late planted, sprayed, oilseed fields; (3) early planted unsprayed, oilseed fields (only two fields sampled); (4) late planted, unsprayed, oilseed fields; 5) early planted, sprayed, confection fields; (6) late planted, sprayed, confection fields; (7) early planted unsprayed, confection fields; and (8) late planted, unsprayed, confection fields. Field were aerial sprayed using 3 GPA, and the insecticide Asana (esfenvalerate, E. I. du Pont de Nemours and Co., Wilmington, DE, USA) applied at 9 fl oz per acre or the insecticide Baythroid XL (beta-cyfluthrin, Bayer Crop Sciences, RTP, North Carolina, USA) applied at 2.8 fl oz per acre. Applications were made at the 10% ray petal stage (or when early instar larvae of banded sunflower moth were present). A total of 23 fields were sampled on 25-26 September 2006. Ten randomly selected sunflower heads were collected at distances of edge (5 m), 40 m, and 150 m from two sides of each field for a total of 60 heads per field. The heads were bagged individually, labeled, and returned to the USDA, ARS laboratory at Fargo. Each head was dried, threshed and subsamples of 100 seeds were evaluated for damage by banded sunflower moth.

The effect of treated and untreated sunflower fields were compared by determining the percent of damaged seed within each sunflower field for both years. In 2005, landscape, parasitoid species richness, percent parasitism, and density of banded sunflower moth larvae also were compared. In 2006, planting date and sunflower type also were also analyzed. Data were evaluated at different sampling distances from the field edge for both years. Data were analyzed using ANOVA and Fisher's Protected LSD to separate means at the 5% significance level. Linear regression was used to determine the relationship between damaged seed in sunflower heads and the number of emerged larvae. Before analysis, banded sunflower moth data for larvae and damaged seeds were square root transformed due to non-normal distributions of residuals and non-homogeneity of variance.

RESULTS

A total of 5,242 tortricid larvae emerged from the 480 sunflower heads collected from the eight field sites in 2005. Thirty-six percent of emerged larvae were identified as *Cochylis* spp., 61 percent were parasitized and the remaining three percent died from unknown factors. Of the *Cochylis* species, 69 percent were *C. hospes* and 31 percent were *C. arthuri*. Of the parasitoids reared from *Cochylis* spp.: 53 percent were *Glypta prognatha* Dasch (Hymenoptera: Braconidae), 45 percent were *Chelonus phaloniae* (Mason) (Hymenoptera: Ichneumondiae), and 2 percent were a hyperparasitoid *Perilampus robertsoni* Crawford (Hymenoptera: Perilampidae).

In this study, untreated fields were monitored for populations of banded sunflower moths and were not treated because population levels were below the economic threshold level. There were significantly lower percent of damaged seed ($F = 1.10$; $df = 1, 82$; $P = 0.2971$), mean number of larvae ($F = 0.37$; $df = 1, 82$; $P = 0.5033$) and percent parasitism ($F = 1.42$; $df = 1, 82$; $P = 0.2369$) in the treated fields compared

to the untreated fields in 2005 (Table 1). There were no significant differences in head diameter between untreated and treated sunflower fields. Results indicate that spraying on edges can successfully reduce moth damaged seed and mean number of banded sunflower moth larvae emerging from the seed. However, these results could vary depending on locality and year-to-year population densities of banded sunflower moth. As anticipated, insecticide spraying had a negative impact on the parasitoid complex, reducing parasitism by 6.9 percent.

Table 1. Effects of spraying edges of sunflower fields on head diameter, percent seed damaged by banded sunflower moth, mean number of larvae and percent parasitism in 2005

Location	Head diameter (cm)	% Damaged Seeds[1]	Mean number of larvae[1]	% Parasitism
Treated[2]	18.6 a	1.6 a	42.4 a	19.1 a
Untreated	17.9 a	2.5 b	74.1 b	26.0 b

Means within a column followed by the same letter are not significantly different (P< 0.05), Fisher's Protected LSD.
[1]Data transformed using square root, untransformed means presented.
[2]Lorsban applied at 1 pt per acre during 10% ray petals by air using 3 GPA, edge spray application.

For 2006, there also were significantly lower percent of banded sunflower moth damaged seed in treated fields compared to the untreated fields for both confection ($F = 88.78$; df = 1, 60; $P =< 0.0001$) and oilseed ($F = 10.54$; df = 1, 60; $P = 0.0019$) sunflower (Table 2). There were no significant differences for head diameter between untreated and treated field regardless of sunflower type. Results indicated that whole field spraying was successful in reducing damaged seed when populations of banded sunflower moth were moderate to high.

Table 2. Effects of spraying whole sunflower fields on head diameter and percent seed damaged by banded sunflower moth in confection and oilseed sunflowers in 2006

Location	Confection		Oilseed	
	Head diameter (cm)	% Damaged Seeds[1]	Head diameter (cm)	% Damaged Seeds[1]
Treated[2]	18.2 a	2.3 a	17.8 a	2.3 a
Untreated	17.6 a	10.6 b	17.7 a	3.1 b

Means within a column followed by the same letter are not significantly different (P< 0.05), Fisher's Protected LSD.
[1] Data transformed using square root, untransformed means presented.
[2] Asana (9 fl oz per acre) or Baythroid (2.8 fl oz per acre) was applied during 10% ray petals by air using 3 GPA, whole field spray application.

Comparison of early versus late-planted sunflower fields indicate that early planting dates had a significantly higher percent of damaged seed ($F = 20.15$, df = 1, 60, $P =< 0.0001$) than late planting dates for oilseed sunflower in 2006 (Table 3). There were no significant differences in head diameter between the two planting dates regardless of the sunflower type.

Table 3. Effects of early versus late planted sunflowers on head diameter and percent seed damaged by banded sunflower moth in confection and oilseed sunflowers in 2006

Location	Confection		Oilseed	
	Head diameter (cm)	% Damaged Seeds[1]	Head diameter (cm)	% Damaged Seeds[1]
Early planted	18.2 a	6.6 a	17.9 a	3.6 a
Late-planted	17.6 a	6.5 a	17.5 a	2.0 b

Means within a column followed by the same letter are not significantly different (P< 0.05), Fisher's Protected LSD.
[1] Data transformed using square root, untransformed means presented.

In 2005, the comparison of sampling locations revealed that the edge sample had significantly higher percent damaged seed ($F = 8.35$; df = 2, 82; $P = 0.0005$) and mean number of larvae emerging from heads ($F = 12.24$; df = 2, 82; $P =< 0.0001$) than the 20 m and 40 m samples (Table 4). There were no significant differences among the sampling locations for head diameter or percent parasitism. When data for treated and untreated sunflower fields were analyzed separately (results not presented), results were identical to the combined analyses. Results indicate that field edges harbor higher numbers of banded sunflower moth larvae than the samples collected at 20 m and 40 m in fields. These data validate why field edge spraying can be effective in controlling banded sunflower moth when populations are low to moderate. Parasitoids

were as proficient in searching for banded sunflower moth larvae in the edge as they were in 20 m and 40 m within fields.

Table 4. Effects of sampling locations from combined (treated and untreated) sunflower fields on head diameter, percent damaged seed by banded sunflower moth, mean number of larvae and percent parasitism in 2005

Location	Head diameter (cm)	% Damaged Seeds[1]	Mean number of larvae[1]	% Parasitism
Edge	18.2 a	3.4 a	102.8 a	23.8 a
20 M	18.2 a	1.6 b	43.6 b	21.0 a
40 M	18.3 a	1.0 b	28.3 b	22.8 a

Means within a column followed by the same letter are not significantly different (P< 0.05), Fisher's Protected LSD.
[1] Data transformed using square root, untransformed mean presented.

Edge samples in 2006 had significantly higher percent damaged seed than the 40 m and 150 m samples for both confection ($F = 8.12$; df = 2, 60; $P = 0.0008$) and oilseed ($F = 5.25$; df = 2, 60; $P = 0.0080$) sunflower (Table 5). There were no significant differences among sampling locations for head diameter. When data for untreated or treated confection or oilseed sunflower fields were analyzed separately (results not presented), results were identical to the combined analyses. The 2006 results were identical to results in 2005 and further support that field edges have higher numbers of banded sunflower moth than the samples collected in the field.

Table 5. Effects of sampling locations from combined (treated and untreated) sunflower fields on head diameter and percent seed damaged by banded sunflower moth in confection and oilseed sunflowers in 2006

Location	Confection		Oilseed	
	Head diameter (cm)	% Damaged Seeds[1]	Head diameter (cm)	% Damaged Seeds[1]
Edge	18.0 a	9.5 a	17.5 a	3.5 a
40 m	17.5 a	5.7 b	17.7 b	2.0 b
150 m	18.2 a	5.3 b	18.0 a	2.6 b

Means within a column followed by the same letter are not significantly different (P< 0.05), Fisher's Protected LSD.
[1] Data transformed using square root, untransformed means presented.

Landscape effects were evaluated only for 2005 (Table 6). Fields without sunflower fields nearby had a significantly higher mean number of banded sunflower moth larvae ($F = 3.61$; df = 1, 82; $P = 0.0611$) than fields that were adjacent to sunflower fields. However, there were no significant differences for percent damaged seeds, percent parasitism or head diameter. When data were analyzed separately by treated and untreated sunflower fields (results not presented), results were identical to the combined analyses. Since fields with non-sunflower fields nearby had higher mean number of larvae, this suggests that the presence of the host plant is a density-dependent factor for banded sunflower moth populations. The opposite was observed for parasitoids with no landscape effects on parasitism, which suggests that parasitoids are not density-dependent on sunflower in the landscape.

Table 6. Landscape effects on head diameter, percent damaged seeds by banded sunflower moth, mean number of larvae and percent parasitism in 2005

Adjacent Fields	Head diameter (cm)	% Damaged Seeds[1]	Mean number of larvae[1]	% Parasitism
Sunflower	17.7a	1.3a	23.8a	17.6a
Non-sunflower	18.3a	2.1a	63.5b	23.3a

Means within a column followed by the same letter are not significantly different (P< 0.05), Fisher's Protected LSD.
[1] Data transformed using square root, untransformed means presented.

Mean number of larvae that emerged from the seed resulted in a significant parameter estimates and a significant relationship to percent damaged seeds [square root of mean number of larvae emerged from seed = 1.4870 (square root of percent damaged seeds) + 1.1415; N = 89, $R^2 = 0.498$, P< 0.0001] (Fig. 1). This indicates a positive relationship between the number of larvae emerging from seed and the percent of damaged seeds.

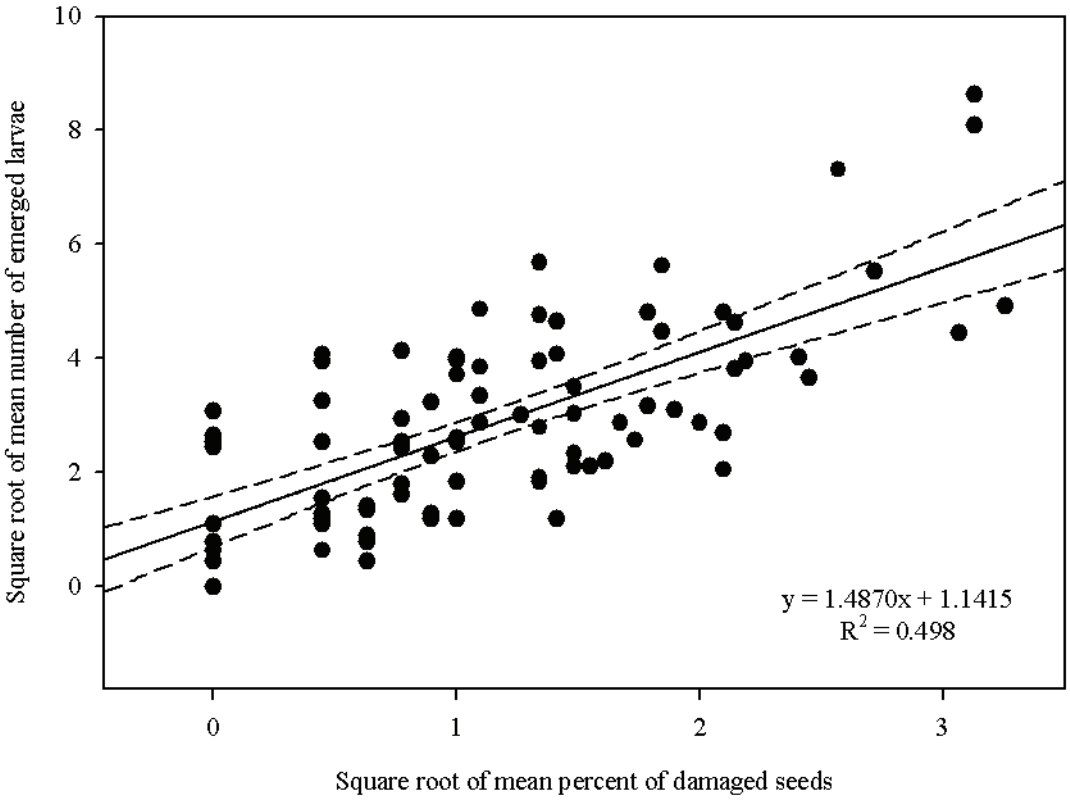

Fig. 1. Relationship between mean percent of damaged seeds and mean number of emerged larvae (n=89) across all locations and treatments. The solid line represents the best fit linear equation. Dashed lines represent 95% confidence intervals.

DISCUSSION

Since the early 1980s, cultivated sunflower fields in North Dakota, Minnesota and South Dakota have had frequent economic damage caused by banded sunflower moth (Charlet and Busacca, 1986; Charlet and Glogoza, 2004). Insecticide spraying decisions are based on sampling for eggs or adults of banded sunflower moth in fields during mid to late July (Knodel and Charlet, 2007; Knodel et al., 2008). In our study, edge spraying was effective in controlling banded sunflower moth because populations of banded sunflower moth were found to be concentrated in field edges. However, edge spraying was only effective in controlling banded sunflower moth when population levels were low to moderate. When populations of banded sunflower moth were higher in 2006, whole field spraying was required to control banded sunflower moths. A positive linear relationship was established between percent damaged seed and the subsequent number of banded sunflower moth larvae emerging from heads. Manipulating planting dates to avoid oviposition minimized damage caused by banded sunflower moth. Late planting sunflower fields into June could provide producers with a cultural control tactic to mitigate banded sunflower moth damage. Oseto et al. (1989) also reported that sunflower planted late (early June) in southeastern North Dakota had fewer damaged seeds than sunflower planted early (first week in May). The presence of sunflower in the landscape had a diluting effect on field densities of banded sunflower moth. In contrast, when sunflower was not present in the landscape, a concentrating effect was observed with higher densities of banded sunflower moths in that sunflower field.

Charlet (1999, 2001) identified several species of parasitoids attacking banded sunflower moth. Sixty-one percent of banded sunflower moth larvae reared were parasitized by two species of parasitoids: *Glypta prognatha* and *Chelonus phaloniae*. Parasitism rates were negatively impacted by insecticide spraying in field edges. Parasitoids were effective in searching from field edges to 40 m into the field and were not dependent on the presence of sunflower in the landscape.

In summary, this research supports the concept of integrating cultural control, biological control and insecticide control, which together can be used effectively to reduce banded sunflower moth damage in cultivated sunflowers in North Dakota.

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REFERENCES

Charlet, L.D. 1999. Biological control of sunflower pests: searching for new parasitoids in native *Helianthus* - challenges, constraints, and potential, p. 91-112. In: L.D. Charlet and G.J. Brewer (eds), Biological Control of Native or Indigenous Insect Pests: Challenges, Constraints, and Potential, Thomas Say Publications in Entomology, Entomol. Soc. Am., Lanham, MD, USA.

Charlet, L.D. 2001. Biology and seasonal abundance of parasitoids of the banded sunflower moth (Lepidoptera: Tortricidae) in sunflower. Biological Control 20:113-121.

Charlet, L.D., and J.D. Busacca. 1986. Insecticidal control of banded sunflower moth, *Cochylis hospes* (Lepidoptera: Cochylidae), larvae at different sunflower growth stages and dates of planting in North Dakota. J. Econ. Entomol. 79:648-650.

Charlet, L.D., and P.A. Glogoza. 2004. Insect problems in the sunflower production regions based on the 2003 sunflower crop survey and comparisons with the 2002 survey. Proc. 26th Sunflower Research Workshop, Natl. Sunflower Assoc., Fargo, ND, 14-15 January 2004. http://www.sunflowernsa.com/research/research-workshop/documents/143.pdf.

Charlet, L.D., and T.A. Gross. 1990. Bionomics and seasonal abundance of the banded sunflower moth (Lepidoptera: Cochylidae) on cultivated sunflower in the northern Great Plains. J. Econ. Entomol. 83:135-141.

Charlet, L.D., G.J. Brewer, and B. Franzmann. 1997. Insect pests, p. 183-261. In. A. A. Schneiter (ed.), Sunflower Production and Technology. Agronomy Monograph 35. ASA, CSSA and SSSA, Madison, WI, USA.

Knodel, J.J., and L.D. Charlet. 2007. IV. Pest Management – Insects. In: D.R. Berglund (ed.), Sunflower Production, NDSU Ext. Serv. A-1331 (EB-25 Revised).

Knodel, J.J., P. Beauzay, M. Boetel, and D. Markle. 2008. 2008 North Dakota Field Crop Insect Management Guide. NDSU Ext. Serv., E-1143.

Mundal, K.D., G.J. Brewer, L.D. Charlet, and J.J. Knodel. 2006. Banded Sunflower Moth. NDSU Ext. Serv. E-823 (revised), November 2006.

Oseto, C.Y., L.D. Charlet, and J.D. Busacca. 1989. Effects of planting date on damage caused by the banded sunflower moth (Lepidoptera: Cochylidae) in the northern Great Plains. J. Econ. Entomol. 82:910-912.

Epicuticular wax content in the pericarp of sunflower fruits (*Helianthus annuus* L.) grown under moderate water deficit

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ABSTRACT

The effect of a moderate water deficit (MWD), imposed on field grown plants in two sunflower hybrids from early anthesis (reproductive stage 6 or R6) to harvest maturity (HM), on the development of epicuticular waxes (epw; mg/g) of the fruit's pericarp, was studied in the present work. The experiment was repeated during two consecutive years. In both hybrids and experiments, plants grown under MWD showed an epw content higher than the 'controls. A decrease in the epw from stage R6 to HM was observed. This could be attributed to the erosive action on the surface of the pericarp by particulate solids carried by wind or rain. These results constitute valuable information for sunflower breeders to further investigate about the mechanisms that regulate wax content in the fruit's pericarp.

Keys words: epicuticular wax - *Helianthus annuus* - pericarp - sunflower - water deficit.

INTRODUCTION

After the sunflower oil has been industrially obtained and cooled, a crystalline sediment can be observed which affects its commercial quality (Rivarola et al., 1988). This sediment is mainly composed of waxes of epicuticular origin (epicuticular waxes or epw). They come from the fruit's pericarp (hull; 83%) (Martin and Juniper, 1970; Morrison, 1983), from the seed teguments (16%) and the embryo (1%) (Morrison et al., 1994).

The amount of waxes passing to the oil during the extraction process depends on the relative hull content of the fruit and the amount of wax it carries. In modern hybrids with high oil content, a thin pericarp is strongly adhered to the seed increasing epw transfer to the oil (Morrison et al., 1984). In these hybrids, fruit's hull content is inversely correlated with oil wax content (Morrison, 1983).

Although waxes constitute a problem for the oil industry, no studies on the development of epw in the sunflower hull are available to date. So, there is no information about the variability in the epw content among hybrids or the effect that different environmental factors and agronomical practices could produce on the epw genesis.

It is known that thermal and water stress can trigger and enhance epicuticular wax synthesis in several plant organs (Premachandra et al., 1992) and that the level of response is phenotipically sensitive and genetically controlled (Koornneef et al., 1989; Jenks et al., 2002). So in this work we have analyzed the evolution of epw content in the pericarp through different developmental stages of two sunflower hybrids grown under two water regimes.

MATERIALS AND METHODS

Plant material

Two sunflower hybrids, Dekasol (DK) 3900 and DK4030, were sown at the Department of Agronomy, UNS, experimental field (Bahía Blanca. Argentina, Lat. S., 38° 45'; Long. W, 62°11') during two consecutive growing seasons (Experiment I: 2003/2004; Experiment II: 2004/2005). The crop was grown under drip irrigation and managed according to recommended conventional agronomical practices (Pereyra and Farizo, 1981). Plant density was adjusted at 5.6 plants/m^2. Fruit samples taken from the capitulum's periphery during reproductive stages R6, R9 and harvest maturity (HM) (Schneiter and Miller, 1981) were analyzed (Table 1).

Treatments
During the reproductive stages R4 to R6 a moderate water deficit (MWD) was generated by interrupting irrigation. It was monitored by measuring the relative water content of plant leaves (RWC_{leaf}) in each treatment at different crop developmental stages.

Determination of epw content
Epw content was measured in the pericarp of the fruits at each sampling stage, for each hybrid and experiment, following the technique described by Franchini and Hernández (2006) using carbon tetrachloride as extracting agent. The epw content was expressed in mass of epw by mass of pericarp dry weight (mg/g).

Experimental design and statistical analysis
Both experiments consisted of complete randomized split plots, with water status assigned to main plots and hybrids to subplots. To determine differences between treatments and hybrids, experimental results were processed by ANOVA and differences between means were evaluated with LSD test.

Table 1. Days from first anthesis to attain reproductive stages R6, R9 and HM (Schneiter and Miller, 1981) in each of the hybrids and experiments HM: harvest maturity

	Experiment I		Experiment II	
	Hybrid		Hybrid	
Stage	DK3900	DK4030	DK3900	DK4030
R6	8	12	13	12
R9	58	48	48	44
HM	71	68	60	56

RESULTS

Plant water status
In both experiments and at different sampling times, an overall decrease of RWC_{leaf} was observed in plants under MWD comparing to control plants (Figs. 1A and 1B). Nevertheless a significant reduction (Fig. 1B; $p<0.05$) in the RWC_{leaf} was only observed 79 days after crop emergence in Experiment II accompanied by a temporary leaf wilting. After irrigation was reestablished, leaves recovered their normal turgor.

Epw content in the pericarp.
In both hybrids and treatments a reduction in epw content was observed from R6 to HM (Figs. 2A and 2B). In fruits of DK3900, during Experiment I, the observed reduction was 28 % ($p<0,05$) from stage R6 to HM (Fig. 2A), while during the Experiment II, the observed reduction was not significant ($p = 0,09$; Fig. 2B).

Although a continuous reduction in the epw content of DK4030 fruits was observed from stage R6 to HM, this was not significant in Experiment I ($p>0,05$; Fig. 2A). In Experiment II, epw content was significantly reduced by 14% ($p<0,05$) from R6 to R9, with no significant differences detected between the latter stage and HM (Fig. 2B).

MWD and epw content
Since there was no hybrid x water regime interaction ($p>0,05$) for the variable epw content, only the average results for both hybrids (Table 2) in each experiment are presented. In both experiments and in each reproductive stage studied, epw of fruits from plants under MWD showed a 33% epw increase compared to control plants (Table 2). Nevertheless, it must be mentioned that during Experiment I water deficit was not as high as expected so the differences between treatments might not be so evident.

Table 2. Average content of epw (mg/g) of the pericarp of the sunflower hybrids DK3900 and DK4030 both in control and under moderate water deficit (MWD). R6, R9: Reproductive stages as described by Schneiter and Miller (1981). HM: harvest maturity.

Stage	Experiment I			Experiment II		
	Control	MWD	S.E.	Control	MWD	S.E.
R6	5,08 a*	6,24 a	0,3	5,25 a	7,08 b	0,3
R9	4,72 a	5,59 b	0,4	4,46 a	6,53 b	0,3
HM	3,64 a	4,44 a	0,4	4,24 a	6,22 b	0,3

* In a row, within each assay, means followed by the same letter are not significantly different at p>0,05. MDW: Moderate Water Deficit. S.E.:Standard error.

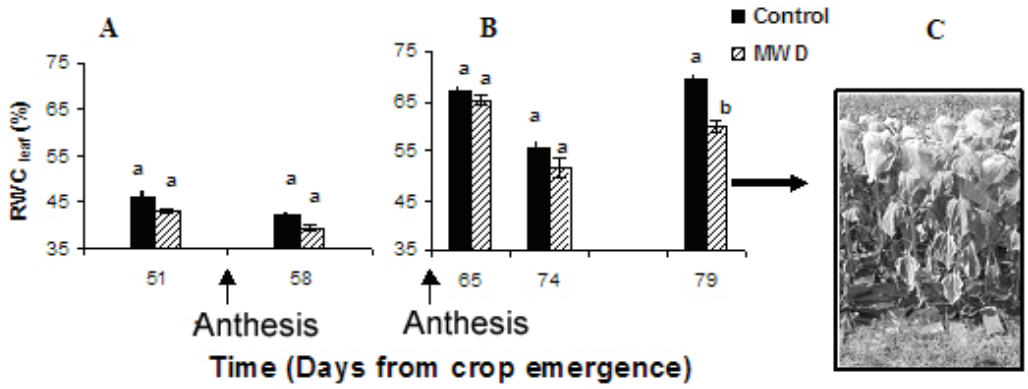

Fig. 1. Leaf relative water content (RWC_{leaf} %) in the sunflower hybrids DK3900 and DK4030 during experiment I (A) and II (B). C. Temporary wilting of leaves of plants under MWD during experiment II, 79 days after crop emergence (24 days after anthesis). Leaves became turgent once irrigation was reestablished. MDW: Moderate Water Deficit. Within each set, bars topped by the same letter are not significantly different at p>0,05.

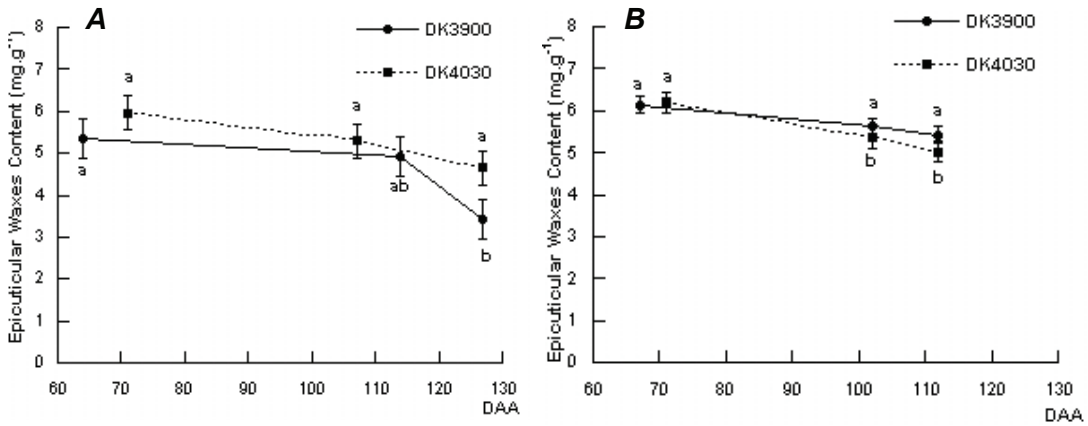

Fig. 2. Changes with time of epw (mg/g) in the pericarp of fruits of the sunflower hybrids DK3900 and DK4030, averaged across water treatments, from R6 to HM. A) Experiment I. B) Experiment II. DAA: Days after anthesis. For each hybrid, values followed by different letters indicate significant differences between sampling dates (p<0,05).

DISCUSSION

Plant water status

The observed RWC_{leaf} magnitudes (Fig. 1) show that, in both experiments, the procedure of irrigation shortage was sufficient to generate a suboptimal water status in the critical developmental stages of the formation of pericarp (stages R5 and R6; Lindström et al., 2000).

Epw content in the pericarp

The observed reduction in epw content from R6 to HM in both hybrids and experiments, could be attributed to the erosive action produced by several environmental factors, among which rainfall and wind are particularly common. They can transport abrasive particulate material removing wax crystals from the pericarp surface. The same effect has been observed in leaves of *Eucalyptus* sp. (Baker and Hunt, 1986), *Brassica* sp. and *Fraggaria* sp. (Neinhuis and Barthlott, 1997). Also, in both hybrids and experiments, the highest content of epw measured in R6, when the pericarp is still young and contains high water concentration (Rondanini et al., 2007), agrees with the phenomenon observed by Neinhuis et al. (2001). These authors demonstrated that cuticular transpiration allows the waxes attached to water molecules to move from the inner regions of the leaf to its outer surface. So, in young epidermis with a thin cuticle, such as that present in undeveloped fruits, with a lesser resistance for the passage of waxes through it compared with mature ones, a higher epw content can be expected.

MWD and epw content

In both experiments and in the three fruit developmental stages (Table 2), the imposed leaf water deficit induced a comparatively higher epw than in the controls. Similar results can be found in leaves of weeping lovegrass (*Eragrostis curvula* Schrad) (Echenique et al., 1986) and sorghum (*Sorghum bicolor* L.) (Premachandra et al., 1992), where a constant water stress led to an increase in the content of epw and a reduction in the cuticular transpiration rate.

CONCLUSIONS

A moderate plant water deficit during fruit development led to an increase of 33 % in the epw content in the pericarp, compared with that of the control plants.

From R6 to HM, epw content decreased, possibly due to the erosive action produced by wind and rain on the fruit surface.

The results shown here can be used as a physiological tool to define the dynamics of wax accumulation in the sunflower fruit pericarp, a variable that can be genetically modified (Jenks et al., 2002). Thus, breeders would be able to manipulate two characters, which are currently antagonists in the sunflower fruit: seed oil and pericarp wax content.

ACKNOWLEDGEMENTS

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REFERENCES

Baker, E.A., and G.M. Hunt. 1986. Erosion of waxes from leaf surfaces by simulated rain. New Phytol. 102:161-173.

Echenique, C.V., M. Poverene, and N.R. Curvetto. 1986. Epicuticular wax and water stress in *Eragrostis curvula* (Schrad) cultivars. Effects of water stress on the quantity and morphology of epicuticular wax. Microscopía Electrónica y Biología Celular 10:91-95.

Franchini, M.C., and L.F. Hernández. 2006. Contenido de ceras epicuticulares del pericarpio de frutos de girasol. Distribución espacial en el capítulo. XXVI RAFV. Chascomús, Buenos Aires, Argentina.

Jenks, M.A. 2002. p. 1-24. In: The Arabidopsis Book. Somerville, C., E. Meyerowitz (Eds). ASPB. http://www.aspb.org/publications/arabidopsis.

Koornneef, M., C.J. Hanhart, and F. Thiel. 1989. A genetic and phenotypic description of *eceriferum* (cer) mutants in *Arabidopsis thaliana.* J. Heredity 80:118-122.

Lindström, L.I., C.N. Pellegrini, and L.F. Hernández. 2000. Anatomía y desarrollo del pericarpio de distintos genotipos de girasol (*Helianthus annuus* L.). p. D13-D18. In: Proc. 15th Int. Sunfl. Conf., Toulouse, France.

Martin, J.T., and B.E. Juniper. 1970. The cuticles of plants. Edward Arnold, London, 347 p.

Morrison III, W.H. 1983. Variation in wax content of sunflower seeds with location and hibrids. J. Am. Oil Chem. Soc. 61:1013-1015.

Morrison III, W.H., R.E. Sojka, and P. Unger. 1984. Effects of planting date and irrigation on wax content of sunflower-seed oil. J. Am. Oil Chem. Soc. 61:1242-1245.

Morrison III, W.H., R.J. Hamilton, and C. Kalu. 1994. Sunflower Oil. p. 133-152. In: R.J. Hamilton (ed.), Development in Oils and Fats. John Moores University, Liverpool, UK.

Neinhuis, C., and W. Barthlott. 1997. Characterization and distribution of water-repelent, self-cleaning plant surfaces. Ann. Bot. 79: 667-677.

Neinhuis, C., K. Koch, and W. Barthlott. 2001. Movement and regeneration of epicuticular waxes through plant cuticles. Planta 213:427-434.

Pereyra, V.R., and C.L. Farizo. 1981. Girasol. Técnicas de Producción. INTA, Balcarce, 27 p.

Premachandra, G.S., H. Saneoka, K. Fujita, and S. Ogata. 1992. Leaf water relation, osmotic adjustment, cell membrane stability, epicuticular wax load and growth as affected by increasing water deficits in sorghum. J. Exp. Bot. 43:1569-1576.

Rivarola, G., M.C. Añon, and A. Calvelo. 1988. Crystalization of waxes during sunflower seed oil refining. J. Am. Oil Chem. Soc. 62:1508-1513.

Rondanini, D.P., R. Savin, and A. Hall. 2007. Estimation of physiological maturity in sunflower as a function of fruit water concentration. Eur. J. Agron. 26:295-309.

Schneiter, A.A., and J.F. Miller. 1981. Description of sunflower growth stages. Crop Sci. 21:902-904.

The pattern of foraging paths of the Honey bee (*Apis mellifera* L.) can also explain the appearance of located regions with incompletely developed fruits in the sunflower capitulum

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ABSTRACT

The occurrence of fruits with absent or poorly developed embryos, also defined as seedless fruits or incompletely developed fruits (IDF), respectively, significantly impacts sunflower yield. Failures in pollination, fertilization and physiological or morphological defects in the ovary and embryo, either genotypic or environment-induced, post-pollination or post-fertilization, are among the most common reasons for the generation of this kind of fruits. A detailed study of the foraging pattern of diurnal pollinators in the sunflower crop, mainly honey bees, showed that there was a significant inverse correlation between the percentage of areas covered by foraging paths (ACP) and the total IDFs counted per capitulum's concentric sector. Almost a complete limitation of visitations in these sectors (0-30% ACP) resulted in poor seed set and IDFs ranging from 9 to 17%. At that level of ACP, a significant inverse correlation (r^2=0.61; p= 0.05) was found between the density of honeybee visitation and the percentage of IDFs. Partial limitation of the insect visitation (30 to 59% ACP) generated 5 to 9 % of IDFs. It is concluded that as much as 60% or more of the capitulum's area must be covered by pollinators to minimize the occurrence of IDFs.

Keywords: *Apis mellifera* - *Helianthus annuus* - pollen - seed set - sunflower.

INTRODUCTION

At maturity, the capitulum of sunflower [*Helianthus annuus* L., var. Macrocarpus (D.C.) Cockerell] usually has fruits with a different degree of pericarp and embryo development. In most of them, the embryo reaches its full size filling the internal cavity of the ovary. These fruits are defined as fully developed (FDF) (Lindström et al., 2006; 2007). On the other hand, many fruits often contain ovules that did not fully develop into seeds. In those fruits, growth processes stop at different moments, leaving the fruits with an incompletely developed pericarp and/or seed so being defined as seedless or incompletely developed fruits (IDF; Alkio et al., 2002; Alkio and Grimm, 2003; Lindström et al., 2004). Generally, IDFs can be seen to be randomly distributed over the capitulum surface (Hernández et al., 2002; Lindström et al., 2004).

The causes of the origin of IDFs are unknown but several proximate mechanisms put forward to explain the low seed to ovule ratio in many species of the *Angiospermae* can be applied to the sunflower. Poor seed set occurs mainly due to inadequate pollination, the competition for resources between developing ovaries, or vascular deficiencies at the ovary-receptacle interface (Birch and van der Sandt, 1985; Durrieu et al., 1985; Hernández and Orioli, 1991; Hernández and Palmer, 1992; Connor and Hall, 1997; Alkio and Grimm, 2003; Cantagallo et al., 2004, Lindström et al., 2006). Several studies have shown that the foraging activity of the honey bee (*Apis mellifera* L.) can increase seed set and yield (Parker, 1981a; Birch and van der Sandt, 1985; Fell, 1986; Skinner, 1987; Medan et al., 2003; DeGrandi-Hoffman and Chambers, 2006). Nevertheless, the bee foraging pattern on the sunflower capitulum has not been deeply studied (Parker, 1981b) and its relationship with seed set has not been totally established.

The aim of the present work was to determine the relationship between the path of daily visits of pollinators on capitula of the cultivated sunflower and the pattern of IDFs.

MATERIALS AND METHODS

The experiment was carried out at the Agronomy Department-UNSur, Bahía Blanca, Argentina (Lat. S. 38°45'; Long. W. 62°11') over one growing season. A low self-fertile experimental sunflower genotype, provided by Dow Agrosciences of Argentina, was sown starting the first week of October on three

successive dates, separated by 5 days, in order to obtain plants at the beginning of flowering (first anthesis [FA]; Schneiter and Miller, 1981) during several consecutive days and study them individually.

At seedling emergence, plant density was adjusted to 5.6 plants/m^2. Weeds, pests and irrigation were adequately controlled. The experimental plot was near (300 m) 20 bee colonies. This ensured that visitation at flowering was highly intense. Daily records of temperature and solar radiation were obtained from a meteorological station located 800 m from the experimental field.

Plant selection and pollinator visits observations

Two plants displaced 4 to 5 days in time for each seeding date were randomly selected in the stand (n=6). Before FA, the selected plants were staked with the florets oriented eastwards. At FA, the capitulum diameter was measured and four landmarks were placed at the periphery using colored pearl head pins. The capitulum of one plant at a time was then continuously recorded using a digital camera. The recording process took 2 to 3 days, from FA until the first 6 to 7 rows of peripheral flowers finished opening. Bee foraging on a head was continuously watched from 8.00 a.m. to 5.00 p.m. Recording was interrupted when visitors were absent. Only honey bees and sporadically carpenter bees (*Xylocopa* sp.) were observed. At dusk, each capitulum was covered with a mesh bag to avoid the action of night pollinators. After the study was completed, the procedure was repeated with another plant that by that time was at FA. The observed capitula were covered during the night until harvest.

Data processing

Digital files for each observed plant (n=6) were processed using the software VideoPoint v.2.5 (Lenox Softworks, Lenox, MA) to define, in Cartesian coordinates, the pattern of foraging routes of the pollinators (Fig. 1). The bee's thorax was the reference point of movement to digitize the route followed by the insect during its visit (arrival-departure) to the capitulum. The landmarks on the capitulum allowed the correct location and correspondence of the recorded paths at anthesis and at maturity (Fig. 1). Each image of the capitulum was then fractionated in 60 sectors and the pixel density corresponding to the foraging routes in each sector was quantified with the software Object-Image v.2.21 (Vischer et al., 1994) in a Macintosh platform. After calculating the area of each sector of the capitulum, the average pixel density was estimated for each sector (pixels% per sector) for each capitulum for each one of the six observed plants (Fig. 2).

At harvest, IDFs were identified on each mature capitulum and its location per capitulum sector defined using the reference landmarks. The IDF proportion was calculated per each capitulum sector and then compared with the intensity of visitations (Fig. 1), defined in this work as area covered by paths or ACP (Fig. 3).

RESULTS

The complete pattern in the capitulum generated by the routes followed during two consecutive visiting days for one of the six studied plants is shown in Fig. 1. Its corresponding density of visits per capitulum sector calculated according to the above methodology is presented in Fig. 2. The relationship between the % of areas covered by paths (ACP%) and the percentage of IDFs per sector in capitula of the six plants observed in this work is presented in Fig. 3.

The main floral visitors in all observations belonged to the order Hymenoptera (100% of total visits): *Apis mellifera* L., (98%), and *Xylocopa sp*. (2%). The path density was not homogeneous, showing zones with quite different densities (Figs. 1-2). There was an inverse relationship between the absence of visits or a low density of visits in a sector and the percentage of IDFs (Fig. 2). From Fig. 3 three intervals for the relationship between the ACP% and the IDF% can be defined. Thus, between 0 and 30% ACP an inverse relationship (r^2= 0.61; n=91; p= 0.05) was observed (Fig. 3). Between 30 and 100% ACPs, IDF magnitudes were distributed in two levels of a broad fluctuation, ranging from 5.0 to 9.0 % IDFs between 30 to 100% ACPs and 0% IDF or 6.0 to 9.5 % IDFs between 56 and 100% ACPs (Fig. 3).

DISCUSSION

Lack of sufficient pollen loads on the stigma to fertilize all the flowers (Zimmerman and Pyke, 1988) and physiological and/or anatomical alterations and source limitations to provide for seed development (Stephenson, 1981; Zimmerman and Pyke, 1988; Connor and Hall, 1997) have been most commonly attributed as causes for a low seed set.

According to the "non-uniform pollination hypothesis" (Thomson, 1989; Berry and Calvo, 1991) the

observed patterns of IDFs in the mature capitula may be attributable to variations in pollen receipt over the flowering period. Specifically, the relatively low seed set in central areas of the sunflower capitulum has usually been attributable to insufficient pollen quantity or pollinator visits.

The reason why some florets are left unvisited in the observed regions of the capitulum is not known. Recently, Giurfa (2004) demonstrated that the honey bee can discriminate color, and, regarding this, it has been noticed that some floret corollas in different capitulum locations of recently open florets, have a different color intensity compared with their neighbors (L.F. Hernández, unpublished). It is also known that honeybees avoid probing flowers that have been recently depleted by conspecifics, presumably repelled by foraging scent marks deposited by the previous visitor (Giurfa and Nuñez, 1992; Gawleta et al., 2005). Probably, if this is the case, the reason to leave some disc florets unvisited (Fig. 1) could be related with its proximity to already visited neighbor florets.

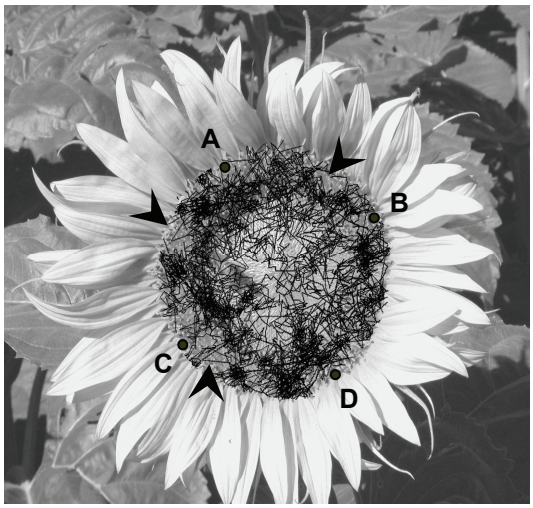

Figure 1. Pattern of routes of daily visits of honeybees (*Apis mellifera* L.) and, in a lesser proportion, carpenter bees (*Xylocopa sp*.) during two consecutive days after FA, on the capitulum of one plant studied in this work. The tracing of the image of 2 pixel width was accomplished after processing the digital images with the software VideoPoint. Arrows show some of the unvisited regions. Circles noted with letters A, B, C and D correspond to the lansmarks defoned with colored pearl head pins. The routes followed towards the central region of the capitulum were not considered in this study because these flowers were not open at the time of the analysis.

In this work, climate conditions during capitulum maturation were optimal. No rain occurred during the observation period, which could induce pollination failures by pollen lixiviation, and air temperature was always near or below 30^{o} C, a thermal level known to affect sunflower pollination (DeGrandi-Hoffman and Chambers, 2006).

Sunflower genotypes vary in their attractiveness to the honey bee. Short corolla length, non pigmented stigmas, many nectaries, and high sucrose content of the nectar are preferred by bees. If the flower was never visited it could be indicating that perhaps the floret *per se* was responsible for the lack of attractiveness due to some intrinsic difference that made it special and "unvisitable", compared with the surrounding ones. Sammataro et al. (1984; 1985) found intragenotypical differences in the quality, quantity and anatomy of nectaries. Perhaps some interplant differences could also exist.

The availability of resources can vary in both space and time for an individual flower, due to local competition for the resources (Stephenson, 1981). Hence, within a single plant, resources may be limited for some flowers but not for others. Nevertheless, perhaps this was not the case for the external flowers in the capitulum. It has been observed that at early anthesis, recently open flowers in the capitulum are not deprived of an assimilate supply (Hernández and Orioli, 1991; Alkio et al., 2002; Alkio and Grimm, 2003). Finally, according to the "architectural effects hypothesis", the pattern of seed production can also be caused in some plants by intrinsic factors, biological or physical, limiting the ripening of ovules located in some inflorescence positions (Diggle, 1995). The proximate causes of these architectural effects are still unknown (Diggle, 1997), although accumulating evidence is showing that it can have an

important effect on the observed pattern of seed production (Medrano et al., 2000 and references therein).

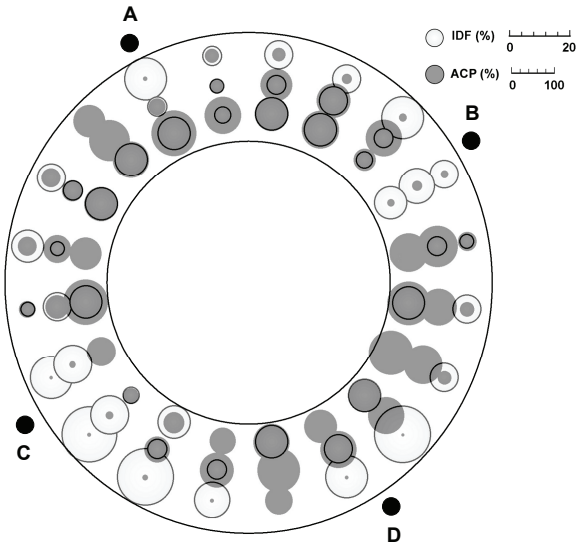

Figure 2. Emerging relationship between the area covered by visit paths (◯ ; ACP%) and the IDF% (⬤) produced in each sector for the capitulum of Fig. 1. The scales indicate the length of the diameter of each circle with the percentage magnitude for each variable. A,B,C and D, as in Fig. 1.

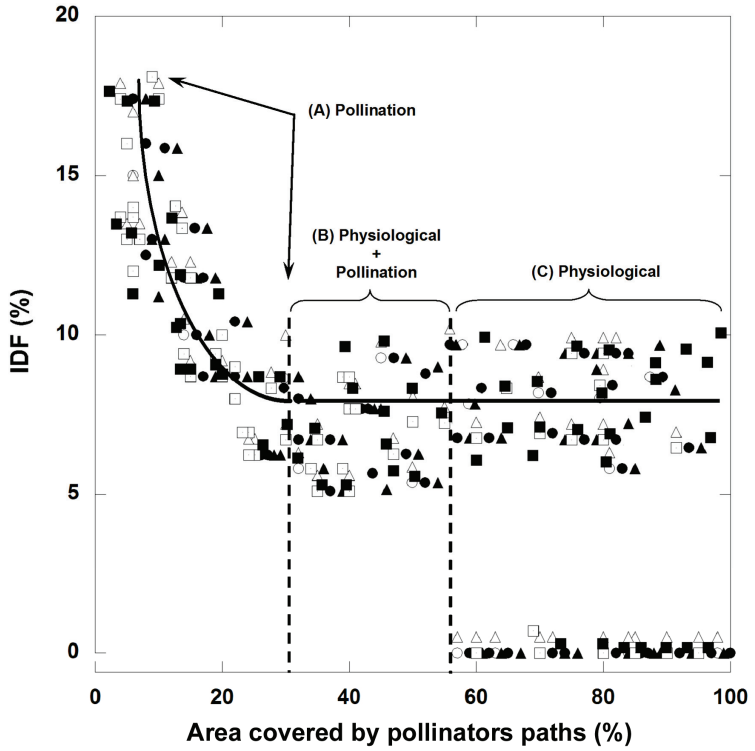

Figure 3. Relationship between the area covered by paths (ACP%) and the percentage of incompletely developed fruits (IDF%) observed in each sector (n=60) in which each capitulum of the 6 sunflower plants was divided for analysis. The sum of observations of IDF% are divided into three intervals ranging from 0% to 30%, 31% to 59 and 60% to 100% of ACP to discriminate the causes acting in the generation of IDF: (A), pollination failures, associated with the lack of pollinator visits: $IDF\% = 31.48 \times ACP^{-0,43}$; $r^2 = 0.61$; n=91; p= 0.05), (B), physiological plus pollination failures and (C), mainly physiological causes. Different markers indicate individual plants.

Considering the conditions under which the experiment and the observations were conducted and assuming that no contribution from other pollinators occurred from anthesis to fertilization, the analysis of Fig. 3 revealed at first glance three intervals which can separate different causes for the generation of IDFs. From 0% to 30% of ACP, the negative correlation found between ACP% and IDF% (r^2= 0.61) suggests that within this range, the lack of visits had a high incidence on fruit set (Fig. 3).

Approximately from 30% of ACP and above this value, the stable level of IDFs, fluctuating from 5 to 10% suggests that we would have to consider other variables. Probably there was a combination of a low occurrence of bee visits and factors related to the floral biology (physiological factors) of the tested genotype (Fig. 3).

Above 60% of ACP, the absence of IDFs (0%) in several sectors (Figs. 1 and 2) and the occurrence of sectors with a fluctuating level of IDF% ranging from 6 to 10% of the total value, would suggest that the IDFs generated in that region were produced by physiological causes, which were neither detected nor studied in this present work. Probably, they were associated with the low self-compatibility of the genotype used. Given the present information, it would be expected that in sunflower genotypes with high self-compatibility, the IDF fraction, although fluctuating, could descend to levels under 5% per sector.

The correlation between the percentage of IDFs per sector and the ACP (%) over 30% was then weak (Fig. 3), probably because the data was masked with other variables, which would act to generate IDFs.

Another weakness is the fact that the ACPs were sampled only after the bees settled during the day, without quantifying the behavior of other pollinators during the night. Nevertheless, the positive relationship between the density of foraging routes and the development of IDFs in several areas of the capitulum, confirms the important role of day-sheltering bees as sunflower pollinators. Unvisited areas are positively correlated with the presence of seedless or incompletely developed fruits at maturity. This suggests that some flowers from those areas are inclined to show an absence or delay in pollination with respect to the adjacent flowers. Due to night covering, we could assume that nocturnal pollinators would substitute the lack of visited sites by areas that bees did not visit during the day. Nevertheless if a deficiency in the number of nectaries or floret functionality occurred, these sites would not be visited at night either.

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REFERENCES

Alkio, M., and E. Grimm. 2003. Vascular connections between the receptacle and empty achenes in sunflower (*Helianthus annuus* L.). J. Exp. Bot. 54:345-348.

Alkio, M., M. Diepenbrock, and E. Grimm. 2002. Evidence for sectorial assimilate supply in the capitulum of sunflower (*Helianthus annuus* L.). New Phytol. 156:445-456.

Berry, P.E., and R.N. Calvo. 1991. Pollinator limitation and position dependent fruit set in the high Andean orchid *Myrosmodes cochleares* (Orchidaceae). Plant Syst. Evol. 174:93-101.

Birch, E.B., and J.C. van der Sandt. 1985. Bee pollination of sunflower. p. 255-260. In: Proc. 11th Int. Sunflower Conf. Mar del Plata, Argentina.

Cantagallo, J.E., D. Medan, and A.J. Hall. 2004. Grain number in sunflower as affected by shading during floret growth, anthesis and grain setting. Field Crops Res. 85:191-202.

Connor, D.J., and A.J. Hall. 1997. Sunflower Physiology, p. 113-182. *In*: A.A. Schneiter, [ed.], Sunflower Technology and Production, Agron. Ser. 35, ASA, CSSA, SSSA, Madison, WI, USA.

DeGrandi-Hoffman, G.,. and M. Chambers. 2006. Effects of honey bee (Hymenoptera: Apidae) Foraging on seed set in self-fertile sunflowers (*Helianthus annuus* L.). Environ. Entomol. 35:1103-1108.

Diggle, P.K. 1995. Architectural effects and the interpretation of patterns of fruit and seed development. Ann. Rev. Ecol. Syst. 26:531-542.
Diggle, P.K. 1997. Ontogenetic contingency and floral morphology: the effects of architecture and resource limitation. Int. J. Plant Sci. 158 (supplement): S99-S107.
Durrieu, G., C. Percie du Sert, and A. Merrien. 1985. Anatomie du capitule de tournesol consequences sur la nutrition des akenes. p. 7-12. In: Proc. 11th Int. Sunflower Conf. Mar del Plata, Argentina.
Fell, R.D. 1986. Foraging behaviors of *Apis mellifera* L. and *Bombus* spp. on oil sunflower (*Helianthus annuus* L.). J. Kansas Entomol. Soc. 59:72-81.
Gawleta, N., Y. Zimmermann, and T. Eltz. 2005. Repellent foraging scent recognition across bee families. Apidologie 36:325-330.
Giurfa, M. 2004. Conditioning procedure and color discrimination in the honeybee *Apis mellifera*. Naturwissenschaften 91:228-231.
Giurfa, M., and J.A. Núñez. 1992. Honeybees mark with scent and reject recently visited flowers. Oecologia 89:113-117.
Hernández, L.F., and G.A. Orioli. 1991. Role of different leaves of the sunflower (*Helianthus annuus* L.) plant during the grain filling period (in spanish). Turrialba 41:330-334.
Hernández, L.F., and J.H. Palmer. 1992. Incorporation of ^{14}C labeled metabolites into the developing sunflower capitulum. p. 564-570. In: Proc. 13th Int. Sunflower Conf. Pisa, Italy.
Hernández, L.F., C.N. Pellegrini, and L.I. Lindström. 2002. Prediction of the generation of seedless fruits in the sunflower capiulum (*Helianthus annuus* L.) (in spanish). XI LatinAmerican Meeting of Plant Physiologists. Punta del Este, Uruguay.
Lindström, L.I., M.E. García, and L.F. Hernández. 2004. Morphology and distribution of incompletely developed fruits in sunflower (*Helianthus annuus* L.) capitula. p. 333-337. In: Proc. 16th Int. Sunflower Conf., Fargo, ND, USA.
Lindström, L.I., C.N. Pellegrini, L.A.N. Aguirrezábal, and L.F. Hernández. 2006. Growth and development of sunflower fruits under shade during pre and early post-anthesis period. Field Crops Res. 96:151-159.
Lindström, L.I., C.N. Pellegrini, and L.F. Hernández, L.F. 2007. Histological development of the sunflower pericarp as affected by pre and early post-anthesis canopy shading. Field Crops Res. 103:229-238.
Medan, D., M. Chamer, M. Devoto, N.H. Montaldo, A.I. Mantese, N.J. Bartoloni, A. Roig-Alsina, and S. Leguizamón. 2003. Do the argentine sunflowers need pollinators? (in spanish). II Argentine Sunflower Association Meeting, Buenos Aires, Argentina, ASAGIR.
Medrano, M., P. Guitián, and J. Guitián. 2000. Patterns of fruit and seed set within inflorescences of *Pancratium maritimum* (Amaryllidaceae): nonuniform pollination, resource limitation, or architectural effects? Am. J. Bot. 87:493-501.
Parker, F.D. 1981a. Sunflower pollination: abundance, diversity and seasonality of bees and their effect on seed yields. J. Apic. Res. 20:49-61.
Parker, F.D. 1981b. How efficient are bees in pollinating sunflowers? J. Kansas Entomol. Soc., 54:61-67.
Schneiter, A.A., and J.F. Miller. 1981. Description of sunflower growth stages. Crop Sci. 21:901-903.
Sammataro, D., P.K. Flottum, and E.H. Erickson. 1984. Factors contributing to honeybee preferences in sunflower varieties. p. 20-21. In: Proc. Sunflower Res. Workshop, Bismark, ND.
Sammataro, D., E.H. Erickson, and M.B. Garment. 1985. Ultrastructure of the sunflower nectary. J. Apic. Res. 24: 150-160.
Skinner, J.A. 1987. Abundance and spatial distribution of bees visiting male-sterile and male-fertile sunflower cultivars in California. Environ. Entomol. 16:922-927.
Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Ann. Rev. Ecol. Syst. 12: 253-279.
Thomson, J.D. 1989. Deployment of ovules and pollen among flowers within inflorescences. Evolutionary Trends in Plants 3:65-68.
Vischer, N.O.E., P.G. Huls, and C.L. Woldringh. 1994. Object-Image: An interactive image analysis program using structured point collection, BINARY, (Bioline), Vol. 6.
Zimmerman, M., and G.H. Pyke. 1988. Reproduction in Polemonium: assessing the factors limiting seed set. Amer. Nat. 131:723-738.

The influence of weather conditions on economic characteristics of sunflower hybrids in macro experiments from 1997 to 2007

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ABSTRACT

Over the period of 11 years (1997-2007), macro experiments with sunflower hybrids were set up at the location of Vinkovci. The experiments included hybrids of recognized world companies, from 13 to 30 per each test. During 11 years, the average head diameter, stem height, disease incidence of *Sclerotinia sclerotiorum*, grain yield, oil yield and oil content were recorded for each hybrid. The highest plant height was recorded in 1997 and 1998 (204 cm), while the lowest plant height was observed in 2006 (169 cm). Average plant height throughout the experiments was 188 cm. Head diameter ranged from 18.1 to 22.9 cm, averaging 19.6 cm. The greatest incidence of white rot disease (*Sclerotinia sclerotiorum*) occurred in 2005 (39.6% of infected plants). The average grain yield was 3 t ha^{-1} and it varied from 1.13 t ha^{-1} in 2005 to 4.56 t ha^{-1} in 2000. The oil content was between 42.05 and 48.17 %, with an average over experiments of 44.42%. The lowest oil yield was obtained in 2005 (0.49 t ha^{-1}) and the highest in 2000 (2.04 t ha^{-1}), with an average of 1.31 t ha^{-1}).

Key words: economic characteristics – sunflower – weather conditions.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the four most significant oleaginous plants, planted on more than 20 million hectares worldwide with grain yields varying from 0.5 to 3.6 t ha^{-1} and an average grain yield of 1.16 t ha^{-1} (FAOSTAT, 2002). Production of sunflower in Croatia has significantly varied over the past 25 years. Average grain yield was between 1.9 to 2.5 t ha^{-1}, depending on the climate conditions of the production year (Liovic et al., 2006). Numerous pathogens can attack sunflower and one of the most important ones is white rot, *Sclerotinia sclerotiorum* (Lib.) de Bary (Duvnjak et al., 2005; Hudec, 2006). The highest disease incidence can be expected in years with cold and wet months during the vegetation period. Sunflower breeding programs must be based on creation of new genetic variability and hybrids with high oil and grain yield potential (Krizmanic et al., 2004; Hunyadi et al., 2007). Through several years of field experiments in Croatia, many hybrids have been tested and only those with the best economic characteristics were recommended to producers. Experiments also gave a direction to breeding program and seed production work. An overview of such experiments is given in this manuscript.

MATERIALS AND METHODS

During 11 years, experiments were set up in the experiment field of PIK Vinkovci. Experiments included hybrids of well-known world companies (Pioneer, RWA, KWS, Monsanto, Agricultural Institute Osijek, Institute for Crops and Vegetables of Novi Sad). The size of the main parcel was 250 m^2. Sowing was done with pneumatic sowing machines with the same crop density for all hybrids (65,000 grains per ha). Common sunflower agrotechnique was used. Protection was given with fungicide Konker (vinclosolin 20% + carbendasim 16.5%) with dosage of 1.5 l ha^{-1}. Spraying was done with field sprayer in R1-R2 sunflower stage (head size 2 cm) (Schneiter and Miller, 1981). The intensity of white rot (*Sclerotinia sclerotiorum*) incidence was determined in stage R8, by counting infected plants (40 of each hybrid). Grain yield was determined after the harvest with electronic measurer (Schrran Engeneering, model 715) and presented as tons of dry grain per hectare (grain moisture 9%, 2% ingredients). Grain moisture was determined with Dickey John measurer, model GAC 2000 (grain analysis computer).

Weather conditions: In Table 1, amount of rainfall and average temperature over the vegetation (April - August) and over the year (1997-2007) are presented. The driest year was 2007, with 94.1 mm of rainfall over the vegetation period and 238.7 mm over the year, average temperature during the vegetation period was 19.8 ºC. The largest amount of rainfall was in 2001, with 939.7 mm over the year and 514.2 mm over

the vegetation period. The lowest average temperature in 2001 (17.8 ºC). The amount of rainfall over the vegetation period 1997-2007 was 149.6 mm lower and temperature 0.8 ºC higher compared to historical records (1970-2005).

Table 1. Amount of rainfall and average temperature over the vegetation period (April - August) and over the year for the Vinkovci location

Year	Amount of rain fall (mm)		Average temperature ºC	
	Vegetation	Year	Vegetation	Year
1997	384.2	672.8	17.9	11.7
1998	375.4	683.9	18.6	11.3
1999	504.7	867.6	18.7	11.5
2000	153.4	315.2	19.7	12.9
2001	514.2	939.7	17.8	11.4
2002	483.6	682.4	18.7	12.3
2003	105.8	513.6	18.5	11.7
2004	312.8	911.9	20.1	10.9
2005	496.7	859.2	18.0	10.6
2006	461.3	639.7	18.2	11.6
2007	94.1	238.7	19.8	12.9
Average 1997-2007	353.3	665.9	18.7	11.7
Average 1970-2005	502.9	628.5	17.9	11.4

RESULTS AND DISCUSSION

Grain and oil yield, oil content, plant height, head diameter, and incidence of white rot disease varied depending on the year of production (temperature, amount and distribution of rainfall) and hybrid (Table 2). Plant height and head diameter were mostly influenced by the hybrid. In the course of the breeding program, head diameter was reduced from 22.9 cm (1997) to close to 18 cm. The objective was to reach a lower stem with smaller head diameter to facilitate a high crop density and reduced risk of lodging due to head weight, especially in wet years. White rot incidence mostly depended on rainfall and temperatures over the vegetation period. In accordance with this, the lowest disease incidence was determined in very dry years (2003 - 2.1% of infected plants; 2007 - 3.4% of infected plants) and the highest disease incidence in wet and cold years (2005 - 39.6% of infected plants; 2001 - 37.2% of infected plants). The average grain yield was 3 t ha^{-1}. The lowest average grain yield was in 2005 (1.13 t ha^{-1}) and the highest in 2000 (4.56tha^{-1}). The average oil content (1997-2007) was 44.42%. Average oil yield was 1.31 t ha^{-1}, the lowest record in 2005 (0.49 t ha^{-1}) and the highest in 2000 (2.04 t ha^{-1}).

Table 2. Results of research on economic characteristics of sunflower hybrids in macro experiments from 1997 to 2007

Year	No. of hybrids	Plant height (cm)	Head range (cm)	*Sclerotinia sclerotiorum* (%)	Grain moisture (%)	Grain yield (tha^{-1})	Oil content (%DMC^{-1})	Oil yield (tha^{-1})
1997	20	204	22.9	12.5	11.3	2.81	42.05	1.18
1998	17	204	21.1	11.1	10. 6	2.76	44.31	1.26
1999	13	184	19.8	12.2	9.7	2.39	44.17	1.06
2000	20	196	19.4	4.1	7.8	4.56	44.77	2.04
2001	24	184	18.1	37.2	11.8	2.31	44.55	0.96
2002	30	196	19.1	5.6	7.4	2.84	43.64	1.24
2003	20	170	18.7	2.1	8.0	4.35	45.32	2.03
2004	26	185	20.1	9.8	13.2	2.94	43.76	1.20
2005	30	181	19.9	39.6	15.7	1.13	42.46	0.49
2006	24	169	18.2	12.4	12.8	2.97	45.47	1.21
2007	25	198	18.3	3.4	8.7	3.89	48.17	1.75
Average	**23**	**188**	**19.6**	**13.6**	**10.6**	**3.00**	**44.42**	**1.31**
Min	13	169	18.1	2.1	7.4	1.13	42.05	0.49
Max	30	204	22.9	39.6	15.7	4.56	48.17	2.04

CONCLUSIONS

Based on the result of a macro field experiment from 1997 to 2007 in Vinkovci, the following conclusions can be reached:

1. Grain and oil yield of sunflower hybrids was significantly influenced by temperatures and the amount and distribution of rainfall.
2. Weather conditions have less influence on plant height, head diameter and oil content.
3. White rot (*Sclerotinia sclerotiorum*) incidence is significantly influenced by weather conditions (rainfall and temperatures)
4. The breeding program reached important results on creation of new hybrids with increased yield and oil potential.

REFERENCES

Duvnjak, T., M. Krizmanic, M. Vrataric, A. Mijic, A. Sudaric, I. Liovic, M. Bilandzic, and K. Vrandecic. 2005. Sunflower inbred line testing on resistance to *Sclerotinia sclerotiorum*. p. 103-105. In: Proc. 12th Congress of the Mediterranean Phytopathological Union, 11-15 June, Rhodes, Breece.

FAOSTAT. 2002. Food and Agriculture Organization of the UN Database (http://faostat.fao.org/site/567/default.aspx).

Hudec, K. 2006. Influence of seed treatment, temperature and origin of inocula on pathogenicity of *Fusarium* species to wheat and barley seedlings. Cereal Res. Comm. 34:1059-1066.

Hunyadi Borbély, E., J. Csajbók, and M. Lesznyák. 2007. Relations between the yield of sunflower and the characteristics of the crop year. Cereal Res. Comm. 35:285-288.

Krizmanic, M., A. Mijic, I. Liovic, M. Bilandzic, Z. Jurkovic, and R. Sudar. 2004. Realisation of genetic potentials of OS sunflower hybrids in different agroecological conditions. p. 179-180. In: 39th Croatian Symposium on Agriculture, Opatija, Croatia.

Liovic, I., V. Kovacevic, M. Krizmanic, A. Mijic, and B. Simic. 2006. Precipitation influence on edible oil production from sunflower in Croatia. Cereal Res. Comm. 34:573-576.

Schneiter, A.A., and J.F. Miller. 1981. Description of sunflower growth stages. Crop Sci. 21:901-903.

The appropriate technique for collecting and measuring the amount of floral nectar in sunflower (*Helianthus annuus* L.)

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ABSTRACT

The available techniques for collecting and measuring the amount of floral nectar are applicable but often found to be unrepresentative. Centrifugation yields larger samples but they also include nectar that is not actually accessible to insects, the capillary method has been described as unsuitable because of possible damage to the nectary tissue, the method including filter paper is considered to be unreliable because of evaporation and nectar extraction methods including washing are considered limited because the solution may include sugars from plant tissue cells. We have found that capillary tubes with inner diameter of 0.25-0.5mm and outer diameter of 0.5-0.75mm are suitable for nectar collection in sunflower. To determine the amount of nectar, we isolate five inflorescences per sunflower line at the start of flowering and collect the nectar two days after the isolation. The capillary tube is inserted between the style and filaments down to the nectary. After the level of nectar stops rising the next flower is processed. The tubes can be measured on an analytical scale and the amount of nectar is obtained as the weight increase in comparison to the empty tube. Faster determination of floral nectar amount can be provided by using calibrated capillary tubes of a known and uniform inner diameter. The appropriate outer diameter of the capillary tubes reduces the risk of tissue damage and allows more precise collecting so that the capillary method is preferable to others for nectar collecting in sunflower.

Key words: capillary technique – nectar quantity – sunflower

INTRODUCTION

Sunflower is one of the plant species that produces pollen which is too heavy for wind dispersal (Putt, 1940). Even though the cultivated sunflower has a reasonable percentage of self-compatibility it still benefits from insect pollination. One of the major components influencing pollinator choice is certainly the production of nectar, whose amount and quality are often studied.

The nectaries in *Asteraceae* family form on top of the ovary and surround the style base (Mani and Saravanan, 1999), (Fig. 1). The nectar can be accessed for quantification purposes by capillary tubes (Hocking, 1953), volumetric centrifugation (Bosi, 1973), filter paper strips (McKenna and Thomson, 1988), flushing of water into the corolla (Cresswell and Galen, 1991) or floating the flowers inverted in water (Manetas, 2000) depending on flower structure. These techniques were used with a variable success, but the overall conclusion is that no single method can be considered satisfactory for all plant species (Mesquida et all, 1988). The capillary method can be used in sunflower but it is necessary to use capillary tubes of appropriate dimensions. If the outer diameter is too large it is not possible to access the nectar without destroying the surrounding corolla tissue (Fig. 1.) and if the tube is too thin then the intake of nectar is slower and the strong capillary force may make the extraction of nectar from the tubes difficult.

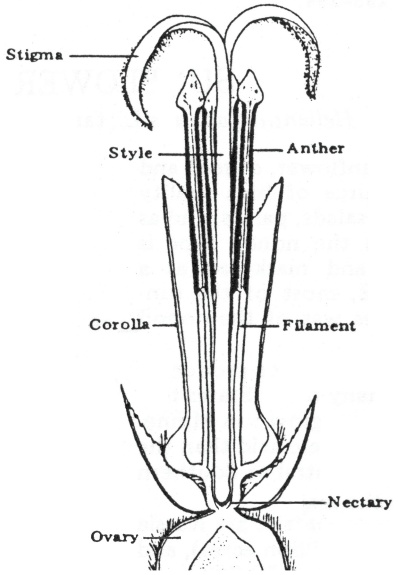

Fig. 1. Longitudinal section of a sunflower disk flower showing the location of the nectary

MATERIALS AND METHODS

We found that capillary tubes with inner diameter of 0.25-0.5mm and outer diameter of 0.5-0.75mm are suitable for nectar collection. The length of the tube then determines the capacity and can be picked for its suitability to the collecting design but should not be smaller than 15 µl.

To determine the amount of nectar, we isolate five inflorescences per sunflower line at the start of flowering with linen bags to prevent insects from collecting nectar. The best moment is when the first two rows of disk flowers have opened. Two days after the isolation, at approximately 8 AM, the inflorescences are cut and taken to the laboratory in a portable refrigerator to minimize evaporation and the change in nectar volume. It is advisable for the transport duration to be as short as possible. The following should be prepared to analyze one inflorescence:

1. Four capillary tubes (previously weighed on an analytical scale) each placed in a separate tube labeled with sample and replication to ease the work of collecting and measuring
2. A clean vegeglass (previously weighed on an analytical scale)
3. A clean HPLC vial with sample label on it filled with 1 ml mixture of AcCN:H_2O in a ratio of 75:25
4. A plastic dish with sample label on it for deep freezing

Four groups of five analyzed flowers are equally far from each other on an inflorescence. We collect nectar from 5 fully opened disk flowers with one non calibrated capillary tube. The tube is inserted between the style and filaments down to the nectary (Fig. 1.). After the level of nectar stops rising the next flower is processed. When a total of 20 flowers are finished, the tubes can be measured on an analytical scale and the amount of nectar is obtained as the weight increase in comparison to the empty tube. Faster determination of nectar amount in flowers can be provided by using calibrated tubes (capillary tubes with a uniform known inner diameter) for nectar collecting, in which case the height of nectar in tubes can be correlated with the nectar volume. This method is suitable when it is necessary to determine the amount of nectar in field conditions, without cutting the sunflower head and taking it to the lab.

The next step in method developing is a qualitative and quantitative HPLC analysis of nectar extracted from a single inflorescence as a collection from 20 disk flowers. For this purpose, ten disk flowers are also pulled off with tweezers, put in a glass and weighed on an analytical scale to obtain the information about the flower mass and possible correlation with nectar production.

The nectar collected in capillary tubes, after weight measurement, can also be kept for subsequent analysis. The contents of all capillary tubes from a single inflorescence are transferred into a HPLC vial (2 ml) filled with 1 ml mixture of AcCN:H_2O in a ratio of 75:25 and placed in a refrigerator for

subsequent HPLC nectar quality analysis. Twenty flowers are pulled out of the disk with tweezers, frozen in liquid nitrogen and then kept at -72ºC.

DISCUSSION

The rest of the techniques cited are applicable but often unrepresentative. Centrifugation yields larger quantities but they also include nectar that is not actually accessible to insects and modified chemical composition due to tissue lesion (Mesquida et al., 1988). The method including filter paper is considered to be unreliable because of evaporation (Livtzieva, 1954). Nectar extraction methods including washing are considered limited because the solution may include sugars from plant tissue cells (Kenoyer, 1917).

A combination of capillary method and filter paper can be used so that the nectar is extracted with capillary tubes and than ejected on to a filter paper, which is measured for total nectar. After the evaporation has finished the amount of sugars is obtained as a difference between wet and dry filter paper.

The capillary method has been used on sunflower (Pham-Delegue et al., 1985) but it has also been described as unsuitable for collecting nectar amounts less than 1 µl and to cause damage to the nectary tissue (McKenna, 1988). The appropriate outer diameter of the capillary tubes reduces the risk of tissue damage and allows more precise collecting so that the capillary method is preferable to others for sunflower nectar collecting.

REFERENCES

Bosi, G. 1973. Méthode rapide pour la détermination par chromatographie en phase gazeuse des glucides du nectar: technique de prélèvement du nectar et de préparation des ethers trimethylsilyles en présence d`eau. Apidologie 4:57-64.

Cresswell, J.E., and C. Galen. 1991. Frequency-dependent selection and adaptive surfaces for floral character combinations: the pollination of *Polemonium viscosum*. Amer. Natur. 138:1342-1353.

Hocking, B. 1953. The intrinsic range and speed of flight of insects. Trans. Royal Entomol. Soc. London 104:223-345.

Kenoyer, L.A. 1917. Environmental influences on nectar secretion. Bot. Gaz. 63:249-365.

Livtzieva, E.K. 1954. Methods used in nectar secretion investigations. (In Russian). Pchelovodstvo, 31(11):33-39.

Mani, M.S., and J.M. Saravanan. 1999. Pollination ecology and evolution in Compositae (Asteraceae). Enfield, NH, USA: Science Publishers Inc.

McKenna, M.A., and J.D. Thomson. 1988. A technique for sampling and measuring small amounts of floral nectar. Ecology 69:1306-1307.

Mesquida, J., R. Marilleau, and M. Pham-Delegue. 1988. A study of rapeseed (*Brassica napus* L. var. oleifera Metzger) flower nectar secretions. Apidologie 19:307-318.

Pham-Delegue, M.H., C. Masson, A. Bonjean, P. Etievant, and R. Marilleau. 1985. Etude des paramètres chimiques impliques dans la pollinisation du tournesol par les abeilles en production de semences hybrides. p. 559-564. In: Proc. 11th Int. Sunflower Conf., 10-13 March, Mar Del Plata, Argentina.

Putt, E.D. 1940. Observations on morphological characters and flowering processes in sunflower (*Helianthus annuus* L.). Sci. Agric. 25:185-190.

Sunflower and peanut emergence: initial development under sugarcane mulch

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ABSTRACT

The research aimed to evaluate the effect of residual sugarcane mulch on sunflower and peanut plant emergence and initial development. Containers of 4.0 L were disposed in a randomized experimental block design, with four replications, in a factorial arrangement of five mulch amounts and three cultivars of each crop. The mulch treatments consisted of four increasing amounts (5, 10, 15 and 20 t ha^{-1}) and a control with no mulch. The sunflower cultivars were the varieties IAC-iarama and Catissol and the hybrid H-358; the peanut cultivars were the runner-type varieties IAC-Caiapó and Runner 88, and the erect type Tatu. The speed emergence index and final emergence percentage, the plant height and shoot dry mass were evaluated. The presence of different levels of sugarcane mulch negatively influenced the emergence and initial plant development mainly in peanut but also in sunflower. The negative effects were particularly stronger for the runner-type cultivars of peanut, while cultivar Tatu was less influenced by the mulch thickness.

Key-words: *Arachis hypogaea* L. - *Helianthus annuus* L – mulch - oilcrops - seedling development.

INTRODUCTION

The sugarcane crop expansion is a reality in Brazil due mainly to the bio-energy or renewable energy concern. This expansion is now associated with the biodiesel agriculture chain, favored by the possibility of sugarcane rotation with oil crops. In Sao Paulo state, sugarcane rotation with peanut crop is a reality (Borsari Filho, 2006), but there is also a great potential for sunflower crop. There is increased interest in the oil crops that can be used for biodiesel production (MAPA, 2006). Thus, together with the sunflower rotation benefits on sugarcane of about 50% in the sugar yield (Ambrosano et al., 2005), the energetic benefits of fuel association are expected, due to the use of the biodiesel produced by the sugarcane chain in the agriculture and transportation vehicles from the sugar mills and farms.

With the implementation of laws that prohibited sugarcane burning, the harvest leaves a large amount of mulch on the soil surface. This presence of mulch can modify physical soil characteristics like water content and thermic extent (Vasconcelos, 2002), which contribute to soil conservation but, on the other hand, can cause problems to crop management (Furlani Neto et al., 1997).

The mulch layer in sugarcane areas can be as high as 10cm in thickness on the soil surface, which corresponds to 20 t ha^{-1} of residues; these form a physical barrier that reduces the light incidence and modifies the local climate conditions (Velini and Negrisoli, 2000). Those alterations are able to affect the emergence and plant development due to the influence on dormancy and seed emergence processes (Trezzi and Vidal, 2004). With the increased use of oilcrops in the sugarcane rotation, the study of the influence of mulch on different crops is a necessity. In this sense, the aim of the present research was to evaluate the influence of sugarcane residual mulch on the emergence and initial development of different sunflower and peanut cultivars.

MATERIALS AND METHODS

A greenhouse experiment was done at Ecophysiology and Biophysics Center of Instituto Agronômico (IAC), Campinas, SP, Brazil. Plastic containers of 4.0 L capacity, filled with 2.7 L of an argilous sieved soil, with the following chemical composition: pH ($CaCl_2$) = 5.2, organic matter = 25 g dm^{-3}, P (resin) = 1 mg dm^{-3}, K = 0.9, Ca = 23, Mg = 6, H+Al = 28, SB=29.9, CTC=57.7, expressed in $mmol_c$ dm^{-3} and V = 52% were used. The soil was amended according to Van Raij et al. (1997).

Before sowing, seeds of sunflower and peanut were physiologically characterized by determining the germination, emergence (MARA, 1992), and speed emergence index according to Maguire (1962).

The treatments were arranged in a factorial scheme (5 x 3), in a randomized block design, with four replications, combining five amounts of sugarcane mulch (0, 5, 10, 15 e 20 t ha^{-1}) and three cultivars of each crop, separately. For sunflower we used the open pollinated cultivars IAC-iarama and Catissol and the hybrid Helio-358, while for peanut the runner type varieties IAC-Caiapó and Runner 88, and the erect type Tatu were used.

In each pot, ten seeds previously treated with Thiram 0.2% were sown at 3cm deep, followed by the addition of the mulch of sugarcane cultivar SP 803280, which was cut into small pieces before scattering it on the soil surface. The layer thicknesses in the containers were 4, 6, 9, and 10 cm, which corresponded to 5, 10, 15, and 20 t ha^{-1}, respectively.

The final plant emergence (EM) was evaluated 15 days after sowing (MARA, 1992). For the speed emergence index (SEI), the number of normal seedling was counted daily up to a constant number, according to Maguire (1962). The initial plant development was evaluated 30 days after sowing by harvesting the plants and measuring the plant height (PH) and, after drying the aerial part in an oven at 65°C through constant mass, the shoot dry mass (SDM) was obtained.

The data were analyzed using the variance analysis with F test. The data in percentage were transformed to arc $\sin\sqrt{x/100}$ before the statistical analysis, although the original means are reported in the tables and figures. The Duncan test was used for the comparison of means among cultivars. For the mulch analysis, a regression analysis was utilized.

RESULTS AND DISCUSSION

The initial characterization of the physiological potential for sunflower and peanut cultivars (Table 1) showed that all cultivars fitted the commercialization patterns. The three sunflower cultivars presented the same physiological level while the peanut cultivar IAC-Caiapó showed a slightly lower level. Those results indicated the adequate physiological quality of all cultivars to be studied.

Table 1. Characterization of physiological potential for the seeds of sunflower and peanut cultivars in relation to initial germination level (G), final emergence (EM), and speed emergence index (SEI).

	G (%)	EM (%)	SEI
Sunflower			
IAC-iarama	99a[1]	100a	1.66a
Catissol	95a	100a	1.74a
Helio 358	98a	100a	1.51a
Peanut			
IAC-Caiapó	88b	86a	0.95a
Runner 886	92a	83a	0.87a
Tatu	97a	84a	1.04a

[1]Means followed by the same letter in column, for each specie, did not differ from Duncan ´s test at 5% probability

For sunflower there were significant interactions between mulch amount and cultivars only for shoot dry mass (SDM), while for peanut the interactions were significant only for seedling emergence (EM). There were almost no variations in the shoot dry mass of any of the sunflower cultivars with the increasing amount of mulch on soil surface (Table 2). The higher data presented by Helio-358 could be associated with the genetic vigour of the hybrid.

Table 2. Means of sunflower shoot dry mass affected by mulch (M) and cultivars (C). Campinas-SP, Brazil

Cultivars	Sugarcane mulch on the soil surface (t ha^{-1})					Adjustment equation and coefficient of determination (%)	
	0	5	10	15	20		
Sunflower –	Shoot dry mass – SDM (g)						
IAC-iarama	1.6b[1]	1.5b	1.6b	1.4b	1.4b	$Y = 1.59 - 0.01x$	r^2=69
Catissol	1.5b	1.4b	1.4b	1.4b	1.7b	$Y = 1.51 - 0.03x + 0.002x^2$	r^2=86
Helio-358	2.0a	2.1a	1.9a	2.0a	1.9a	not significant	
M x C	0.05*						

[1]Means followed by the same letter in column, for each species, did not differ from Duncan ´s test at 5% probability; *Significant at P=0.05.

The seedling emergence of all peanut cultivars was negatively affected by the mulch presence (Table 3). The cultivar Tatu was the least affected by the mulch on the soil surface, which indicates that this cultivar would be the one most indicated for the ploughed out sugarcane areas.

Table 3. Means of peanut seedling emergence affected by mulch (M) and cultivars (C). Campinas-SP, Brazil.

Cultivars	Sugarcane mulch on the soil surface (t ha^{-1})					Adjustment equation and coefficient of determination (%)	
	0	5	10	15	20		
Peanut -	Seedling emergence - EM (%)						
IAC-Caiapó	64.6a[1]	37.8b	23.2a	8.4b	2.6b	Y = 51.04 – 2.20x	r^2=99
Runner 886	60.8a	27.0b	16.5a	7.2b	7.3b	Y = 50.40 – 3.91x	r^2=99
Tatu	60.5a	57.1a	25.9a	48.4a	33,9a	Y = 51.22 . 0.97^x	r^2=68
M x C	442.11**						

[1]Means followed by the same letter in column, for each species, did not differ from Duncan ´s test at 5% probability
**Significant at P=0.01.

The isolated effect of mulch amount and cultivars interfered with the evaluated parameters both for sunflower and peanut. The sunflower seedling emergency percentage (EM) and the speed emergency index (SEI) were directly affected by the mulch increasing on the soil surface (Fig. 1). The greater seedling emergence reduction occurred with the introduction of 5 t ha^{-1} (4 cm) of mulch on soil surface in relation to the tester with no mulch at all; between 15 t ha^{-1} and 20 t ha^{-1} the values did not change. The SEI followed the same pattern of reduction presented by seedling emergence (Fig. 1B). According to Teasdale (1996), the mulch deposition on soil surface can cause chemical, physical and biological alterations in the environment and, depending on the plant species, it can affect the seedling emergence and plant development. Mulch deposition is responsible for delay in the soil heat absorption, which interferes in the thermic difference between day and night, leading to a delay in the speed emergence index, in same cases, like what happened in the present research.

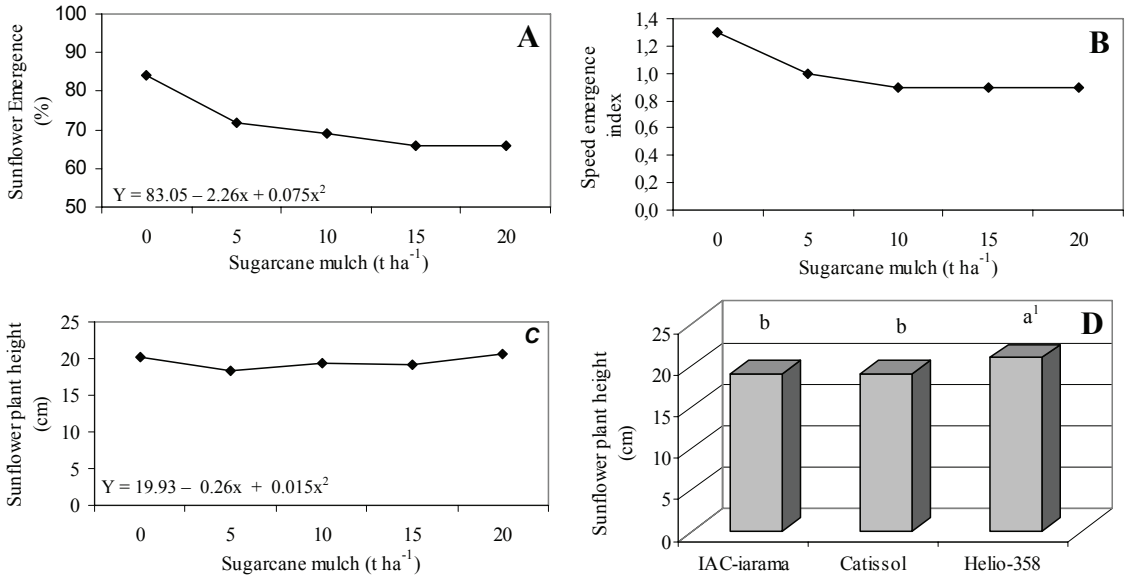

Fig. 1. Sunflower percentage of emergence (A), speed emergence index (B) and plant height (C) affected by the presence of sugarcane mulch on the soil. Sunflower plant height in the studied cultivars (D). Campinas-SP, Brazil.

Differently from other parameters, the plant height of sunflower (Fig. 1C) showed a small increase with the mulch thickness increase. This was an expected behavior since with the increasing of mulch thickness the seedling elongates in the sunlight direction (Carvalho and Nakagawa, 2000). In field conditions, plant shading leads to less biomass accumulation and the plants become more sensitive to lodging (Correia and Durigan, 2004). There were also differences between cultivars (Fig. 1D), with Helio-358 presenting the highest values for plant height in comparison to IAC-iarama and Catissol, which did not differ between each other. The better performance of Helio-358 could be related to its genetic vigour. Both seedling emergence and SEI did not vary between cultivars.

In relation to peanut crop (Fig. 2), there was a significant negative effect of the sugarcane mulch on SEI (Fig. 3A), plant height (Fig. 2C), and dry shoot mass (Fig. 2E). SEI was negatively influenced by the thickness of the mulch layer; with mulch 10-cm thick, the SEI was 78% lower. In peanut, the mulch negative effect was much more pronounced than in sunflower, probably due to the higher temperature necessary for seedling emergence because this is a tropical species whose center of origin is Brazil. Also, the DSM was reduced with the mulch layer increasing up to 10t ha^{-1}, being constant after this thickness. Like sunflower, the peanut plant height was positively influenced by increasing the mulch layer, with the elongation of the plants, already described by Carvalho and Nakagawa (2000) for seedlings under light deficits.

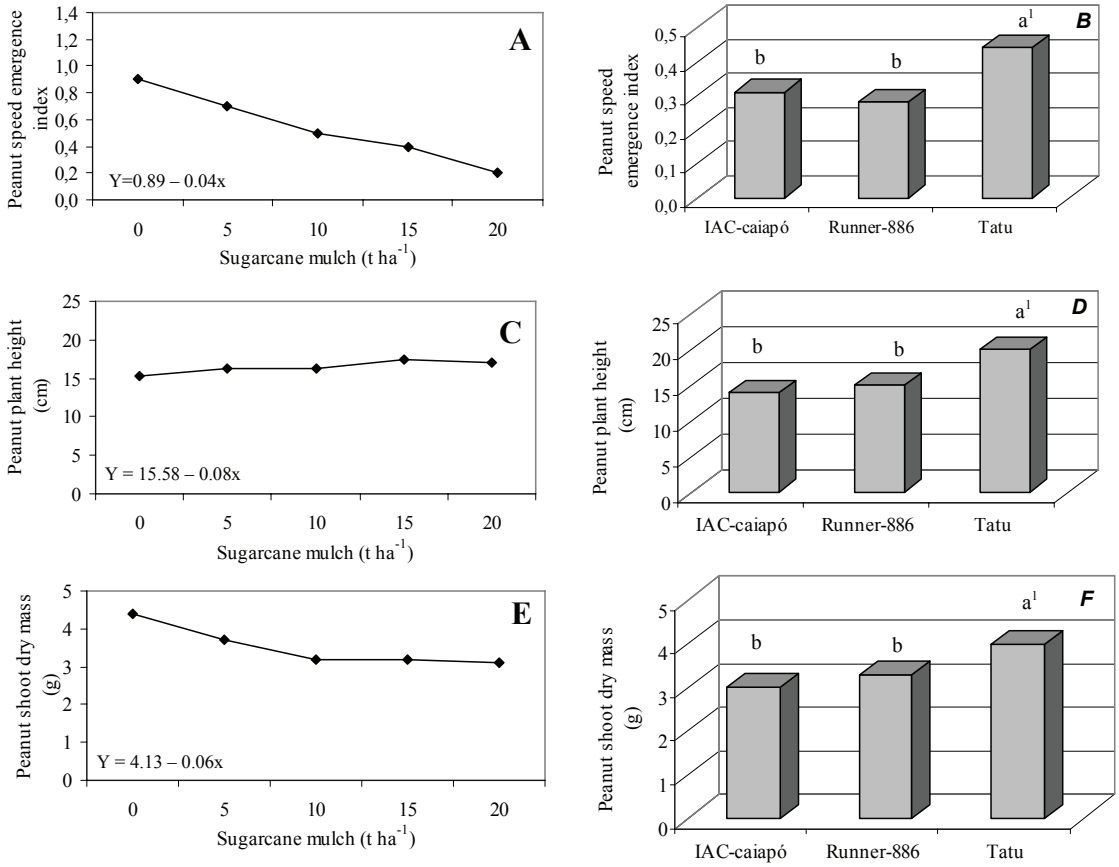

Fig. 2. Means of peanut speed emergence index (A and B), plant height (C and D) and shoot dry mass (E and F) affected respectively by sugarcane mulch (A, C and E) and cultivars (B, D, and F). Campinas-SP, Brazil.

The isolated effect of cultivars is also presented in Fig. 2. The cv. Tatu presented the best performance in comparison to IAC-caiapó and Runner-886 for SEI (Fig. 2B), HP (Fig. 2D), and DSM (Fig. 2F). This superiority could be related to its growing habit classified as erect, while IAC-Caiapó and Runner 886 are classified as runner type. The erect plants have a tendency to grow up faster than the

runner type. In the present research work, cultivars with different growing habits were evaluated in order to verify if the erect cultivars would perform better initially in comparison to the runner type which has a tendency to be more productive than the erect cultivar. In the past, the erect type was the one most cultivated in Brazil and it will likely become an option for the ploughed out sugarcane areas with the mechanical harvest obligation.

The research showed that both emergence and initial sunflower development was less negatively influenced by the presence of sugarcane mulch than peanuts. So, in the first approach, sunflower seems to be under better conditions for giving a good performance in areas with high levels of sugarcane mulch. Otherwise, it would be of interest to carry out field evaluations in the future.

CONCLUSION

Under greenhouse conditions it is possible to conclude that the presence of different levels of sugarcane mulch on the soil surface can negatively influence both sunflower and peanut emergence and initial plant development. The negative effects are stronger for peanut cultivars, especially for the runner type; the cultivar Tatu was less influenced by the mulch thickness.

REFERENCES

Ambrosano, E.J., N. Guirado, M.R.G. Ungaro, H. Cantarella, P.C.D. Mendes, F. Rossi, G.M.B. Ambrosano, R.H. Sakai, and E.A. Schammass. 2005. Vantagens da utilização da rotação com girassol e outras leguminosas em áreas de reforma de canavial em Piracicaba, São Paulo. p. 92-94. In: Documentos Embrapa Soja n.261, 2005, Londrina, Brazil.

Borsari Filho, S. 2006. Potencial da cultura do amendoim como fonte de matéria-prima para o Programa Nacional de Produção e Uso de Biodiesel. p. 43-55. In: Câmara, G.M.S.; Heiffig, L.S. Agronegócios de plantas oleaginosas – matérias-primas para biodiesel. FEALQ – Piracicaba, Brazil.

Carvalho, N.M., and J. Nakagawa. 2000. Sementes: ciência, tecnologia e produção. FUNEP, Jaboticabal, Brazil. 650 p.

Correia, N.M., and J.C. Durigan. 2004 Emergência de plantas daninhas em solo coberto com palha de cana-de-açúcar. Planta Daninha 22:11-17.

Furlani Neto, V.L., T.C. Ripoli, and N.A. Vila Nova. 1997. Biomassa de cana-de-açúcar: energia contida no palhiço remanescente de colheita mecânica. Stab – Açúcar, Álcool e Subprodutos 15:24-27.

Maguire, J.D. 1962. Speed of germination aid in selection and evaluation for seedling and vigor. Crop Sci. 2:176-177.

MAPA. 2006. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Produção de Agroenergia Plano Nacional de Agroenergia 2011-2006. 2 ed.rev. Embrapa Informação Tecnológica, Brasília, DF, Brazil.

MARA. 1992. Ministério da Agricultura e Reforma Agrária. Regras para análise de sementes. Agiplan, Brasília, Brazil, 365 p.

Teasdale, J.R. 1996. Contribution of cover crops to weed management in sustainable agricultural systems. J. Prod. Agric. 9:475-479.

Trezzi, M.M., and R.A. Vidal. 2004. Potencial de utilização de cobertura vegetal de sorgo e milheto na supressão de plantas daninhas em condição de campo: II – Efeitos da cobertura morta. Planta Daninha 22:1-10.

Van Raij, B., H. Cantarella, J.A. Quaggio, and A.M.C. Furlani. 1997. Recomendações de adubação e calagem para o estado de São Paulo, 2ª ed. Instituto Agronômico, Fundação IAC, Campinas, Brazil. 285 p.

Vasconcelos, A.C.M. 2002. Desenvolvimento do sistema radicular da parte aérea de socas de cana-de-açúcar sob dois sistemas de colheita: crua mecanizada e queimada manual. PhD Thesis, Universidade Estadual Paulista, Jaboticabal, Brazil, 140 p.

Velini, E.D., and E. Negrisoli. 2000. Controle de plantas daninhas em cana-crua. p. 148-164. In: Congresso Brasileiro da Ciência das Plantas Daninhas, Foz Do Iguaçú, Brazil.

La agricultura de conservación como sistema viable para combatir el jopo en el girasol

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RESUMEN

Con objeto de estudiar la influencia que tienen dos sistemas de manejo de suelo (laboreo y no laboreo) sobre el desarrollo y propagación de las infestaciones de jopo en el cultivo del girasol y su producción, se ha llevado a cabo durante tres años un experimento utilizando tres variedades de girasol (Olimpia, Vanko y Peredovik). Los resultados obtenidos muestran que tanto el número de plantas de girasol atacadas por jopo, como el número de jopos por planta son significativamente menores en aquellas parcelas con el tratamiento de no laboreo. Estas diferencias se suavizan cuando se repite el cultivo de girasol más de dos años en la misma parcela. La producción de las tres variedades resultó siempre mayor en los tratamientos de no laboreo. Sin embargo la producción de la variedad tolerante al jopo (Olimpia) no mostró diferencias significativas con los tratamientos de laboreo.

Palabras clave: agricultura de conservación - girasol - jopo- manejo del suelo - susceptibilidad – tolerancia.

ABSTRACT

A study has been carried out to assess the influence of soil management systems on the occurrence and propagation of broomrape in the sunflower crop under dry-farming. Three sunflower varieties, Olimpia, Vanko and Peredovik have been cultivated during three years in a heavy clay soil of Andalusia under direct drilling and conventional tillage. The results of the last two years indicate that both the number of infested plants and the density of parasites per plant are significantly reduced in the direct drilling system with respect to the conventional tillage system. The differences between treatments are reduced when the sunflower crop is maintained during the three years in the same plot. The highest sunflower yield was obtained under the direct drilling system. Nevertheless, yield differences were not significant with the broomrape-resistant sunflower variety Olimpia.

Keywords: conservation agriculture - soil management - sunflower cropping - susceptibility - tolerance

INTRODUCCIÓN

El girasol (*Helianthus annuus* L.) es una planta oleaginosa cultivada en España desde la década de los 60 y que se caracteriza por su adaptabilidad a una gran diversidad de medios ambientes. Es un cultivo de primavera-verano, y con rendimientos bastante aceptables aunque dependen en gran medida de las temperaturas y pluviometria en el periodo que va de floración a maduración.

El jopo (*Orobanche cumana* Wallr.) es una planta parásita que ataca el sistema radicular del girasol y depende completamente de éste para su nutrición y desarrollo, siendo tal su agresividad que ha puesto en peligro la supervivencia del cultivo en amplias zonas de Andalucía, causando siempre perdidas económicas de importancia.

Las plantas de jopo florecen y maduran a la misma vez que el girasol. Los jopos alcanzan una altura variable en su único tallo que tiene escamas y brácteas y en cuyas axilas se forman flores coloreadas, que dan lugar a cápsulas que al madurar liberan miles de pequeñas semillas.

Las semillas de jopo germinan en el suelo en respuesta a los exudados radicales del girasol y los tubos germinativos penetran en las raíces del girasol estableciendo conexiones vasculares. Las células de esta zona responden intensificando su división con lo que la parte atacada de la raíz aumenta de tamaño. Estas conexiones vasculares entre las raíces del girasol y el jopo permite al parásito quedar integrado en la

fisiología de su huésped, tomando de éste nutrientes y agua por lo que reduce su vitalidad y su capacidad productiva (Melero y Alonso, 1988).

Las plantas atacadas forman capítulos pequeños, y con muchas de las semillas vacías. Si el ataque es muy intenso, las plantas parasitadas se marchitan ya que se incrementa la transpiración y disminuyen las reacciones de oxi-reducción.

Las pérdidas que ocasiona el jopo en el cultivo del girasol varían según la severidad de la infección y ésta a su vez depende de la cantidad de semilla de jopo que se encuentra en el suelo y del nivel de susceptibilidad o resistencia genética de la variedad. En variedades muy susceptibles, la pérdida de cosecha puede ser total ya que la planta no llega incluso a florecer.

El cultivar un híbrido con resistencia al jopo es el medio más eficaz y económico para prevenir o controlar la infestación.

No obstante, la continua aparición de nuevas razas de jopo del girasol, cada vez más virulentas, está poniendo en evidencia la vía de la resistencia genética ya que cuando se obtienen nuevos genes de resistencia para razas inéditas, no tardan en aparecer otras razas que vencen la nueva resistencia introducida por aquellos.

La posibilidad de utilizar material vegetal resistente a un herbicida, cuya resistencia no es de origen transgénico, aporta una nueva vía de lucha contra el jopo del girasol.

Adicionalmente se puede recurrir a los sistemas de manejo del suelo. Considerando que con la Agricultura de conservación (siembra directa) el lecho de siembra permanece casi intacto, las semillas de jopo tendrán más dificultades en alcanzar niveles más profundos del suelo para localizar las raíces del girasol. Por ello se ha planteado este experimento para estudiar la influencia que la agricultura de conservación y más concretamente la siembra directa, ejerce en el desarrollo y expansión de las nuevas razas de jopo, así como en el rendimiento del cultivo de girasol.

MATERIALES Y MÉTODOS

El estudio se ha realizado en la Estación Experimental de Tomejil, perteneciente al IFAPA Centro Las Torres- Tomejil en la provincia de Sevilla. Las coordenadas del punto central de la finca son 37º 24´ 07´´ N y 05º 35´ 10´´ W, localizada en la Vega de Carmona. El suelo es muy arcilloso, bujeo en la denominación local, clasificado como Chromic Haploxerent en el sistema de taxonomía del USDA (Ordóñez y col. 2007). Por su elevado contenido en arcilla, superior al 60%, la mayor parte expansible, el suelo retiene el agua durante la estación seca, lo que le hace adecuados para los cultivos de primavera (Giráldez y González, 1995).

El ensayo ha consistido en la prueba de tres variedades de girasol bajo dos regímenes diferentes de manejo de suelo: laboreo tradicional y siembra directa. Se ha realizado durante las campañas agrícolas 2005/06 y 2006/07. El experimento se comenzó en la campaña 2004/05, pero no se obtuvieron resultados ya que apenas aparecieron plantas de jopo en ninguna variedad y en ningún tratamiento.

Este ensayo se incluye dentro de los ensayos que realiza la Red Andaluza de Experimentación Agraria (RAEA) de girasol y cuyos resultados se publican anualmente en las revista serie RAEA y están disponibles en la dirección www.ifapa.cice.junta-andalucia.es.

Para la realización del ensayo se han usado dos híbridos de girasol: Olimpia (tolerante a la raza F de jopo y con rendimientos muy regulares en años anteriores, según los resultados de los ensayos de la RAEA de girasol) y Vanko (muy susceptible a la raza F de jopo, pero con unas producciones muy altas en zonas sin infestaciones del parásito, según las fuentes citadas anteriormente), y una variedad población Peredovik, adaptable a diversos ambientes y susceptible a la raza F de jopo (García Ruiz, 2003, 2004 y 2005).

La preparación del terreno en el ensayo con labor, en ambos años, consistió en un pase de chisel en el mes de septiembre del año anterior, un pase de cultivador en enero, un pase de vibrocultivador en marzo para incorporar el herbicida (Trifluralina 1,5 l/ha) y a continuación un pase de rulo.

En el ensayo de siembra directa se aplicó un tratamiento de 0,5 L/ha de glifosato + 0,5 L/ha de MCPA en presiembra.

La siembra de ambos ensayos se realizó con una sembradora de experiencias a alta densidad. La semilla se depositó a chorrillo y posteriormente se realizó un aclare manual (cuando las plantas tenían dos pares de hojas verdaderas) dejándose 4 plantas por metro lineal. En el momento de la siembra se incorporo junto con la semilla un insecticida de suelo (Clorpirifos 5%).

La parcela elemental estaba formada por cuatro líneas de siembra de 10 m de longitud y 0.70 m de separación entre ellas. El diseño experimental fue de bloques al azar con 8 repeticiones en dos sistemas de cultivos (laboreo y no laboreo).

El análisis conjunto de los resultados de los dos años de ensayos se ha realizado como el de un experimento factorial de bloques al azar combinado con años (Mcintosh, 1983).

La campaña agrícola 2006/07, se ha caracterizado por una precipitación abundante, 560mm concentrada durante los meses de invierno y primavera (Fig. 1), especialmente en el mes de mayo, que con 123 mm, representa el 23% del total anual, lo que favorece el desarrollo y producción del cultivo del girasol.

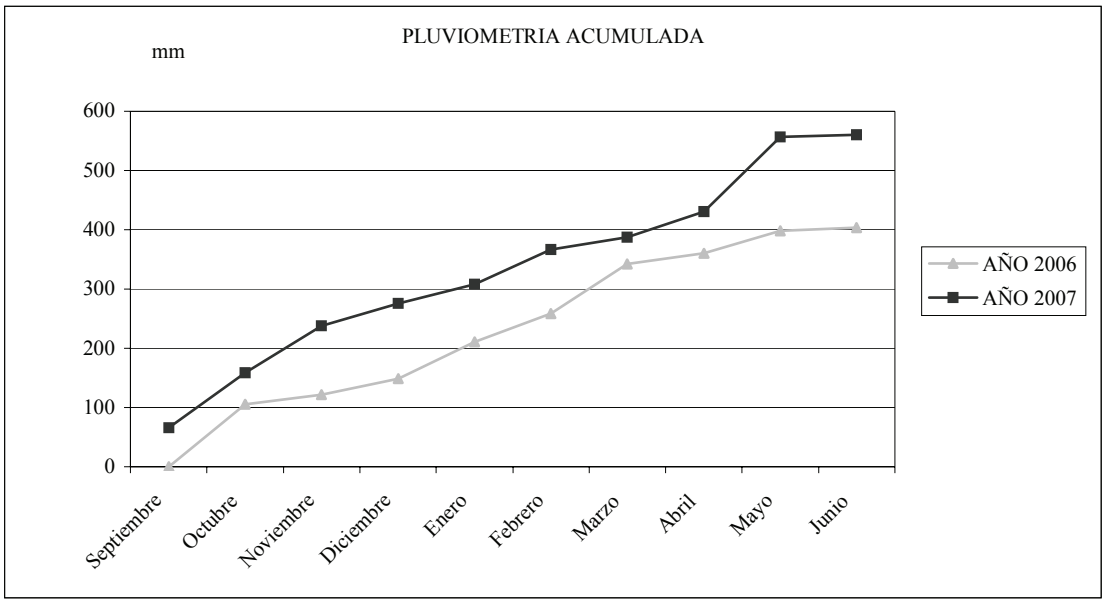

Fig. 1. Distribución de la pluviometría en los años agrícolas 2005/06 y 2006/07.

RESULTADOS Y DISCUSIÓN

La Fig. 2 representa la evolución en el porcentaje de plantas atacadas por jopo para las tres variedades, los dos sistemas de manejo de suelo y las dos campañas agrícolas consideradas en el experimento.

En dicha figura se puede apreciar, independientemente de la variedad y del sistema de manejo del suelo utilizado, un aumento significativo en el porcentaje de plantas infestadas por jopo en el año 2007 y, de forma especial en el tratamiento de no laboreo, con respecto a este mismo tratamiento en el año 2006.

En el sistema de laboreo tradicional, los aumentos han sido muy importantes, aunque la variedad Vanko ya presentaba en el año 2006 un 90% de plantas atacadas, pero Peredovik ha pasado del 56 al 97,5%(un aumento del 74%) y Olimpia ha pasado del 2.5 al 9% (lo que representa un incremento del 261%).

En el sistema de siembra directa, el incremento de plantas infestadas en el año 2007 ha sido muy alto alcanzándose cifras muy parecidas a las obtenidas con el sistema de laboreo tradicional. La variedad Peredovik ha pasado a tener del 23 al 89% de plantas infestadas, Vanko de 25 al 100% y Olimpia del 0 al 8%.

Se observa como en el primer año de ensayo, el factor no laboreo evita el aumento del número de plantas atacadas por jopo, manifestándose en la variedad Olimpia con 0 plantas y en Vanko con una disminución del 65%. Sin embargo considerando los dos años en conjunto se observa, que en el segundo año, además de existir un aumento significativo del porcentaje de plantas con jopo, de manera especial en las variedades susceptibles, desaparecen las diferencias entre los dos sistemas de manejo de suelo observada en el primer año. Esto se podría deber a un aumento considerable del inóculo de jopo en el suelo por haber mantenido el cultivo continuado de girasol durante tres años.

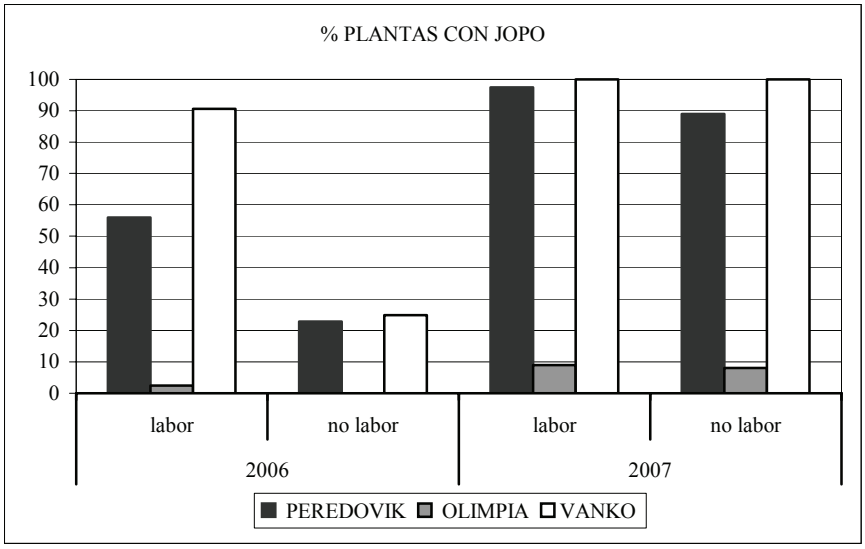

Fig. 2. Evolución en los dos años del porcentaje de plantas de girasol atacadas por jopo para las tres variedades y los dos sistemas de manejo del suelo.

La Fig. 3, representa el número de jopos por planta en cada una de las tres variedades, los dos años de ensayos y para los dos sistemas de manejo de suelo. En ella se puede apreciar cómo el mayor número de jopos por planta en ambos años se produce en el sistema de laboreo tradicional.

Tanto en el primer como en el segundo año al cambiar del sistema de laboreo tradicional a siembra directa se produce un drástico descenso en el número de jopos por planta, un 84, 91 y 100 % para las variedades Peredovik, Vanko y Olimpia, respectivamente, en el año 2006, y un 63, 49 y 48% en el año 2007.

Durante el año 2007 se aprecia un aumento considerable en el número de jopos por planta en ambos sistemas de manejo del suelo (Fig. 3).

La siembra durante tres años consecutivos de girasol en la misma parcela de ensayo ha producido una mayor cantidad de inóculo en el suelo, lo que ha podido tener incidencia no sólo en el número de plantas con jopo sino también en el número de jopos por planta.

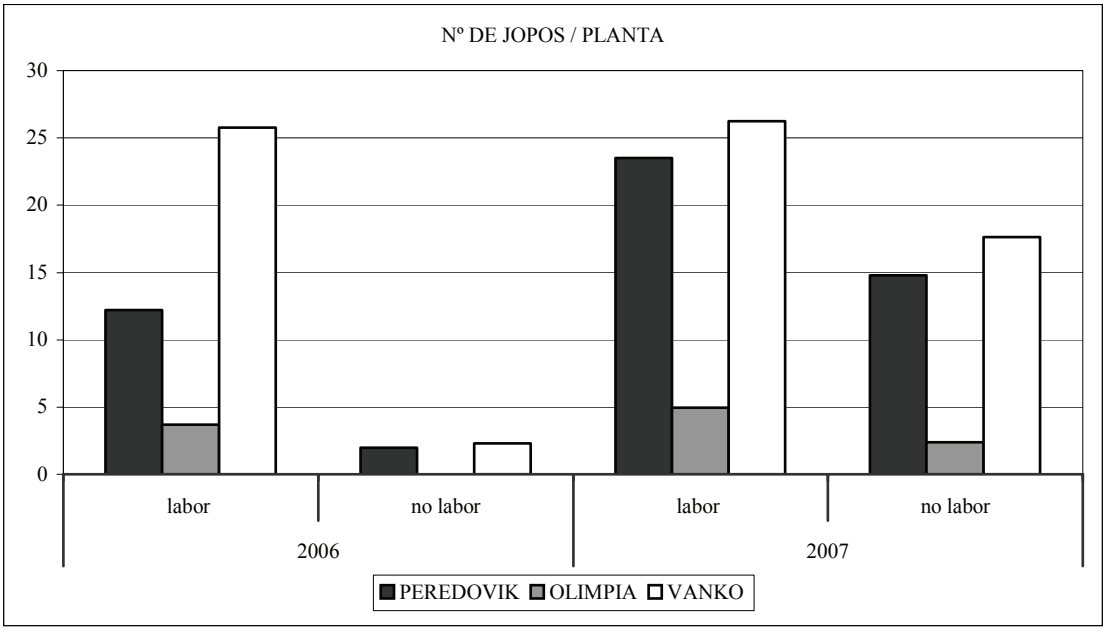

Fig. 3. Evolución del número de jopos por planta para las tres variedades y los dos sistemas de manejo del suelo.

El análisis del rendimiento medio obtenido por cada variedad en los dos años de ensayos y en los dos sistemas de manejo del suelo (Fig. 4), muestra que la siembra directa mejora la producción media en todos los casos, sin diferencias significativas en la variedad Olimpia y con diferencias significativas en la producción en las variedades Peredovik y Vanko.

Los buenos resultados de la variedad Olimpia, pueden ser debidos, por un lado a que el porcentaje de plantas afectadas por el jopo es menor en el tratamiento de siembra directa que en el tratamiento de laboreo tradicional, lo que explicaría su mayor producción en este tratamiento, y por otro a su tolerancia al jopo en suelos con altas infestaciones donde variedades susceptibles ven muy mermada su producción.

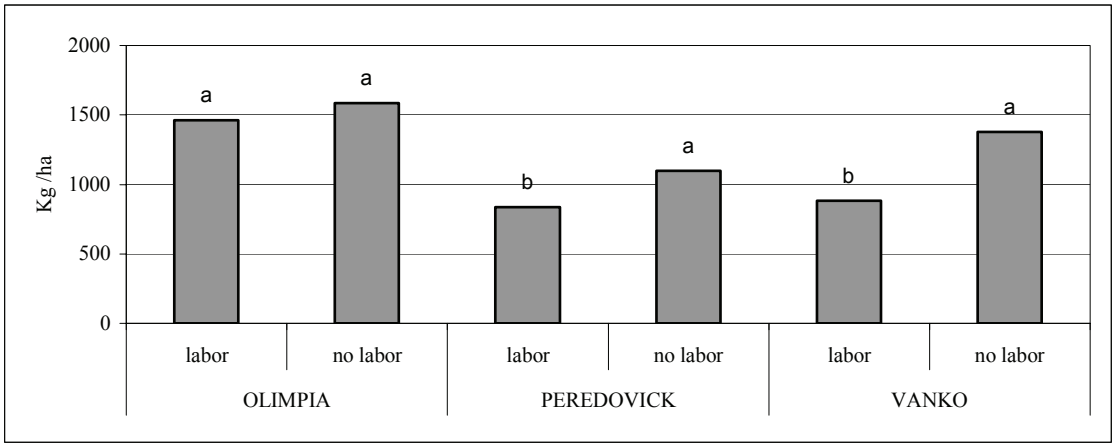

Fig. 4. Rendimiento medio en los dos años de las variedades usadas en el ensayo según el sistema de manejo del suelo.

El sistema de siembra directa es más efectivo reduciendo la infestación con variedades no tolerantes o susceptibles, ya que al eliminar la redistribución del suelo, supone una barrera para la incorporación del jopo a los horizontes inferiores donde se desarrollan las raíces del girasol.

En el análisis de los rendimientos medios de los dos años (Fig. 5), se observa que las tres variedades, independientemente del sistema de manejo de suelo utilizado, han disminuido su producción en el año 2007 de forma significativa con respecto a las obtenidas en el año 2006. Así, Olimpia ha disminuido su producción en un 19%, Peredovik en un 31% y la variedad Vanko en un 52%.

Esta disminución general de producción parece estar en contradicción con la precipitación del año 2007 (Fig. 1), que ha sido superior en 150 mm a la registrada en el año 2006.

Una posible explicación es la reiteración del monocultivo de girasol durante tres año en la misma parcela que favorece el empobrecimiento de los horizontes del suelo que exploran las raíces de la planta y aumenta la densidad inóculo de jopo.

Esto explicaría que en la variedad Vanko, que es muy susceptible a la raza "F" de jopo, sea la más afectada en la disminución de su rendimiento productivo y Olimpia, que es la más tolerante, sea la que mantenga una mayor producción.

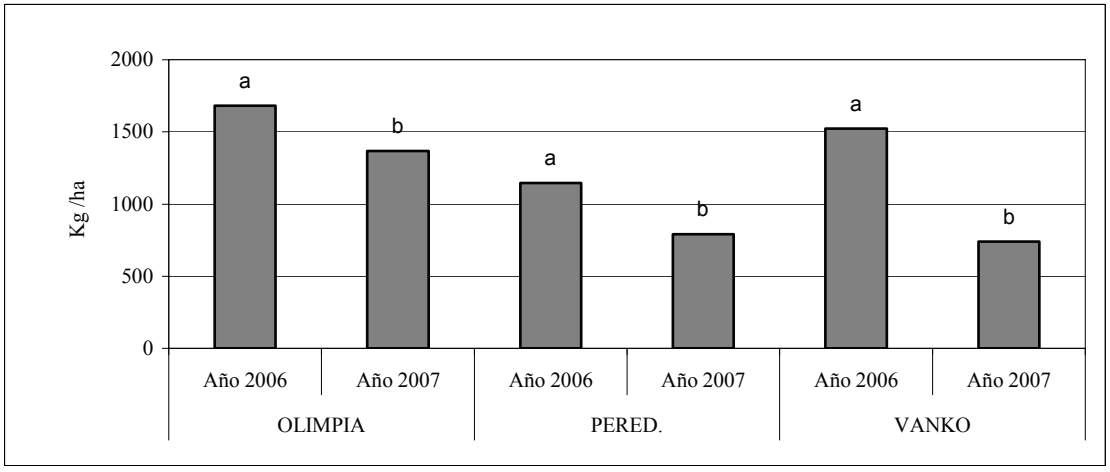

Fig. 5. Rendimiento medio de las tres variedades en los dos años de ensayos.

CONCLUSIONES

Los resultados obtenidos de los experimentos realizados en estos dos años de ensayos con objeto de estudiar la influencia de la siembra directa en la expansión del jopo, parecen indicar que:

1.- Las infestaciones de jopo (% plantas con jopo y numero de jopos por planta) son significativamente menores en el sistema de siembra directa que en el de laboreo tradicional, pero estas diferencias disminuyen notablemente cuando se repite el cultivo de girasol en la misma parcela durante dos o más años.

2.- Las producciones obtenidas son significativamente superiores en el sistema de siembra directa, salvo cuando se siembran variedades tolerantes, que aunque siguen manteniendo las diferencias en producción a favor del sistema de siembra directa, éstas no son significativas.

Por ello se aconseja introducir en el manejo del cultivo la rotación. Esta operación permite disminuir los riesgos y detener el ciclo de enfermedades, plagas y malezas, al renovarse anualmente el horizonte superficial, del suelo. Además, desde el punto de vista de la fertilidad química del suelo, una rotación de cultivos bien planificada favorece un uso más equilibrado de los nutrientes. En siembra directa las rotaciones también tienen un efecto favorable sobre la estructura de los suelos, debido a que las raíces de los cultivos implantados exploran diferentes estratos del perfil, generando una mejor distribución y estabilidad de los agregados.

REFERENCIAS

García Ruiz, J.R. 2003. Resultados ensayos de girasol 2003. Serie RAEA

García Ruiz, J.R. 2004. Resultados ensayos de girasol 2004. Serie RAEA

García Ruiz, J.R. 2005. Resultados ensayos de girasol 2005. Serie RAEA

Melero J.M., and L.C. Alonso. 1998. Enfermedades y daños de los herbicidas en el cultivo del girasol. Koipesol (Ed.) Madrid.

Giráldez, J.V., and P. González. 1995. No tillage in clay soils under mediterranean climate: physical aspects. p. 111-117. In: F. Tebrügge and A. Böhrnsen (eds), Procceddings of the Workshop: Experience with the Applicability of No-tillage Crop Production in the West-European Countries, June 27–28, Giessen, Germany.

McIntosh, M.S. 1983. Analysis of combined experiments. Agron. J. 75:153-155

Ordoñez, R., P. González, J.V. Giráldez, and F. Perea. 2007. Soil properties and crop yields after 21 years of direct drilliing trials in southern Spain. Soil Till. Res. 94:47-54

Changes in seed oil content of sunflower (*Helianthus annuus* L.) as affected by harvesting date

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ABSTRACT

The paper studied the effect of harvesting date on seed oil accumulation in three sunflower genotypes. Harvesting began seven days after pollination and continued at three to four day intervals until ten harvests were carried out in all. Parallel to this, seed moisture content was determined as well. The trial's locations were India and Serbia. In both locations, the genotypes ranked the same for oil content, but all three produced considerably higher values in Serbia. The trial locations differed as to how the minimum and maximum air temperatures fluctuated in them. Regression analysis revealed that in all three genotypes in both locations the highest oil contents were produced with seed moisture at harvesting at around 30%.

Key words: air temperature – moisture content – oil content – sunflower.

INTRODUCTION

Oil is the main reserve substance in sunflower seeds. Seed oil accumulation begins relatively early, several days after pollination, as soon as the space for oil storage forms. Seed oil percentage depends more on weather conditions in the first part of the seed filling stage, while absolute seed weight depends more on the conditions in the second. Higher temperatures in the early stages of seed fill lead to a higher seed oil content (Škorić et al., 1988). The question is, when does oil accumulation end? This is important because of the need for an earlier harvest, especially when chemical desiccation of sunflower is planned. The moment when seed oil content peaks could be expresseed as days after the end of pollination, but the length of this period depends greatly on weather conditions. In Russia, for example, seed oil content may reach its peak anywhere between 30 and 60 days after flowering (Šepetina and Rogoževa, 1971). Seed moisture content is a better indicator of the maximum oil content. Depending on the author, peak seed oil levels are achieved when seed moisture is at 60% (Role et al., 1976), 45% (Dedio, 1985), 33-50% (Chervet and Vear, 1989), 26-30% (Miklič, 2001), etc. Such diverse findings may be a result of differences in weather conditions or of different genotypes used in the trials.

The objective of this paper was to determine how the same set of sunflower genotypes behaves in different agroecological conditions with respect to oil accumulation rate.

MATERIALS AND METHODS

The trial was first carried out in India (Hyderabad, Andra Pradesh) in 1999 and then in Serbia (Rimski Šančevi) the following year. The usual crop tending measures were applied and a randomized block design with three replications was used. The following sunflower genotypes were studied:

1. Ha-Ns-26
2. Ocms-98
3. Ocms-74

The harvesting of sunflower heads began seven days after the end of flowering and continued therafter at three to four day intervals until a total of ten harvesting dates was reached. Three heads were taken from each replicate. The seed moisture content was determined right after harvesting using the common method of drying the seed in a dryer at 105°C to a constant weight. The seed oil content was expressed in relative terms and was determined by leaving the seed to dry naturally and then using nuclear magnetic resonance (NMR) to measure oil levels. Data on the minimum and maximum daily air temperatures during ripening were taken from the local weather stations.

Data were processed with the MSTATC statistical package and the results were interpreted using two-factor ANOVA and regression analysis.

RESULTS AND DISCUSSION

In India, the Ocms-98 genotype had the highest and Ocms-74 the lowest average seed oil content (Table 1). Differences between the genotypes were highly significant. The highest average oil content was recorded on the last harvesting date, with an average seed moisture (ASM) of 10.75%. From the sixth harvesting date on (at 43.27% ASM), there was no significant increase in seed oil content observed.

Table 1. Seed oil content (%) as affected by harvesting date in three sunflower genotypes in India

Genotype	Harvesting date										Average
	1	2	3	4	5	6	7	8	9	10	
Ha-Ns-26	6.9	15.0	29.1	34.5	36.9	37.3	36.3	36.8	35.7	37.3	30.6
Ocms-98	13.3	22.8	31.4	40.0	38.0	37.8	39.9	38.0	38.1	37.1	33.6
Ocms-74	5.5	1.4	4.7	24.5	20.9	28.5	31.8	33.5	34.1	35.8	22.1
Average	8.5	13.1	21.7	33.0	31.9	34.6	36.0	36.1	36.0	36.7	28.8

LSD	Genotype	Harvesting date	Genotype x Date
5%	1.68	3.08	5.33
1%	2.24	4.09	7.09

The Ha-26 genotype had the highest oil contents on the sixth and tenth harvesting dates (at 34.66 and 11.00% ASM), although from the fourth date (52.14% ASM) onwards, there was no statistically significant increase in the oil content. In Ocms-98, the highest seed oil content was found on the fourth harvesting date (51.14% ASM) and there were no significant changes in this parameter from then on. In Ocms-74, the highest seed oil content was observed on the tenth harvesting date (10.27% ASM), with no significant increases from the seventh date (41.00% ASM) onward.

In Serbia, the highest average seed oil content was found in Ocms-98 and the lowest in Ocms-74 (Table 2). Differences between the genotypes were either significant or highly significant. The highest average seed oil content was recorded on the ninth harvesting date (at 19.18% ASM). From the fourth date (50.11% ASM) forth, the value of this parameter did not increase significantly.

Table 2. Seed oil content (%) as affected by harvesting date in three sunflower genotypes in Serbia

Genotype	Harvesting date										Average
	1	2	3	4	5	6	7	8	9	10	
Ha-Ns-26	32.5	34.9	40.2	37.6	36.1	40.5	37.9	36.5	44.9	46.4	38.8
Ocms-98	29.6	33.8	41.1	43.5	51.2	51.4	48.1	52.6	50.8	50.2	45.2
Ocms-74	15.6	28.3	32.9	39.8	40.9	39.4	41.2	40.6	44.0	41.8	36.5
Average	25.9	32.3	38.1	40.3	42.7	43.8	42.4	43.2	46.6	46.1	40.2

LSD	Genotype	Harvesting date	Genotype x Date
5%	1.85	3.38	5.85
1%	2.46	4.49	7.78

The highest seed oil content of Ha-Ns-26 was achieved on the tenth harvesting date (8.87% ASM), with no significant increase being recorded after the ninth date (12.45% ASM). In Ocms-98, the highest oil content was recorded on the eighth date (33.61% ASM), and there was no significant increase in this parameter from the fifth date (54.59% ASM) on. Ocms-74 had the highest oil content on the ninth harvesting date (22.93% ASM) and no significant increase after the fourth date (50.10% ASM).

A strong relationship between seed moisture content and seed oil content at harvesting was found. The regression curves below show increasing oil content with decreasing seed moisture. In most cases, maximum oil levels were achieved with seed moisture at about 30% (at any time the coefficient of determination was around 0.9 or higher) (Fig. 1.). The coefficients of determination were high, ranging from 0.62 to 0.96.

Minimum and maximum daily temperatures at ripening varied a lot between the two locations (Fig. 2).

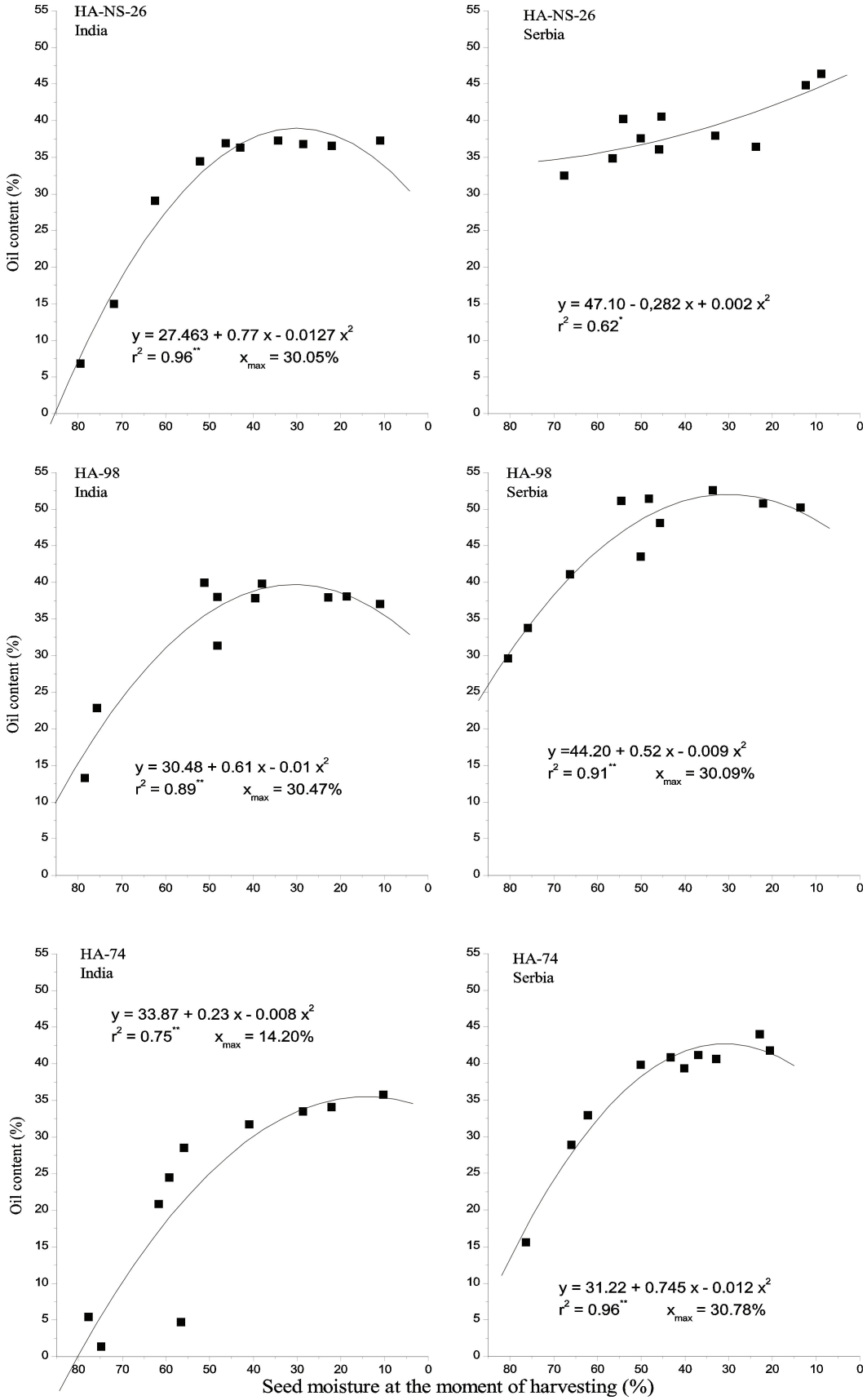

Fig. 1. Seed oil content as affected by seed moisture content at harvesting

Minimum and maximum temperatures varied a lot less in India than in Serbia. In India, the maximum temperatures were initially below 30°C and then they kept increasing slightly for much of the rest of the season, whereas the minimum temperatures increased steadily and significantly from the beginning. In Serbia, the maximum and minimum temperatures were considerably higher in the early stages of ripening, after which they kept decreasing steadily, albeit with large fluctuations. There was no significant precipitation in either location during the period. Day length was considerably greater in Serbia.

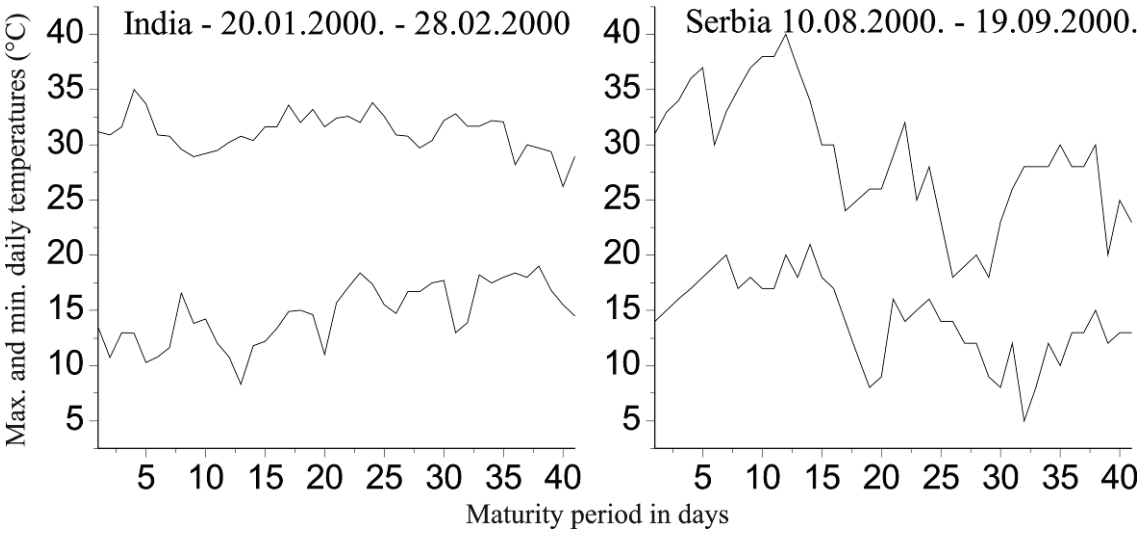

Fig. 2. Minimum and maximum temperatures during maturation

In both locations, therefore, the maximum oil content was reached with seed moisture at about 30%, which is in agreement with the results of Miklič (2001). In most cases, however, statistically significant increases in oil content were already absent with seed moisture at around 50%, which is in agreement with the findings of Chervet and Vear (1989). Considerably higher oil contents were obtained in Serbia than in India. Given that the same set of genotypes was used in both locations, this could be attributed to weather conditions. In Serbia, these conditions were better suited to producing higher oil levels, because minimum and maximum air temperatures at seed fill were higher than in India. Higher temperatures in the first part of the seed filling stage will result in a higher oil content (Škorić et al., 1988). In some cases, minor drops in seed oil content were observed in the closing days of maturation. Rodrigues Pereira (1978) attributes this to the transfer of oil from the kernel to the husk and to dissimilation of accumulated reserves in the absence of inflowing assimilates once the connection between seed and the mother plant has ceased.

CONCLUSIONS

The Ocms-98 genotype had the highest oil content in both locations.

The highest average oil content at both sites was recorded on late harvesting dates, but in most cases no significant differences were recorded once the seed moisture content dropped down to 50% or thereabouts.

Regression analysis showed that in the majority of cases the oil content reached its theoretical maximum with a seed moisture level of 30%.

Considerably higher oil levels were achieved in Serbia than in India, most likely as a result of the more favorable weather conditions in the former.

REFERENCES

Chervet, B., and F. Vear. 1989. Evolution des caracteristiques de la graine et du capitule chez le tournesol au cours de la maturation. Agronomie 9:305-313.

Dedio, W. 1985. Effects of seeding and harvesting dates on yield and oil quality of sunflower cultivars. Can. J. Plant Sci. 65:299-305.

Miklič, V. 2001. Uticaj momenta desikacije na semenski kvalitet i prinos suncokreta. Magistarski rad. Poljoprivredni fakultet, Novi Sad.

Rodrigues Pereira A.S. 1978. Influence of temperature on seed growth and seed ripening in sunflower. p. 199-211. In: Proc. of 8th Int. Sunflower Conf.

Role, M., et al. 1976. (Роле, М., Бюргар, П., Шане, М. 1976). Изменение характеристик семян и корзинки подсолнечника в процесе созревания. p. 374-376. In: Proc. of 7th Inter. Sunflower Conf.

Šepetina F., and M. Rogoževa, 1971 (Шепетина, Ф., Рогожева, М. 1971). Когда лучше убирать подсолнечник в Тамбовской области. Зерновые и масличные культуры, Но 9, 17-19

Škorić, D. 1989. Dostignuća i daljnji pravci u oplemenjivanju suncokreta. p. 285-392. In: Suncokret (monografija), Nolit, Belgrad, Serbia.

Sunflower yield and root system development under water stress in tropical conditions

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ABSTRACT

Field experiments were carried out in Limeira, SP, in 2001, 2002 and 2003, to evaluate the sunflower cv. M 742 root system and yield under water stress conditions. A modified absence and presence method was used to evaluate the root system. The experimental design was a randomized block with four replications. The treatments were: (i) always irrigated, (ii) irrigated in critical periods, and (iii) rainfed. Sunflower plants developing under severe water stress showed higher root number, which also grew deeper than in plants under slight or no water stress. Rainfed sunflower showed two to four times more roots at 30 to 80 cm deep than the irrigated treatments. Sunflower cv. M 742 showed a grain yield reduction of about 30% under hard water stress. Under moderate water stress, with water supplementation at budding and grain filling, sunflower showed 17.2% grain yield reduction in relation to no water stress.

Key words: rainfed – root development – root system evaluation – seed yield.

INTRODUCTION

Temperature and water stress are the most important factors for the sunflower crop development and yield, although this plant adapts well to water stress periods (Choné, 1983). The plant is highly sensitive to soil acidity, mainly when a low pH occurs in subsurface levels; in those cases the taproot bends and there is less secondary root development which leads to smaller plants and less grain yield (Ungaro et al., 1985).

There are many studies about sunflower water stress under temperate and semi-arid conditions. Olalde et al. (2001), under sub-humid and semi-arid climate conditions, found different patterns of sunflower development, yield and yield components. These authors suggested that the greater soil humidity led to greater soil nutrient absorption, which resulted in greater grain and oil yield under warm sub-humid climate conditions. The data agreed with those obtained by Asri et al. (2000), who observed that the water supplementation during grain filling period increased the grain yield up to 1500 kg/ha and produced a greater oil content in the seeds. Singh and Singh (2000) found that water stress during flowering and grain filling period negatively influenced the final seed yield.

Bona et al. (2000) argue that sunflower is more tolerant to water stress than other plant species and that the deep root system is the main factor responsible for this trait. The root system is responsible for fixing, absorption, storage and nutrient and water translocation. The root density or volume usually shows a direct influence on the plant growth (Gomes, 1996). The sunflower roots develop fast at the beginning of the plant life cycle, when it is more important than the aerial part. At this time, the roots represent 20 to 25% of the total dry matter; but this proportion drops progressively to 15% at the end of the life cycle (Merrien and Milan, 1992).

Bona et al. (2000) report that sunflower is tolerant to water stress in comparison to other crops due to morphological and physiological characteristics, to its deep root system and also to some metabolic modifications that can be induced by less water availability in the soil. The transpiration index can be used as a metabolic sign which is strongly linked to the plant's physiological process. Under temperate conditions, these authors verified that one of the morphological effects of water stress was the leaf area reduction, which can cause a potential photosynthesis reduction. Foliar development reduction was observed before the transpiration decrease, which demonstrates that sunflower plant is able to tolerate the water stress by limiting foliar development without any transpiration reduction. Sunflower plant subjected to progressive water stress is able to adapt itself and be more efficient than a plant under late stress.

The aim of the study was to evaluate the sunflower grain yield and root development under different water stress regimes.

MATERIALS AND METHODS

Field experiments were carried out at Campo de Pesquisa Hidroagrícola do Pinhal, in 2001 and 2002, and at Horto Municipal Florestal, in 2003, both in Limeira, SP, using the sunflower cultivar M 742, in a winter sowing. Liming was performed only in the first year. The soil was fertilized with 300 kg/ha of 4-20-20 in 2001 and 2002; in 2003, 375 kg/ha of 4-14-8 was used. Twenty days after emergence 40 kg N/ha and 2 kg B/ha was applied, according to Quaggio and Ungaro (1996). Normal spraying irrigation was used. The experimental design was a randomized block with three treatments and four replications. The treatments were: (i) *Always irrigated*; (ii) *Irrigated in critical periods*; (iii) *Rainfed.*

One trench with 1.0 m depth and 1.0 m width was dug between plant rows to expose the sunflower root system in each replication and for the three years of observation. A thin layer of soil (1-2cm) was carefully removed from the wall along the whole trench. The root evaluation was made by the presence and absence methodology, according to Bohm (1979), modified by the authors.

A wire-wood frame with a grid of 0.2 x 0.2 m was pressed against the trench wall and the presence and absence of roots in each grid were recorded. As this method underestimates the root system because it does not consider the number and diameter of the roots, a method modification was tested, consisting of counting the number of roots in each grid.

For the evaluation of the total root number in each layer interval, the number of roots in each grid of a specific layer was summed and added to the results obtained in each replication of the same treatment for the three years. For the seed yield determinations, samples were taken at plant physiological maturation from an area of 10.8 m^{-2} in each replication during the three years and for all treatments.

Data of number of roots were transformed to log (x+1) before analysis of variance. Turkey test at the p=0.05 level was used for mean comparisons.

RESULTS AND DISCUSSION

Sunflower root distribution in the soil profile is dependent on soil conditions. According to Reichardt (1981), the main factors for little deep rooting in tropical soils are: the low pH, high exchangeable aluminum, compaction, inadequate aeration, and low retention and diffusion of water. In the present study, the soil texture in Campo de Pesquisa had less sand than Horto Florestal (Table 1) and, consequently, showed higher water retention in the 1-m profile. Soil pH was 5.2; 5.4; 5.8; 6.1 in Campo de Pesquisa, and 5.8; 5.9; 5.9 and 5.8 in Horto Florestal at 0-0.25 , 0.25-0.50 , 0.5-0.75 , 0.75-1.0 m depth, so both soils presented good conditions for normal sunflower development.

Table 1. Soil chemical and physical characterization

Hidroagrícola do Pinhal								
Soil depth	MO	P	K	Ca	V%	B	DS	Soil type
	g/dm^3	$mmol/dm^3$			%	mg/dm^3	g/cm^3	
0-25	28	6	2.8	41	59	0.22	1.34	Lime
25-50	17	2	1.7	26	56	0.20	1.29	Lime
50-75	11	1	0.8	27	63	0.07	1.16	Lime
75-100	8	1	0.6	24	62	0.06	1.22	Lime
Horto Florestal								
0-25	26	217	3.6	46	78	0.28	1.76	Lime
25-50	16	117	1.1	36	76	0.18	1.76	Lime
50-75	8	48	1.1	23	69	0.15	1.78	Clay to lime
75-100	8	22	1.1	20	69	0.15	1.78	Clay to lime

DS= Soil Density

Fig. 1 shows rain precipitation during 2001, 2002, and 2003 between June and October, 2003 being the dryest year. In 2001 and 2003 the rain occurred at the beginning of flowering and in the seed filling, which should have diminished the water stress symptoms in the *Rainfed* treatment. With the soil drying process, the upper layers are the first to dry. The plant exhibits a predominantly superficial root system under no water stress (Table 3), as shown in the *Always irrigated* treatment, and a larger number of roots in the *Rainfed* treatment in the deep layers in comparison to the irrigated treatments. The root growing towards humid soil of deeper layers can be considered as a sunflower defense against water stress.

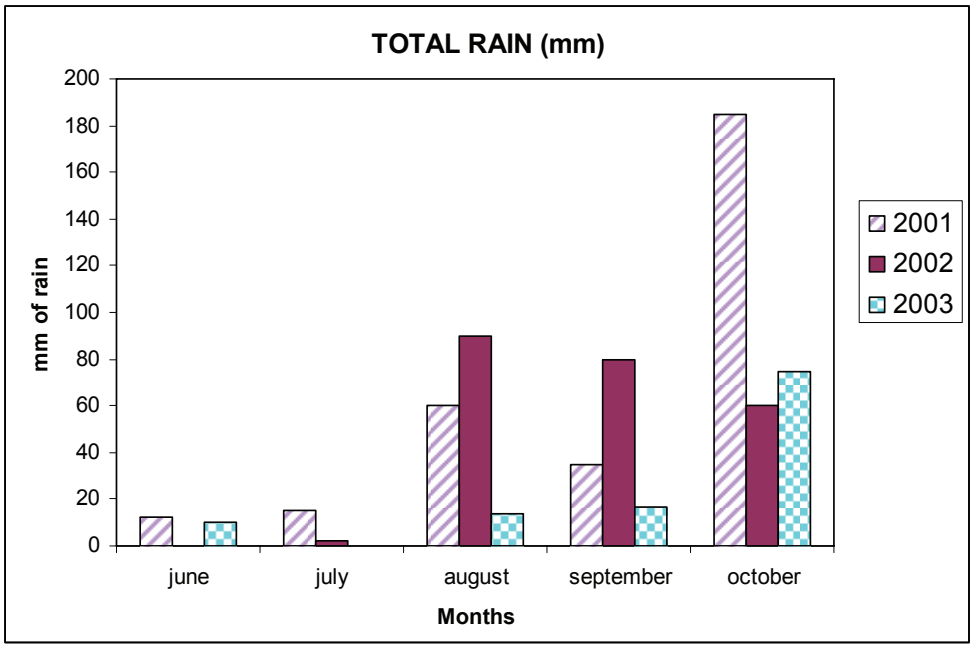

Fig. 1 Total month water precipitation for the years of 2001, 2002, and 2003.

Table 2 shows a significant difference between the average roots per grid of *Rainfed* treatment in relation to *Always irrigated and Irrigated in critical periods* by Turkey test at 5% significance. There was an increase in the number of roots per grid in the *Rainfed* treatment.

Table 2. Average number of roots/grid in the irrigation treatments, observed in the three years.

Treatments	Average roots/grid		
	2001	2002	2003
Always irrigated	7.54 b	13.70 b	6.39 b
Irrigated in critical phases	8.16 b	7.20 c	6.03 b
Rainfed	13.01 a	22.51 a	8.34 a
CV%	28.96	24.43	23.09

[1]Means followed by the same letter in column did not differ from Tukey at 5%.

According to Taiz and Zeiger (2004) moderate water deficits negatively influence the development of the root system. The ratio between root biomass and shoot apex seems to be governed by a functional balance between the root water absorption and shoot photosynthesis. This functional balance can be altered if the water supply decreases. The foliar expansion is affected by water shortage early on, but the photosynthesis activity is less affected. The inhibition of foliar expansion reduces carbon and energy consumption and a high proportion of vegetable assimilates can be distributed to the underground system to support the future growth of the roots. Those factors lead to a root growing priority to humid soils, as shown in the *Rainfed* treatment. With the increasing water shortage, the upper soil layers are the first to dry up.

Table 3 shows the total root number obtained by the sum of roots observed in each grid disposed in each layer of the treatments *Always irrigated*, *Irrigated in critical periods* and *Rainfed* using the adapted presence and absence methodology. Greatest total root number was observed in the *Rainfed* treatment. The total root number at each layer was affected by the water regime; *Rainfed* also showed between 2 to 4 times more roots in the deeper layers, between 30 and 80cm, than those of the irrigated treatments. In the *Irrigated in critical periods* and *Always irrigated* the roots were mainly in the more superficial layers.

Table 3 also shows a more superficial root system when the soil moisture is high, as verified in the treatment *Always irrigated*; when the upper layers dry, there is a root proliferation in the deeper layers in the *Rainfed* treatments. This root growing towards the humid soil according to Merrien and Milan (1992) and Connor and Hall (1997) can be considered a natural sunflower defense against water stress.

Table 3. Total root number found in each treatment in the different soil layers in the four replications.

Soil depth	*Always irrigated*	*Irrigated in critical phases*	*Rainfed*
00 – 10	6,763	7,201	6,814
10 – 20	2,267	1,947	2,148
20 – 30	415	215	382
30 – 40	154	36	250
40 – 50	39	46	157
50 – 60	43	76	114
60 – 70	66	18	65
70 – 80	6	3	22
80 – 90	5	0	3
90 –100	0	0	0
Total	9,542	9,758	9,955

Table 4 shows the grain yield obtained in each treatment and year. It is interesting to observe that the data shows the same yield level in the three years and in the two soil types, although 2003 was much drier with only 48mm of rain during the whole sunflower cycle, while 2001 and 2002 presented 90mm and 180 mm, respectively. The better soil characteristics of 2003 must have positively influenced the grain yield. The higher water stress in the *Rainfed* treatment resulted in a 30% yield reduction in relation to the treatment with no water stress. The moderate water stress presented by *Irrigated in critical phases* treatment reduced grain yield by about 17%.

Table 4. Results of the grain yield obtained in the three treatments, in 2001, 2002, and 2003, and the percentage of yield reduction in relation to *Always irrigated* treatment.

Treatment	2001	2002	2003	Average	%reduction
Always irrigated	1732 a	1604 a	1860 a	1732 a	0
Irrigated in critical phases	1483 b	1425 ab	1541 b	1483 b	17.2
Rainfed	1122 c	1112 b	1131 c	1121 c	29.8
Average	1446	1380	1511		

Means followed by the same letter in column did not differ from Turkey at 5%.

CONCLUSIONS

- The irrigation treatments showed no differences in relation to root number and distribution in the soil profile while *Rainfed* treatment developed two to four times more roots at 30 to 80cm deep;
- Under strong water stress the sunflower plant increases the number and the depth of the roots in order to minimize the lack of water;
- Sunflower cv. M 742, under hard water stress showed a grain yield reduction of about 30%;
- Under moderate water stress, with water supplementation at budding and grain filling, sunflower showed 17.2% grain yield reduction in relation to no water stress;

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REFERENCES

Asri, M.E., A. Essahat, A. Bouniols, and M. Mondies. 2000. Rendement et qualite des graines du tournesol cultive sous contrainte hydrique. Resultats des essais en cooperation au Maroc et dans le sud-ouest de la France. p. C127-C132 (vol. 1). In: Proc. 15th Int. Sunflower Conf., Toulouse, France.

Bohn, W. 1979. Methods of studying root systems. Ecological studies. Berlin, Springer, Verlag, 189 p.
Bona, S., G. Mosca, A. Cantele, and T. Vamerali. 2000. Response of sunflower to progressive water stress. In: Proc. 15th International Sunflower Conference, Toulouse, France, International Sunflower Association, vol. I: D58-D63.
Choné, E. 1983. Physiologie de la formation du rendement chez le tournesol. Informations Techniques, CETIOM, Paris 83:3-72.
Connor, D.J., and A.J. Hall. 1997. Sunflower Physiology. p. 113-182. In: Schneiter, A. Sunflower Techonology and Production. American Society of Agronomy. Madison, WI, USA.
Gomes, E.M. 1996. Estudo comparativo do sistema radicular de citros sob três tipos de irrigação. Campinas, FEC, UNICAM, 1996. Tese Mestrado, Faculdade de Engenharia Civil, Universidade Estadual de Campinas.
Merrien, A., and M.J. Milan. 1992. Phyiologie du Tournesol. Paris. Centre Technique Interprofessionnel des Oléagineux Metropolitáins, Racines et feuilles. p. 21-27.
Quaggio, J.A., and M.R.G. Ungaro. 1996. Recomendações de Adubação e Calagem para o Estado de São Paulo. Boletim Técnico, 100. Campinas, IAC, 198 p.
Reichardt, K. 1981. Soil physico-chemical conditions and the development of roots. p. 103-114. In: R. S. Russel, R. S., K., Igue, Y.R., Mehta (eds), The soil/root system in relation to brazilian agriculture**.** IAPAR, Londrina, Brazil.
Robinson, R.G. 1978. Production and culture. p. 89-143. In: J.F. carter (ed.), Sunflower Science and Technology. American Society of Agronomy. Madison, WI, USA.
Singh, D.A. and S.M. Singh. 2000. Impact of irrigation on sunflower productivity. p. C109-114 (vol. 1). In: Proc. 15th Int. Sunflower Conf., Toulouse, France.
Taiz, L., and Zeigeir. 2004. Fisiologia Vegetal. Porto Alegre: Artmed, 719 p.
Ungaro, M.R.G., J.A. Quaggio, P.B. Gallo, S.C.F. Dechen, F. Lombardi Neto, and O.M. Castro. 1985. Comportamento do girassol em relação à acidez do solo. Bragantia, Campinas, 44(1):41-48.

Insecticide residues cross-contamination of oilseeds during storage

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ABSTRACT

Pesticide residues are found in oilseeds and crude oils: they are mainly organophosphate insecticides (pirimiphos-methyl, dichlorvos, malathion) used in empty storage facilities and for application to stored cereal grains. Even if pests are found in stored oilseeds, French regulations do not permit the use of these insecticides on stored oilseeds, as they have an affinity for these lipophilic substances. These residues arise from cross-contamination from storage bins and facilities, and not from illegal use. This uptake of insecticide residues from their storage environment by oilseeds can lead to levels that exceed regulatory limits. An investigation of 11 grain storage companies allowed us to follow the course of 27 sunflower seeds batches, from reception at the storage facilities to outloading. Samples from each of these batches, made at outloading, were analysed by ITERG (French Technical Institute for Oil Industry), looking for insecticide residues. Traceability of sunflower seeds established by storers allowed us to identify cross-contamination sources. Substances discovered were dichlorvos, pirimiphos-methyl and malathion (and one case with chlorpyriphos-methyl). Pirimiphos-methyl was most commonly detected, but most cases of non-accordance with regulatory levels were caused by dichlorvos and malathion. Main cross-contamination hazard resulted from treatment of cereals at outloading, just before sunflower seeds were outloaded, especially when these cereal treatments were frequent on that elevator. Other situations led to cross-contamination, but generally at lower levels: outloading of sunflower seeds after outloading of treated cereal, sunflower seeds stored in bin that contained previously treated cereals, empty bins and handling equipment treated before receipt of sunflower seeds.

Key words: cross-contamination – insecticide – oilseeds – pesticide residues – storage.

INTRODUCTION

Post-harvest insecticide residues are frequently found on oilseeds at low levels, although no insecticide is allowed to be applied directly to oilseeds during storage. Consequently, maximum residue levels (MRLs) allowed by European regulation are very low (mostly at the lower limit of analytical determination): 0.01 mg/kg for dichlorvos (still authorised during this study, but forbidden now), and 0.05 mg/kg for pirimiphos-methyl. No MRL exists for malathion, so it should not be found beyond the analytical limit of quantification. These insecticide treatments are authorised on stored cereals and corn as a grain protectant, and on empty storage and handling equipment as a control agent for residual insect populations in empty granaries. Dichlorvos, malathion and pirimiphos-methyl were the substances most employed during this study (storage season 2006-2007, regulations changed later).

So, we can hypothesise that cross-contamination phenomena can exist, between these various kinds of seeds, cereals and oilseeds, sharing the same grain handling and storage system. This phenomenon has already been demonstrated in Canada on rapeseed (Watter and Nowicki, 1982, 1985; White, 1983), when empty bins were treated with organophosphorous insecticides (bromophos, malathion, fenitrothion). Canadian storers were warned that treating bins before storing rapeseed could lead to residues above the maximum allowable limits.

Uptake of pirimiphos-methyl by a single-layer of rapeseed or wheat on galvanized-steel surfaces was demonstrated in a laboratory study (Dauguet et al., 2006, 2007). It was shown that, for small bins (less than 50 tons), it could lead to residue quantities above regulatory limits. But in big elevators, insecticide uptakes by seeds can also occur at other stages: conveyor belts, handling of oilseeds after cereals had been treated in the same circuits, outloading bin, etc. Therefore we cannot rule out either risk for grain storage companies.

In order to improve our knowledge about this post-harvest insecticide cross-contamination, especially in big elevators, an investigation was carried out with the collaboration of several French grain storage companies. Real cases were observed, with an accurate traceability of sunflower seeds lots all along their route inside storage facilities (from receipt to outloading) to find where the insecticides were taken up by the oilseeds.

Results presented in this article were obtained in the first year of the investigation, concentrating on sunflower seeds during the storage season 2006-2007. This investigation will continue on rapeseed during the next storage season.

MATERIALS AND METHODS

The process adopted for this survey was:

- Identifying with storage operators sunflower lots that could be "traced" (recording of each step from receipt to outloading):11 grain storage companies agreed to collaborate, and allowed us to follow 27 sunflower seed bins. These companies were situated throughout the French sunflower crop area.
- Making a mean sample from each batch representative of sunflower seeds arriving at the storage facilities ("first sample") and preserving it. These samples were analysed only when residues were found in the final sample, in order to know if contamination occurred before receipt of grain. These "first samples" were analysed for 4 batches of seeds.
- Making a mean sample representative of outloaded sunflower seeds, "final sample", when the traced lot is commercialized (from one to eight months after harvesting). These "final samples" were always analysed. In one case, we had 2 samples for one sunflower batch, so that we analysed 28 final samples. The sampling method used was based on a standard method (moving seeds, for contaminant with heterogeneous distribution determination, prEN ISO 24333:2006): 25 elementary samples for 500 tons evenly distributed during the outloading (one elementary sample each 20 tons). This method was usually well observed by the commercial operators.
- Filling in a questionnaire called "traceability" which recorded each step from receipt to outloading. Operators had to indicate if treatments were applied on empty bins or handling equipment, or if cereals were treated at their receipt or outloading and if these cereals used the same conveyer circuit inside the storage facilities just before the sunflower seeds.
- Determination of insecticide residues in all the "final samples": the analytical laboratory of ITERG conducted these determinations, using the "common method" developed three years ago by a group of about twenty French laboratories (public and private) coordinated by CETIOM and ITERG: Soxhlet extraction of oil with hexane (NF EN ISO 659) was followed by analysis of organophosphorous residues by gas chromatography with NPD detection.

RESULTS

Twenty-eight samples were analyzed (Table 1). The insecticides used on cereals and for storage facilities treatment were detected: dichlorvos, pirimiphos-methyl, malathion and chlorpyriphos-methyl (only one case). Most commonly detected substance was pirimiphos-methyl: detected in 61% of samples and quantified in 39% of samples. But, malathion and dichlorvos were more frequently above MRL: 21% and 18% of cases, respectively.

On the whole, final samples were slightly contaminated as half of them contained less than 12 µg/kg of insecticide residues (sum of residue median), and 90% of them contained less than 120 µg/kg (sum of residues 9th decile).

Table 1. Analytical results (expressed in µg/kg) on the 28 final samples[1]

	LQ	MRL	Mean	Median	Standard deviation	9th decile	Maxi	% samples ≥ LD	% samples ≥ LQ	% samples > MRL
Dichlorvos	10	10	21	0	79	27	422	32%	29%	21%
Pirimiphos-methyl	10	50	19	5	55	29	295	61%	39%	4%
Chlorpyriphos méthyl	10	50	0	0			10	4%	4%	0%
Malathion	10	-	8	0	25	17	125	18%	18%	18%
Sum of residues			48	12	102	120	427			

[1]LQ: limit of quantification; LD: limit of detection; MRL: maximum residues limits in sunflower seeds; Sum of residues: a value of 5 µg/kg is given when a substance is detected but below the limit of quantification, and zero value if under the limit of detection.

Analytical results for each substance (Fig. 1, Fig. 2, Fig. 3): Pirimiphos-methyl - Only one sample had a very high level (T12: 295 µg/kg). The other samples were always below the MRL: 4 between 20 and 50 µg/kg, 12 between 10 and 20 µg/kg, and 11 below the limit of quantification; Dichlorvos - Only one sample had a very high level (T9: 422 µg/kg). Three other samples were between 20 and 50 µg/kg.

Five samples were near the MRL or below it. It was not detected in 19 samples; Malathion - Only one sample had a high level (T28: 125 µg/kg). Four samples were between 10 and 50 µg/kg, 12 between 10 and 20 µg/kg. It was not detected in 23 samples.

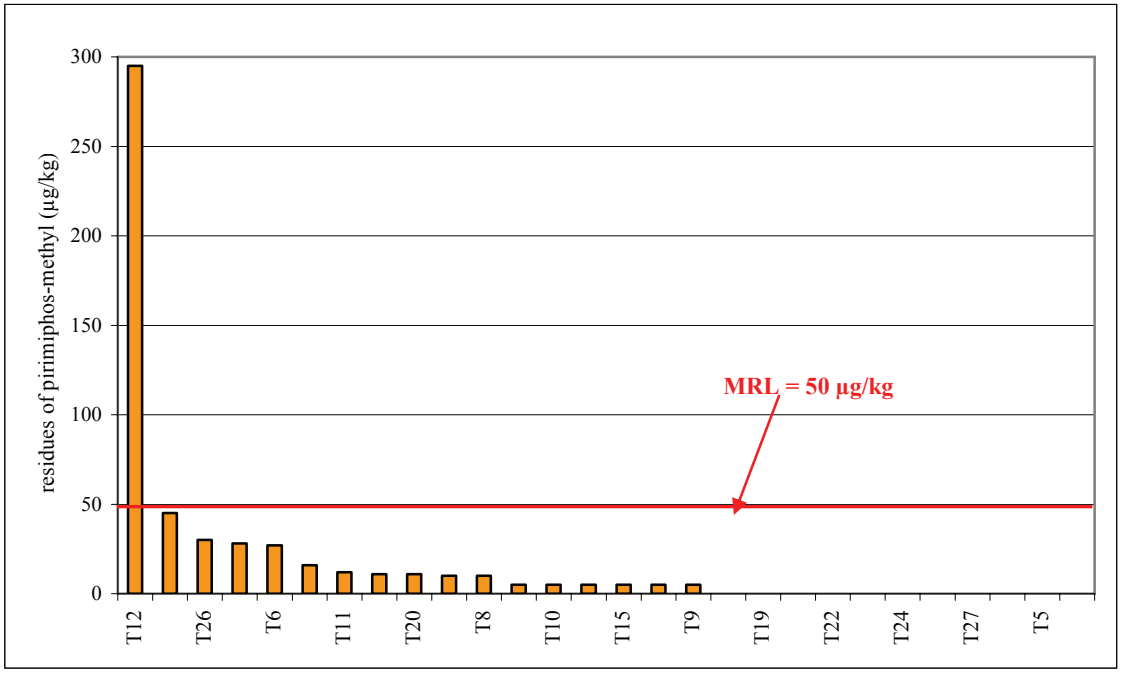

Fig. 1. Individual analytical results for pirimiphos-methyl

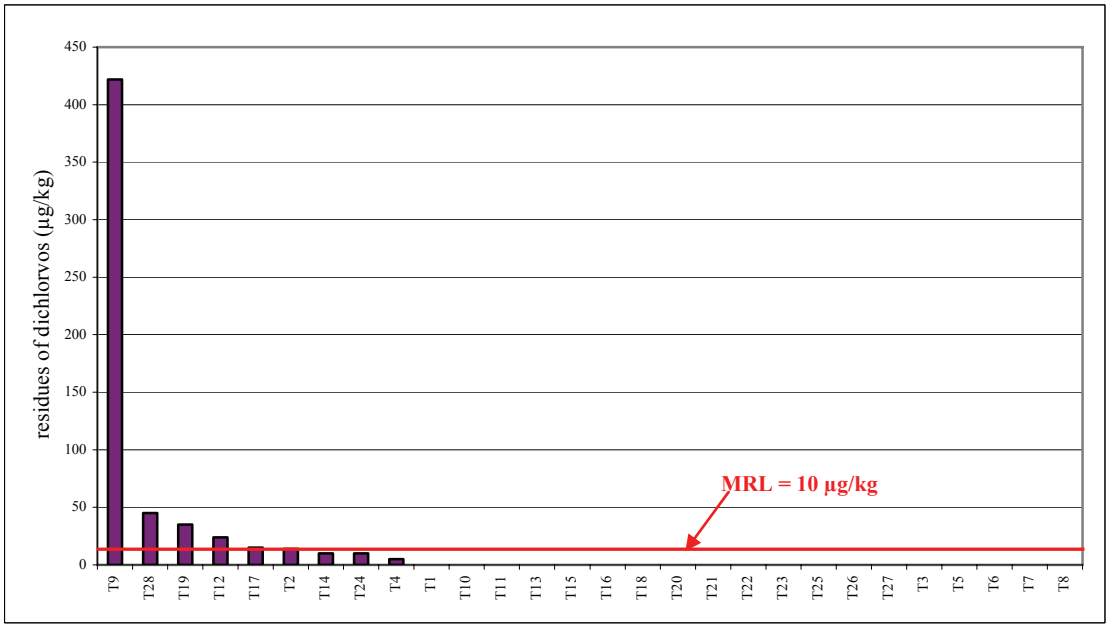

Fig. 2. Individual analytical results for dichlorvos

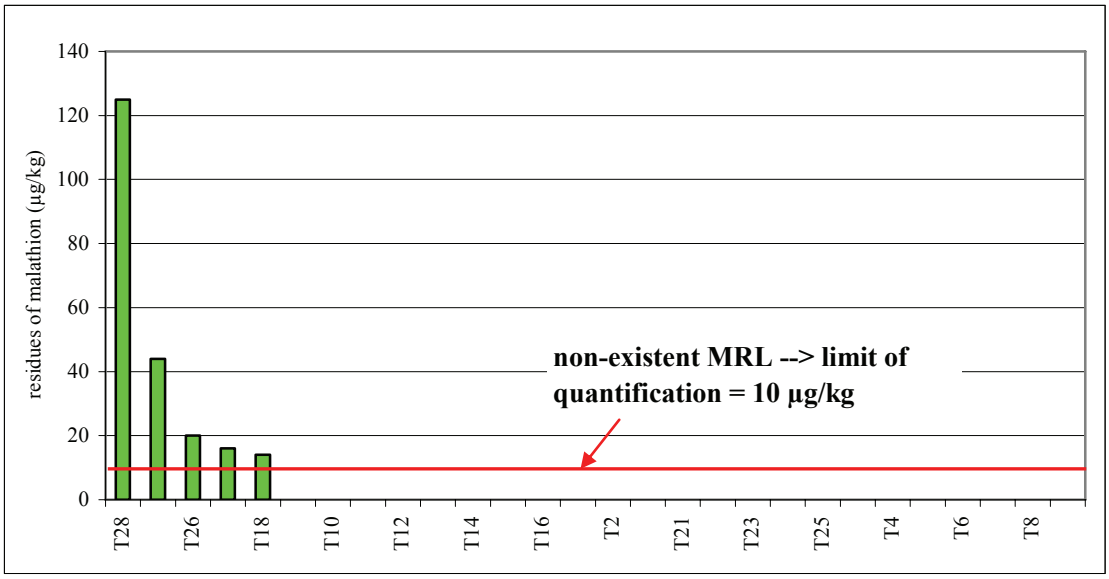

Fig. 3. Individual analytical results for malathione

In order to explain the contamination, when large residues were detected, the "first samples" (taken at receipt of the sunflower seeds at the storage facilities) were analyzed for the batches T4, T12, T19 and T28. The insecticide residues on each "first sample" were too low to explain the residues found at the end, in the "final samples". So, the explanation had to be found in the route of the sunflower seeds inside the elevator.

Four cases leading to cross-contamination were identified:

- K1: treatment of cereals at outloading, just before outloading of sunflower seeds
- K2: outloading of cereals, treated at their receipt, just before outloading of sunflower seeds
- K3: storage of treated cereals in the same bin just before storage of sunflower seeds
- K4: treatment of empty bin and of handling equipment before receiving sunflower seeds

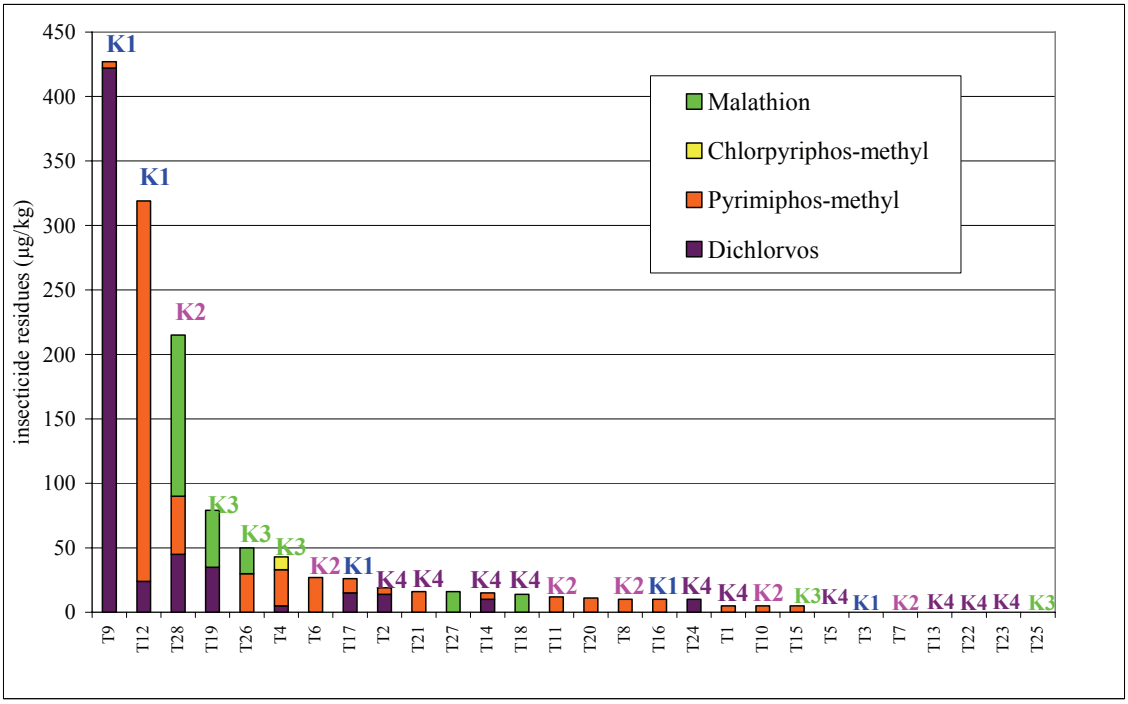

Fig. 4. Distribution of the four cases (K1, K2, K3, K4) for each sunflower lot, and sum of insecticide residues (µg/kg) for each sunflower final sample

It would seem that the biggest cross-contamination occurred with the case K1. Studying the circumstances of K1, the risk was higher when treatment of cereals at outloading was frequent or systematic. In the worst case (T9), the sunflower seeds batch was transported by lorry over a period of two weeks. Cereals were also sent during this period, and treated before outloading, and using the same outloading circuit as the sunflower (conveying belts, outloading bin). The other cases, K2, K3, K4, may also lead to a slighter cross-contamination. For one lot, there were two cases of a cross-contamination risk, and this could only have worsened the contamination.

CONCLUSIONS

Our study in real situations showed that cross-contamination of oilseeds by post-harvest insecticide residues exists, and can sometimes lead to residues above the regulatory limits.

The highest risk of contamination appeared when cereals were systematically treated at outloading, just before outloading of oilseeds, using the same conveyor circuits. The other identified cases may also lead to a slighter contamination. But, silo operators should concentrate on the accumulation of several risky cases, which could worsen the contamination.

Other sources of insecticide residues can occur in storage facilities, but we could not check them in this investigation. They include leak of insecticide from the application equipment, use of sampling equipment contaminated by pesticides. Another situation that we did not meet in our study was cross-contamination or accidental treatment on-farm of oilseeds subsequently delivered to a commercial store.

This investigation will be continued with rapeseed. This will allow us to check if rapeseed can be affected by the same cross-contamination as sunflower. The new work will be carried out in the new regulatory context in which dichlorvos and malathion are forbidden for cereal treatment. Thus, storage operators will certainly have new grain protection strategies, which could lead to a different cross-contamination risk for oilseeds.

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REFERENCES

Dauguet, S., F. Lacoste, B. Ticot, J.P. Loison, J. Evrard, B. Bouchtane, and B. Soulet. 2006. La filière oléagineuse se mobilise autour de la problématique des résidus d'insecticides. OCL 13(6):373-377.

Dauguet, S., J. Evrard, J. Fritsch, and J.P. Loison. 2007. Accumulation of pesticide residues in oil during the storage of rapeseed. 12th International Rapeseed Congress, Wuhan Hubei, China, March 26-30.

Watters, F.L., and T.W. Nowicki. 1982. Uptake of bromophos by stored rapeseed. J. Econ. Entomol. 75:261-264.

White, N.D.G, and T.W. Nowicki. 1985. Effects of temperature and duration of storage on the degradation of malathion residues in dry rapeseed. J. Stored Prod. Res. 21:111-114.

White, N.D.G, T.W. Nowicki, and F.L. Watters. 1983. Comparison of fenitrothion and malathion for treatment of plywood and galvanized steel surfaces for control of the red flour beetle and the rusty grain beetle. J. Econ. Entomol. 76:856-863.

Evaluating irrigation performance of sunflower in an irrigation scheme of Southern Spain

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ABSTRACT

Current water resource scarcity implies a change in the water management in the Spanish irrigation schemes. Assessment of irrigation performance over long time periods is a prerequisite for improving water use in the agricultural lands to respond to the water scarcity. We carried out a comprehensive assessment of the irrigation performance by documenting the water use of fields cultivated with sunflower at the Genil–Cabra Irrigation Scheme (GCIS), located in Andalusia, Southern Spain, from 1991 to 2007. We have used a model that simulates water balance for every field and three performance indicators to assess the performance of irrigation water use and management in the GCIS. Among the performance indicators, the average ratio of measured irrigation supply to the simulated optimum demand (ARIS) for sunflower ranged between 0.09 and 0.34, indicating that the crop was under water stress. The average Irrigation Water Productivity (IWP) provided low values for this crop, although in the last years it has reached higher values due to the recent increase of international market prices, with similar values to maize or wheat. Remote sensing techniques, based on using a satellite-based energy balance called METRIC, were used to obtain actual evapotranspiration maps during the 2004/05 irrigation season. Seasonal ET variability was found to be large between sunflower fields and also within sunflower fields in the GCIS. The current policies of subsidies in sunflower force the farmers to obtain a maximum profit from the irrigation water rather than a maximum yield, a scenario that must be considered when planning programs of water conservation.

Key words: deficit irrigation – evapotranspiration – irrigation management – water allocation – water supply.

INTRODUCTION

Sunflower occupies a large surface of the area cultivated in Andalusia. The high volume of crop water demand and a reduced water supply force farmers to use different strategies from irrigation water management. With the constant increase in water demand from other sectors of society, assessing water management is indispensable for proposing improvements in irrigation management and for quantifying water productivity (Molden and Sakthivadivel, 1999). There are different performance indicators for irrigation water use that require some information related with the irrigation applied by the farmers. However a detailed water-use record from each plot is often not available, which makes it impossible to carry out a good analysis of water management. Records of irrigation water use are required to obtain additional information, helping to estimate all water-balance components in fields, and which can be evaluated using a simulation model, hereafter named LORMOD, which can be employed to assess the actual performance and water management (Lorite et al., 2004a).

Most of the consumption of irrigation water corresponds to evapotranspiration (ET), the loss of water from the earth's surface through the combined processes of evaporation and transpiration. Therefore, the spatial and temporal quantification of ET is essential in agricultural water management. As a recent remote sensing technique, accurate ET estimations have been obtained using a satellite-based energy balance (Bastiaanssen et al., 1998; Allen et al., 2007). METRIC (*Mapping EvapoTranspiration with high Resolution and Internalized Calibration*) is an ET estimation model (Allen et al., 2007) based on the SEBAL (*Surface Energy Balance Algorithms for Land*) model of Bastiaanssen et al. (1998).

The objective of this work was to conduct a comprehensive assessment of the irrigation performance of sunflower, compared with maize and wheat, in an area using on-farm water-use information and a simulation model, as well as an ET estimation model (METRIC). The area selected was the Genil–Cabra Irrigation Scheme (GCIS) located in Andalusia, Southern Spain. This irrigation scheme was chosen because it disposes of accurate information on water use and on the cropping patterns of individual plots since the start of its operations (1990/1991) until present.

MATERIALS AND METHODS

The study area was located within the Genil-Cabra Irrigation Scheme (GCIS), in Cordoba province, Southern Spain (37º 31' N, 4º 51' W). The climate in this area is typically Mediterranean with an annual average precipitation of 606 mm and a rainless summer. The average air temperature ranges from 10 ºC for the coldest month to over 27 ºC for the warmest.

The study was carried out during 16 irrigation seasons (1991/1992 to 2006/2007). Daily meteorological data to estimate Penman–Monteith ASCE reference evapotranspiration (ET_0) and rainfall were obtained from a meteorological station located within the GCIS. Information about the cumulative water-meter for each plot was obtained by individual readings four/five times per irrigation season. Likewise, the information about irrigation practices, water supply and sowing dates was provided by the irrigation scheme manager or directly from farmers (Lorite et al., 2004a). Only the plots with a single crop were selected for this study.

A water-balance model was developed by Lorite et al. (2004a) to simulate water use in the GCIS. LORMOD is composed of sub-models that calculate the different water-balance components and estimate the effects of water stress on crop yield. It calculates the soil water balance components for each computation unit on a daily basis, generates optimum irrigation schedules and compares the optimum schedules for each field against the actual irrigation schedules, which were simulated by basing them on water-meter readings.

For each field, to assess the evolution of irrigation management and benchmarking, the Annual Relative Irrigation Supply (ARIS) was chosen, defined by Malano and Burton (2001) as:

$$\mathrm{ARIS} = \frac{\text{Annual volume of irrigation water inflow}}{\text{Annual volume of crop irrigation demand}} \quad (1)$$

Another indicator computed here was the Crop Yield Ratio (CYR; Bos et al. 1994), that relates the actual crop yield to the intended yield, defined as the attainable crop yield with optimum economic irrigation, defined as:

$$\mathrm{CYR} = \frac{\text{Actual crop yield}}{\text{Intended crop yield}} \quad (2)$$

To evaluate the productivity of the water used in irrigation in this area, the indicator considered was the Irrigation Water Productivity (IWP; Lorite et al., 2004a), defined as:

$$\mathrm{IWP} = \frac{\text{Increase in Annual Value of Agricultural Production due to Irrigation}}{\text{Annual Volume of Irrigation Water Inflow}} \ (€/m^3) \quad (3)$$

In this indicator, the numerator is computed as the difference between actual crop yields under irrigation minus rainfed yields. It is assumed that management does not change much as the grower shifts from rainfed to irrigated conditions, which is probably the case for the GCIS.

Satellite-based energy balance estimation of crop ET (METRIC)

Eleven Landsat 5 TM images were processed using the METRIC energy balance computation procedure of Allen et al. (2007) to obtain daily ET for each image date. The model METRIC estimates ET as a residual of the energy balance at the surface:

$$LE = R_n - G - H \quad (4)$$

where LE is the latent energy consumed by ET, R_n is net radiation, G is sensitive heat flux into the soil, and H is sensitive heat flux to the air. Details of the METRIC model are given in Allen et al. (2007) and Tasumi et al. (2005).

We define a crop coefficient, $K_{c\ act}$, as the ratio between actual ET estimated by METRIC, and the grass reference ET (ET_o) calculated following the ASCE standardized Penman-Monteith method (ASCE-EWRI, 2005). This $K_{c\ act}$ differs from the standard K_c (Allen et al., 1998) in that our actual ET estimate is usually below the maximum ET due to agronomic factors. Weather data for calculating ET_0 were provided by five automatic weather stations located close to the GCIS. These weather stations are part of the Agroclimatic Information Network of Andalusia (Gavilán et al., 2006).

RESULTS AND DISCUSSION

Throughout the period of study it was observed that the water applied to sunflower was different from year to year, due to variations in the precipitation and water availability (Fig. 1). Thus, the average annual rainfall in the periods 1991-1995 and 1999-2005 was smaller than 500 mm. Nevertheless, in the first period the water supply was very restricted (≈ 700 m^3 ha^{-1}), whereas in second the average water supply was three times bigger (≈ 2600 m^3 ha^{-1}) than in the first period. These policies importantly affected the volume of available water, that was little or null during the period 1992-1995 and increased significantly in the period from 1999 to 2005. In 1998/1999 irrigation season, the low precipitation forced all farmers, including those who did not usually apply water to sunflower, to increase the irrigation applied, favoured by the available water supply of that year, which was higher than average (Lorite et al., 2004a). For this reason, the variation between the fields in the annual volume of water applied, quantified by the coefficient of variation, diminished considerably in 1998/1999 irrigation season.

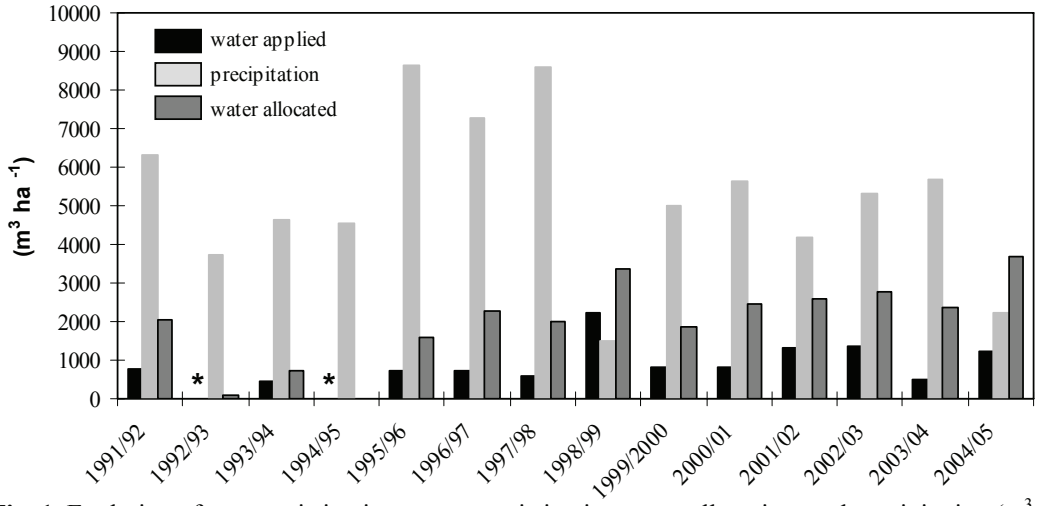

Fig. 1. Evolution of average irrigation water use, irrigation water allocation, and precipitation (m^3 ha^{-1}) from 1991/1992 until 2004/2005 irrigation seasons. * During 1992/1993 and 1994/1995 irrigation depth applied was practically null.

The limited water supply available during the period 1991-1995 caused an increase in sunflower and wheat areas (Fig. 2). However, during the following years the absence of restrictions caused a reduction in those areas, whereas there was a continuous increase in the one devoted to maize.

Performance indicators of irrigation water use

The average ARIS for sunflower and for wheat was very low during the whole period (0.23 and 0.26 respectively; Fig. 3A) with respect to maize, where the ARIS experimented a constant increase up to values close to 1, which represented an optimum irrigation. The low values of ARIS for sunflower and wheat suggest that these crops received below 30% of the maximum potential evapotranspiration. In dry seasons (e.g. 1998/1999), the values of ARIS increased for sunflower and wheat, although this increase does not signify a linear relation between ARIS and the amount of precipitation, as shown by García-Vila

et al. (2008). However, these authors observed that an increase in precipitation caused a clear decreasing of ARIS in cotton, sugar beet and garlic. Low values of the ARIS in sunflower show that most of the farmers consider that this crop could be cultivated as a rainfed crop, or with a small irrigation supply. On the other hand, the low ARIS values observed in the 16 seasons of study (smaller than 0.25; Fig. 3A) suggest that the crop remained under a constant water stress. One of the reasons for this water management is that the current policies of subsidies in sunflower are based on the cultivated area and not on yield. Therefore, for farmers it is more beneficial to obtain a maximum profit from the irrigation water rather than at maximum yield (Lorite et al., 2004b). The ARIS for sunflower showed a high variability, with an average variation coefficient of 1.25, although in the driest year of the period (1998/1999) it descended to 0.88 (Fig. 3B).

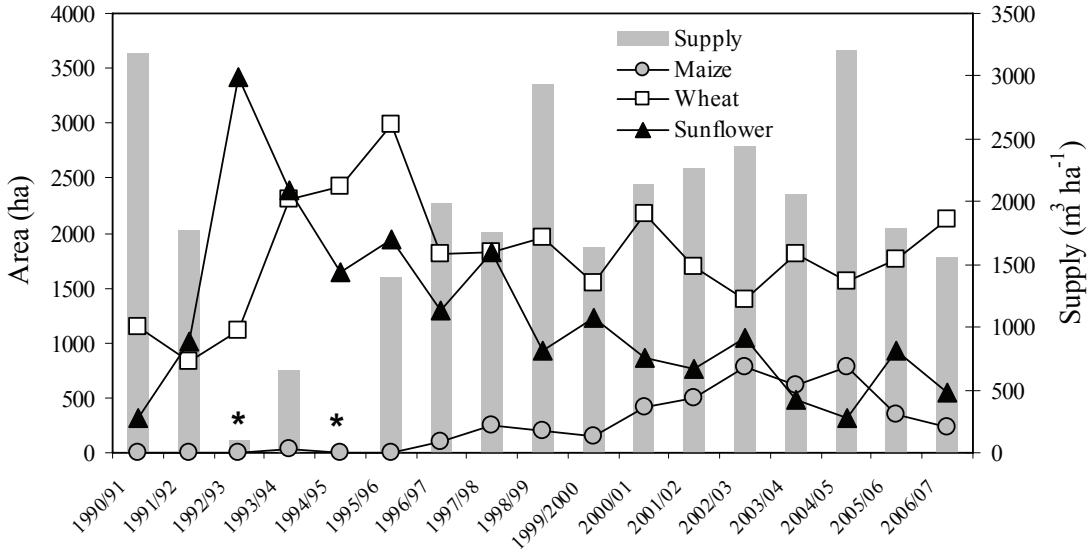

Fig. 2. Evolution of the area cultivated with maize, wheat and sunflower (ha) and irrigation water allocated (m^3 ha^{-1}) in GCIS from 1991/1992 until 2006/2007 irrigation season. *During 1992/1993 and 1994/1995 irrigation depth applied was practically null.

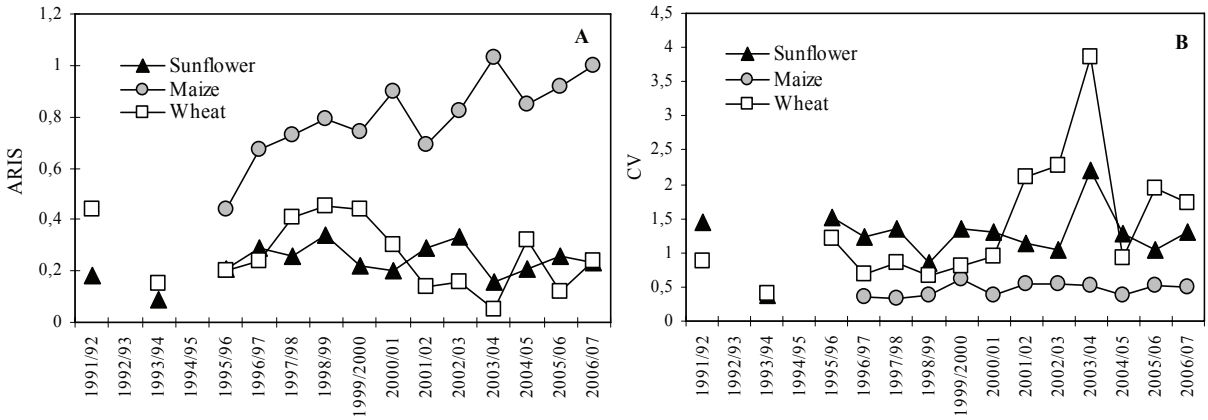

Fig. 3. Evolution of ARIS (A) and coefficient of variation (B) values for sunflower, wheat and maize from 1991/1992 to 2006/2007 irrigation season.

CYR values for sunflower ranged between 20% and 70% depending on the annual rainfall, although the average was 40%, evidencing that the production obtained during the whole irrigation season was smaller than 50% of that attainable, as observed by Lorite et al. (2004b). This confirms that the farmers are not interested in obtaining the maximum yield, and prefer to allocate the available water to other crops such as cotton or maize.

The IWP for sunflower, wheat and maize is presented in Fig. 4A. The average values were low in the three crops (sunflower, 0.19 €/m^3; wheat, 0.26 €/m^3; maize, 0.24 €/m^3), indicating that the application of irrigation here did not generate an increment in gross income, compared with rainfed production. However, in the last few years sunflower values have reached higher values due to its increase in international market prices. Comparing Fig. 3A and 4A, sunflower obtained similar values of IWP compared with maize, but with a significantly lower irrigation consumption. Thus, a deficit in irrigation for sunflower could be considered as a correct alternative compared with other crops such as maize.

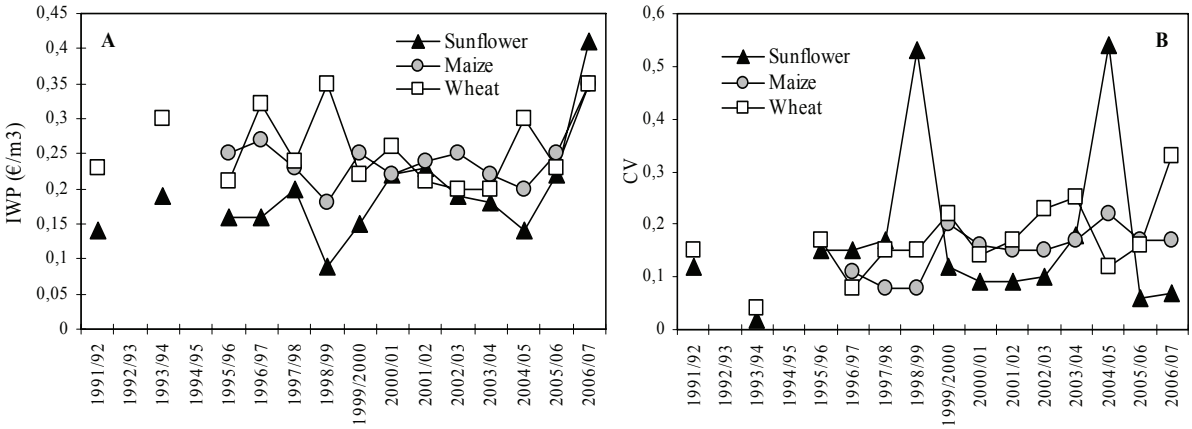

Fig. 4 Evolution of IWP values and coefficient of variation for sunflower, wheat and maize from 1991/1992 to 2006/2007 irrigation seasons.

Seasonal ET variability and crop coefficients for sunflower in the GCIS

The seasonal ET estimated with METRIC for all the plots in the GCIS for the 2004/05 irrigation season ranged from more than 1000 mm for well-irrigated fields, to almost zero for non-agricultural areas (Santos et al., 2008). Crop coefficients for individual fields were estimated as the ratio between METRIC ET and ET_0. Fig. 5A shows real crop coefficient values for different sunflower fields within GCIS, obtained with ET estimated by METRIC.

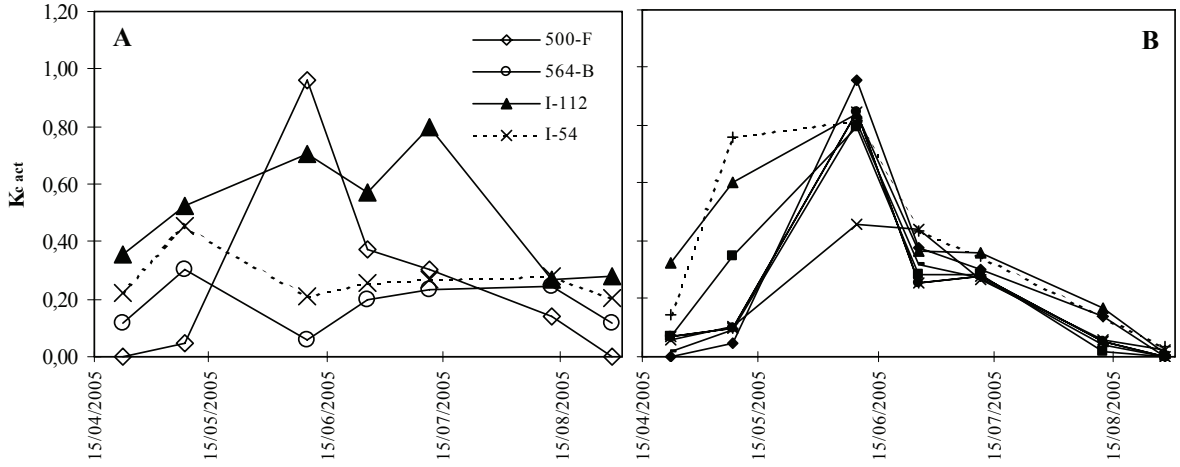

Fig. 5. Real crop coefficient curves for four sunflower fields within GCIS (A) and for one sunflower field having enough size to contain more than one thermal pixel with valid METRIC ET estimates (B).

ET variability was high in sunflower, with a variation coefficient of 0.28, while average ET was low (378 mm). This high variability in actual ET can be explained by the plot to plot variability in irrigation and crop management in the GCIS, as characterized previously by Lorite et al. (2004b).

In the plots with enough size to contain more than one thermal pixel with valid METRIC ET estimates, the ET variability within fields was assessed (Fig. 5B). The variation coefficient within fields

for sunflower was 0.13, which means higher variations than 160 mm (44% of seasonal ET) within a sunflower field, caused by emergence problems, very limited irrigation applied, etc.

In conclusion**,** the study of performance indicators for sunflower at the GCIS showed that this crop is frequently under a clear water stress, and this irrigation management has an impact on yield, which is usually lower than expected. The performance indicators analysed indicated a high variability during the irrigation seasons and between different sunflower fields. The high variability was confirmed with remote sensing techniques using the METRIC model to obtain actual ET measures.

In spite of the low level of inputs provided to the crop, irrigation productivity for sunflower in the last few years has provided similar values than for other crops such as maize, but with very low irrigation requirements.

ACKNOWLEDGEMENTS

We wish to express our gratitude to Mr. J. Berlanga (District Manager), Mrs. S. Carmona (District Technician) for their support. We also wish to thank the farmers of the Genil–Cabra Irrigation Scheme for the information provided.

REFERENCES

Allen, R.G., L.S. Pereira, D. Raes, and M. Smith. 1998. Crop evapotranspiration: guidelines for computing crop water requirements. Irrigation and Drainage paper nº 56. FAO, Rome, Italy.

Allen, R.G., M. Tasumi, and R. Trezza. 2007. Satellite-based energy balance for mapping evapotranspiration with internalized calibration (METRIC)-Model. J. Irrig. Drain Eng. ASCE 133:380-394.

ASCE-EWRI. 2005. The ASCE standardized reference evapotranspiration equation. Environmental and water resources institute of the ASCE standardization of reference evapotranspiration task committee. American Society of Civil Engineers, Reston, 216 p.

Bastiaanssen, W.G.M., M. Menenti, R.A. Feddes, A.A.M. Holtslag. 1998. A remote sensing surface energy balance algorithm for land (SEBAL): 1. Formulation. J. Hydrol. 212-213:198-212.

Bos, M.G. 1997. Performance indicators for irrigation and drainage. Irrig. Drain. Syst. 11:119-137.

García-Vila, M. I.J. Lorite, M.A. Soriano, and E. Fereres. 2008. Management trends and responses to water scarcity in an irrigation scheme of Southern Spain. Agric. Water Manage. (In press).

Gavilán P., I.J. Lorite, S. Tornero, and J. Berengena. 2006. Regional calibration of Hargreaves equation for estimating reference ET in a semiarid environment. Agric. Water Manage. 81:257-281.

Lorite, I.J., L. Mateos, and E. Fereres. 2004a. Evaluating irrigation performance in a Mediterranean environment. I. Model and general assessment of an irrigation scheme. Irrig. Sci. 23:77-84.

Lorite, I.J., L. Mateos, and E. Fereres. 2004b. Evaluating irrigation performance in a Mediterranean environment. II. Variability among crops and farmers. Irrig. Sci. 23:85-92.

Malano, H., and M. Burton. 2001. Guidelines for benchmarking performance in the irrigation and drainage sector. (International Programme for Technology and Research in Irrigation and Drainage) FAO, Rome.

Molden, D.J., and R. Sakthivadivel. 1999. Water accounting to assess use and productivity of water. Water Res. Devel. 15:55-71.

Santos, C., I.J. Lorite, M. Tasumi, R.G. Allen, and E. Fereres. 2008. Integrating satellite-based evapotranspiration with simulation models for irrigation management at the scheme level. Irrig. Sci. 26:277-288.

Tasumi, M, R. Trezza, R.G. Alle, and J. Wright. 2005. Satellite-based energy balance to assess within-population variance of crop coefficient curves. J. Irrig. Drain. Eng. ASCE 131:94-109.

Size reduction of ornamental sunflowers by the application of daminozide

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ABSTRACT

The expansion of floriculture in Brazil in the last years is due to the development and structuring of new markets, professionalization of the components of the production chain and the more extensive purchasing of flowers and ornamental plants. The sunflower (*Helianthus annuus* L.), a species used for oil production, for bird feed and as silage for livestock, has gained a place and distinction among ornamental plants for cut flowers. In order to make ornamental sunflowers suitable for commercial production, technologies are needed that can adapt the species to greenhouse cultivation. Sunflowers are naturally tall plants, which is unsuitable for ornamental purposes. In order to facilitate their production in a protected environment as well as in the field, inhibitors of gibberellin synthesis can be utilized to reduce the size of sunflower plants. The aim of this study was the reduction in size of the ornamental sunflower hybrid BRS Oasis by the application of daminozide (B-Nine 850 PS™) at fifteen days after planting, testing different concentrations. The concentrations evaluated were 4,000, 6,000 and 8,000 $mg.L^{-1}$, which were compared to a control using water. The results obtained demonstrated that the size of the plants treated with the three concentrations of daminozide was smaller than that of the control. Therefore, for economical reasons, the use of 4,000 $mg.L^{-1}$ of daminozide is suggested.

Key words: B-Nine – floriculture – gibberellin inhibitor – *Helianthus annuus* – plant growth regulator – ornamental plant.

INTRODUCTION

The Brazilian market for flowers and ornamental plants has shown a substantial growth in demand and has been expanding in the last few years with the improved quality of products and an increased commercialized volume. It has responded positively to the offering of new products, thereby stimulating research into breeding and cultivation treatments. Sunflowers have a great potential as an ornamental plant because of their short growing cycle and easy propagation, but mainly because they have attractive inflorescences that are much sought after as cut flowers (Dasoju et al., 1998; Anefalos and Guilhoto, 2003). In the local market, although there is no official classification, ornamental sunflower inflorescences of 8-11 cm in diameter are commercialized as small flowers, those of 12-16 cm in diameter as medium flowers, and those more than 16 cm in diameter as large flowers (NAIR MIE NOMI, 2007).

The ornamental sunflower BRS Oasis, developed by Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA), has inflorescences with ornamental characteristics (Oliveira and Castiglioni, 2003), which allow it to be used as a cut flower. However, its size, which can reach up to 3 m in height, is unsuitable for its eventual purpose. To have a production and offering of ornamental sunflowers as cut flowers during the whole year in regions with a temperate climate, where frost occurs, it is necessary to grow the plants in a protected environment or greenhouse, demanding the development of techniques to reduce the size of the plants.

Inhibitors of gibberellin synthesis are widely used in floriculture to reduce the size of various ornamental plants (Whipker, 2001). One the most common agents for this purpose is daminozide (N-dimethylaminosuccinamic acid) (Weaver, 1972; Fahl et al., 1985). The ideal concentration of the plant growth regulator depends on the plant species, variety, number of applications made and size of the plant at the time of application (Lopes, 1977). Daminozide has been recommended for the size reduction of ornamental plants at concentrations varying from 2,000 to 8,000 $mg.L^{-1}$ (Hertwig, 1977; Nell et al., 1980).

The aim of this study was to reduce the size of ornamental sunflower plants, variety BRS Oasis, by the application of daminozide (B-Nine 850 PS™) at fifteen days after planting, testing different concentrations.

MATERIALS AND METHODS

This investigation was carried out in the period of February to May of 2006 in the municipality of Fazenda Rio Grande (PR, Brazil), located 25º37'32"S and 49º15'29" W and having an altitude of 910 m. The effect of the application of the plant growth regulator daminozide (B-Nine 850 PS™) was studied at concentrations of 4,000, 6,000 and 8,000 mg.L^{-1}, in the BRS Oasis hybrid of the ornamental sunflower, *Helianthus annuus* L., which is single-headed with male sterility, and has yellow disc florets and brownish ray florets. In the control treatment, water was applied under the same conditions in which the plant growth regulator was applied. The application of the regulator was effected at fifteen days after planting, when the plants showed two pairs of definitive leaves.

A complete randomized block design was used with four treatments and four replications, where ten plants were studied per parcel. The variables analyzed were: plant height, determined from the level of the soil to the point on the stem of the inflorescence; the stem diameter, determined at fifty centimeters below the inflorescence; and the head diameter. These variables were evaluated when 50% of the plants were with completely expanded ray florets and all the disc florets visible, corresponding to phenological stage R5.5 (Schneiter and Miller, 1981). The data obtained were submitted to analysis of variance. Initially, the variances of the treatments were determined with respect to their homogeneity by Bartlett's test. All variances were shown to be homogeneous, where the transformation of the data was not necessary and the means of the treatments were evaluated using the F test. When the results revealed the existence of significant differences between the means of the treatments, these were compared by Tukey's test at a significance level of 5%.

RESULTS AND DISCUSSION

The evaluations were carried out at 62 days after planting. There was a significant difference among the treatments for all the variables analyzed (Table 1). Although there was a significant difference in the height of the plants treated with the three concentrations of daminozide versus the control, no difference was seen between concentrations. The results agree with those of various authors who obtained a significant reduction in size of ornamental plants utilizing concentrations between 4,000 and 8,000 mg.L^{-1} (Cathey, 1975; Nell et al., 1980; El-Keltawi et al., 1996). Similarly, these findings agree with authors who obtained significant size reductions in ornamental plants utilizing an application of daminozide in *Ruellia colorata* L. at a concentration of 4,000 mg.L^{-1}, achieving a decrease in stem height of 13.85% (Carlucci, 1991) and in *Viola* × *wittrockiana* L applying daminozide at a concentration of 5000 mg.L^{-1}, obtaining a decrease of 18.94% in stem height (Gliožeris et al., 2007).

Table 1. Stem height (SH), stem diameter (SD) and head diameter (HD) of the ornamental sunflower BRS Oasis, after application of daminozide at different concentrations 15 days after planting (Fazenda Rio Grande, PR) in May 2006**.**

Concentration of daminozide (mg.L^{-1})	SH[1] (m)	SD[1] (cm)	HD[1] (cm)
0	2.300 a	1.274 a	9.20 a
4000	2.010 b	1.185 b	8.33 b
6000	1.986 b	1.144 b	8.21 b
8000	1.861 b	1.119 b	8.13 b
DMS	0.12	0.50	0.4

[1]Means followed by different letter in the column differ statistically, based on Tukey's test at the 5% level of probability.

Height diameter (Table 1) at all the concentrations tested was smaller than that of the control. Although there are no published reports on the effect of reducing stem diameter on the quality and life of post-harvest flowers of ornamental sunflower, this has been determined for chrysanthemum (*Dendranthema grandiflora* Tzevelev) (Nardi et al., 2001).

The head diameter (Table 1) at all the concentrations tested was smaller than that of the control. Considering that the ornamental sunflower market pays differently for flowers of smaller diameter, this could be a problem. However, according to the informal local standard for classification of ornamental

sunflowers (NAIR MIE NOMI, 2007) the diminution of the diameter of the flowers would permit them to be classified as medium inflorescences.

Considering that the reduction in the size of the plants was similar at the three concentrations of daminozide tested, it is suggested that, after the evaluation of the post-harvest quality of the flowers, 4,000 mg L^{-1} of daminozide be utilized for economic reasons.

REFERENCES

Anefalos, L.C., and J.J.M. Guilhoto. 2003. Estrutura do mercado brasileiro de flores e plantas ornamentais. Agricultura em São Paulo 50(2):41-63.

Carlucci, M.V., J.I. Fahl, and L.A. Matthes. 1991. Efeito de retardantes de crescimento em *Ruellia Colorata.* Revista Brasileira de Fisiologia Vegetal 3(2):103-106.

Cathey, H.M. 1975. Comparative plant growth-retarding activities of ancymidol with ACPC, phosfon, chlormequat, and SADH on ornamental plant species. HortSci. 10:204-216.

Dasoju, S., M.R. Evans, and B.E. Whipker. 1998. Paclobutrazol drenches control growth of potted sunflowers. HortTechnol. 8:235-237.

El-Keltawi, N.E., G.T. MOUSA, and B.S. Makary. 1996. Regulation of chrysanthemum growth using GA_3 and Alar to overcome salinity depressions. In: International Symposium on Medicinal and Aromatic Plants. Acta Horticulturae 426:657-669.

Fahl, J.I., S.L.F. Cattaneo, and J.E. Soares. 1985. Efeitos do paclobutrazol no crescimento e na floração de crisântemo *(Chrysanthemum moriflorum* Ramat). Planta Daninha 9(1/2):52-55.

Gliožeris, S., A. Tamošiūnas, and L. Štuopytė. 2007. Effect of some growth regulators on chlorophyll fluorescence in *Viola* × *wittrockiana* 'Wesel Ice'. Biologija 53(2):24-27.

Hertwig, K.V. 1977. Manual de herbicidas desfolhantes, dessecantes e fitorreguladores. Ed. Agronômica Ceres, São Paulo, Brazil. 480 p.

Lopes, L.C. 1977. O Cultivo do Crisântemo. Boletim de extensão, 22. Universidade Federal Viçosa, Brazil. 12 p.

NAIR MIE NOMI, 2007. Begônia Flor e Arte, Curitiba, Paraná, Brazil.

Nardi, C., R.A. Bellé, C.M. Schmidt, and K.A. Toledo. 2001. Qualidade de crisântemo (*Dendranthema grandiflora* Tzevelev.) cv. Snowdon em diferentes populações e épocas de plantio. Ciência Rural, Santa Maria vol. 31, n.6.

Nell, T.A., G.J. Wilfred, and B.K. Harbaugh. 1980. Evaluation of application methods of ancymidol and daminozide for height control of Chrysanthemum. HortSci. 15:810-811.

Oliveira, M.F., and V.B. Castiglioni. 2003. R Girassol Colorido para o Brasil. EMBRAPA - CNPSO, dez/2003. Londrina, PR, Brazil.

Schneiter, A.A., and J.F. Miller. 1981. Description of sunflower growth stages. Crop Sci. 21:901-903.

Weaver, R.J. 1972. Plant Growth Substances in Agriculture. W.H. Freeman, San Francisco, USA. 594 p.

Whipker, B.E., J.L. Gibson, and T.J. Cavins. 2001. Diagnosing problems due to plant growth regulators. Commercial Floriculture Research and Extension, North Carolina State University 1:1-5.

Participatory on-farm sunflower variety evaluation in northern and eastern Uganda

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ABSTRACT

Sunflower genotypes were evaluated on farmers' fields between 2005 to 2007 growing seasons in eastern and northern parts of Uganda. The evaluation covered five districts. Researchers worked with extension and private sector staff whereby the latter identified the farmers and also helped in monitoring the trials. Each genotype was planted in a single plot of 5m in length with 4 to 6 rows per farmer field. For each variety, the plot was divided into two blocks so that one block received N60 P30 kg/ha using urea and single superphosphate as source of nitrogen and phosphorous, respectively; meanwhile, the other block had no fertilizer application to compare the effect of fertilizer application on the different genotypes. The main data recorded included seed yield (kg/ha), plant height, head diameter, 1000 seed weight, uniformity, vigour, lodging and maturity. Results showed that the better genotypes were sunflower hybrids from South Africa which included Pan 7351 from Pannar Seed Company; AGSUN 8251 and AGSUN 5383 from AGRICOL Seed Company; DKF68-22 and DK4040 from Monsanto Seed Company. It was observed that plots where fertilizers were applied improved their seed yield, plant height and head diameter significantly. The highest seed yield was by DKF68-22 with 3,556 kg/ha from Bunambutye, in Sironko district. In most areas, hybrids such as DK4040, DKF68-22, and AGSUN 8251 performed better than PAN7351, which was officially released in Uganda in 2003. Because of their good performance from these trials on farmers' fields, those three new varieties were also officially released for commercial production in Uganda in 2007.

Key words: farmer participation – sunflower– variety evaluation

INTRODUCTION

The cultivated sunflower (*Helianthus annuus* L.) ranks with soybean, rapeseed and groundnut as one of the four most important annual crops in the world grown mainly for edible oil. Sunflower is grown in around 20 million hectares in the world. Average grain yield in the world is around 1.1 t/ha, varying from 0.5 to 3.6 t/ha (Krizmanic et al., 2006).

In Uganda, sunflower has become the most important oilseed crop. The other oilseed crops are sesame, soybean, groundnut, and oil palm. It is mainly grown in the eastern and northern parts of the country, where it has become an integral part of the farming system. The crop is primarily cultivated for its grain, which is used for oil extraction and production of animal feeds. The extracted oil is generally used as a cooking medium and in the manufacture of soap.

Uganda's interest in sunflower production dates back to the late 1940's (Bua and Molo, 1985). However, research activities were minimal and varietal evaluation was on imported hybrids and open-pollinated varieties. Up to the late 1990's, evaluation of sunflower varieties and hybrids were done on trial verification centers (TVCs) located across regions in the country. These trial centers, owned by the government, although good for understanding the performance of the different varieties across locations, do not present the attitude and criteria of selecting varieties by farmers. Selection of varieties by farmers themselves leads to a wider acceptability of that variety. Researchers in most cases rely on results from on-station research when evaluating variety performance. The problem is that on-station conditions rarely mirror the farmer's production constraints, that include demands of manual cultivation, input shortages and limited labor supply and yet they have to grow the crops (Laker-ojok, 1994). The Vegetable Oil development Project (VODP) impact assessment report (VODP, 2007) indicated that the reasons for choice of type of sunflower variety demanded by farmers in Uganda were higher yields (60%), tolerance to weather and diseases (18%), high oil content (13%) and fast maturity period (9%).

The objective of this paper is to present the results and the methodology used in the participatory evaluation of the sunflower varieties planted together with the farmers in their own fields.

MATERIALS AND METHODS

The evaluation trial undertaken on farmers' fields was carried out between 2005 to 2007 growing seasons in eastern and northern parts of Uganda. A collaborative approach was undertaken whereby agricultural extension staff and private seed sector staff working in the localities of the farms identified the farmers or farmer groups who were active in cultivating sunflower in their area. Each variety was planted in single plots of 4 or 6 rows at a length of 5 m. In some cases, two blocks were organized so that one block was applied with fertilizer at a rate of N60 P30 kg/ha using urea and single super phosphate as source of nitrogen and phosphorous, respectively, whereas the remaining block had no fertilizer application. Spacing followed was 75 x 30 cm with one plant per hole.

Farmers participated in planting together with the extension staff of the area and the researchers involved. Weeding and thinning were the responsibility of the farmer. Planting was done in at least four districts per season and in at least two farmer fields per district. Farmers also participated in selecting criteria for identifying better varieties before the actual yield was recorded. The main districts covered were Lira, Apac in northern Uganda, Soroti, Kumi, Bukedea and Sironko in eastern Uganda. Data recorded were: head diameter, plant height, uniformity, lodging, vigour of the plot, leaf spot disease, 1000 seed weight, and yield per plot converted to yield per hectare. To avoid damage by birds, the locals were encouraged to scare the birds. Head diameter, plant height, uniformity, lodging and vigour were recorded during physiological maturity, while leaf spot was recorded two weeks after flowering. 1000 seed count and yield were recorded in the laboratory after seed cleaning. Ten to fifteen plants were measured for plant height and head diameter.

The scales used for scoring sunflower data if no actual measurement taken were:

Plant height:	1= Very short	9= Very tall
Lodging:	1= No lodging	9= Completely lodged
Head diameter:	1= Very big head	9= Very small head
Vigour:	1= Very vigorous	9= Very poor vigour
Maturity:	1= Very early	9= Very late
Uniformity:	1= Very uniform	9= Very variable
Leaf spot disease	1= immune	9= completely diseased

RESULTS AND DISCUSSION

In the evaluation undertaken in the second season of 2005 in Apac district, the best hybrid was PAN7351 in the plots where no fertilizer or some fertilizer was applied (Table 1). Where the fertilizer was applied, it had yields of 2,067 kg/ha. Other hybrids that also did well were DK4040, DKF68-22 and AGSUN 8251, which originated from South Africa. All these hybrids have already been released officially for commercial production in Uganda. PAN 7351 was also the tallest hybrid (183 cm) except for AGSUN 8251 which was the tallest under the fertilized condition with 186 cm. Where fertilizer was applied, there was a significant yield increase in some hybrids, especially the high yielding hybrids. Fertilizer increased plant height and head diameter (Table 1).

In Lira district, the hybrid DKF68-22 recorded 2,333 kg/ha as the highest yielder where no fertilizer was applied and AGSUN 8251 recorded the highest yield with 2,600 kg/ha where fertilizer was applied. These were indicators of good hybrids to compete with PAN 7351. Fertilizer also increased plant height, head diameter and vigour of the plants.

Table 1. Performance of yield and other components of ten sunflower hybrids/varieties as affected by fertilization at Loro (Apac District) in the second season of 2005.

Genotype	Country of origin	Seed yield (kg/ha)[1]		Plant Height (cm)		Head diameter (cm)		Vigour[2]		Uniformity[3]		Lodging[4]	
		N0[5]	N1	N0	N1	N0	N1	N0	N1	N0	N1	N0	N1
PAN 7351	South Africa	1,600 (1)	2,067 (1)	183.5	183.5	14.0	14.1	4.5	4.5	3.0	4.0	2.0	2.0
DK 4040	South Africa	1,233 (5)	1,567 (4)	143.5	160.5	13.8	14.8	4.5	3.5	2.0	2.5	1.5	1.5
DK 68-22	South Africa	1,567 (2)	1,800 (3)	167.8	166.5	15.0	14.3	5.0	4.5	2.5	2.0	2.0	1.5
NSH 160	India	1,100 (6)	1,367 (6)	139.8	156.8	14.6	15.8	5.5	5.0	3.5	2.5	4.0	4.0
S 3503	India	967 (8)	967 (9)	163.5	170.3	16.9	17.6	5.5	4.0	2.5	3.0	4.0	2.0
AGSUN 4683	South Africa	633 (10)	767 (10)	134.5	148.8	12.9	15.3	6.5	4.0	3.5	3.0	2.0	2.5
AGSUN 5551	South Africa	1,400 (4)	1,567 (4)	148.3	176.0	14.5	15.6	4.5	3.0	2.5	1.5	2.0	1.5
ASUN 8251	South Africa	1,467 (3)	1,867 (2)	137.3	186.4	13.3	16.4	4.5	3.0	2.0	2.0	1.5	1.5
8998	Kenya	734 (9)	1,067 (8)	125.8	160.0	12.2	15.2	6.0	5.0	3.5	4.0	4.0	2.5
SUNFOLA	Uganda	1,000 (7)	1,267 (7)	162.3	175.5	12.6	13.6	5.5	45	4.0	3.5	4.5	2.5
Mean		**1,170**	**1,430**	**150.6**	**168.4**	**14.0**	**15.4**	**5.2**	**4.1**	**2.9**	**2.8**	**2.8**	**2.2**

[1]Numbers in brackets indicate the ranking of the genotypes at each treament
[2]From 1= Very vigorous to 9= Very poor vigour
[3]From 1= Very uniform to 9= Very variable
[4]From 1= No lodging to 9= completely lodged
[5]N0=no fertlization, N1=fertilization with N60 P30 kg/ha

Table 2. Performance of yield and other components of ten sunflower hybrids/varieties as affected by fertilization at Bar Apwo (Lira District) in the second season of 2005.

Genotype	Country of origin	Seed yield (kg/ha)[1]		Plant Height (cm)		Head diameter (cm)		Vigour[2]		Uniformity[3]		Lodging[4]	
		N0[5]	N1	N0	N1	N0	N1	N0	N1	N0	N1	N0	N1
PAN 7351	South Africa	2,267 (2)	2,267 (3)	224.0	223.4	18.8	18.4	4	3	4	3	2	1
DK 4040	South Africa	2,267 (2)	2,267 (3)	198.0	217.0	16.8	17.0	3	3	3	3	1	1
DK 68-22	South Africa	2,331 (1)	2,400 (2)	209.0	218.0	13.6	18.0	4	4	3	2	2	1
NSH 160	India	2,067 (5)	1,667 (7)	154.0	174.0	13.0	16.2	5	5	2	2	1	2
S 3503	India	1,733 (7)	1,933 (6)	190.0	204.0	14.4	14.4	5	4	3	2	1	1
AGSUN 4683	South Africa	1,000 (10)	1,000 (10)	157.0	168.0	15.4	13.2	5	6	3	3	1	2
AGSUN 5551	South Africa	2,133 (4)	2,100 (5)	164.0	186.0	12.2	13.4	5	4	2	2	1	2
ASUN 8251	South Africa	1,067 (9)	2,600 (1)	158.0	185.0	14.4	16.6	5	3	2	2	1	1
8998	Kenya	2,000 (6)	1,333 (9)	137.0	149.0	13.4	9.2	6	6	4	3	1	2
SUNFOLA	Uganda	1,200 (8)	1,400 (8)	168.0	192.0	16.4	14.6	5	5	4	5	2	2
Mean		**1,807**	**1,897**	**175.9**	**191.6**	**14.9**	**15.7**	**4.6**	**4.3**	**3.8**	**2.7**	**1.2**	**1.4**

[1]Numbers in brackets indicate the ranking of the genotypes at each treament
[2]From 1= Very vigorous to 9= Very poor vigour
[3]From 1= Very uniform to 9= Very variable
[4]From 1= No lodging to 9= completely lodged
[5]N0=no fertlization, N1=fertilization with N60 P30 kg/ha

During the first season of 2006 (Table 3) at Kasoka in Bukedea district, DKF68-22 had the highest yield of 2,067 kg/ha and it had also good vigour and head diameter. The hybrids that showed very good plant vigour were PAN7351, DK4040, DKF68-22 and AGSUN 5383, recording a value of one.

Table 3. Evaluation of sunflower hybrids/varieties at Kasoka (Bukedea District) during the first season of 2006.

	Yield (kg/ha)			Plant height (cm)		Head diameter (cm)		Vigour[3]		Maturity[4]	
Hybrid/Variety	N0[1]	N1	Mean[2]	N0	N1	N0	N1	N0	N1	N0	N1
PAN 7351	1,822	1,200	1,511 (3)	160	166	27.9	23.0	2	1	5	6
DK 4040	1,222	1,311	1,267 (8)	135	152	22.4	24.0	1	1	5	6
DKF 68-22	2,000	2,133	2,067 (1)	166	167	20.6	21.7	3	1	5	5
NSH 160	800	1,600	1,200 (10)	148	149	19.3	19.4	4	4	4	4
S 3503	1,267	1,644	1,456 (6)	147	172	19.3	21.5	4	3	4	4
8998	711	1,111	911 (15)	130	161	18.0	19.7	4	3	4	4
AGUSUN 5282	1,044	1,311	1,178 (11)	163	199	17.0	18.6	3	1	6	6
AGSUN 5383	1,333	1,644	1,489 (4)	153	179	15.4	19.9	3	3	4	4
AUSIGOLD 4	1,644	1,244	1,444 (7)	170	171	21.2	19.1	3	4	4	4
AGSUN 8251	1,689	1,244	1,467 (5)	193	161	17.0	18.2	4	4	4	4
Hysun 33	1,711	1,556	1,634 (2)	172	173	18.0	18.2	3	4	6	5
Hysun 39	622	844	733 (16)	166	172	20.0	17.8	4	4	5	6
Hysun 44	533	489	511 (17)	151	152	17.6	18.9	5	4	6	5
Sunrise 1	978	1,511	1,245 (9)	154	173	13.0	19.3	4	5	4	4
Sunrise2	889	1,022	956 (14)	157	168	12.6	15.9	5	5	4	3
Sunrise 3	978	978	978 (13)	147	148	13.2	19.4	4	4	4	4
Sunfola	889	1,200	1,045 (12)	162	190	13.0	18.4	5	4	4	4
Mean	**1,184**	**1,297**	**1241**	**157**	**167**	**18.0**	**19.6**	**4**	**3**	**5**	**5**

[1]N0=no fertlization, N1=fertilization with N60 P30 kg/ha
[2]Numbers in brackets indicate the ranking of the genotypes at each treament
[3]From 1= Very vigorous to 9= Very poor vigour
[4]From 1= Very early to 9= Very late

In Table 4, evaluation of sunflower was undertaken at Bunambutye in Sironko district in the first season of 2006. This site recorded the highest seed yield. The genotype DKF68-22 again had the highest seed yield of 3,556 kg/ha followed by Sunrise 1 with 3,333 kg/ha. Due to high soil fertility in this area, plant height, head diameter, and 1,000 seed weight were high for most genotypes. Plant heights of over 270 cm and head diameter of over 28 cm were recorded in this area.

Table 4. Evaluation of sunflower hybrids/varieties at Bunambutye (Sironko District) during the first season of 2006.

	Seed yield (kg/ha)			Plant height (cm)		Head diameter(cm)		1000-seed weight (g)		Vigour[1]		Maturity[2]			
Hybrid/Variety	N0[3]	N1	Mean[4]	N_0	N_1	N_0	N_1	N_0	N_1	N0	N1	N0	N1	DF[5]	DM[5]
PAN 7351	2,444	2,556	2,667(4)	224	232	20.9	21.3	67.8	66.0	3	1	4	3	58	93
DK 4040	2,000	2,111	2,222(8)	180	203	22.9	23.2	81.4	80.4	2	2	3	2	55	98
DK F 68-22	3,556	3,556	3,556(1)	229	227	20.4	19.3	58.6	58.8	1	1	1	1	59	100
NSH 160	2,222	2,222	2,222(8)	204	207	20.5	19.1	70.2	64.6	5	5	3	5	47	83
S 3503	2,667	2,667	2,667(4)	230	227	23.3	21.5	64.6	59.0	3	3	2	2	53	89
8998	1,556	1,556	1,556(13)	188	177	20.6	20.2	69.2	71.0	5	6	4	5	52	89
Sunf. SAARI	1,778	1,889	2,000(11)	238	252	21.1	21.3	62.0	68.4	4	2	4	7	51	86
Sunf. UOSPA	1,778	1,667	1,556(13)	231	280	22.4	20.6	69.8	75.8	3	2	4	3	55	89
Hysun 33	2,667	2,889	3,111(3)	247	247	20.2	19.1	64.2	69.8	2	1	2	3	59	97
Hysun 39	2,222	2,445	2,667(4)	262	280	20.2	21.9	69.0	59.0	1	1	3	2	60	99
Hysun 44	2,222	2,045	1,867(12)	251	219	24.2	28.8	49.6	62.6	1	1	4	2	63	102
Sunrise 1	3,778	3,556	3,333(2)	265	241	20.0	20.7	74.0	71.8	3	3	2	3	55	96
Sunrise2	2,667	2,572	2,477(7)	212	198	18.7	19.7	60.4	64.6	5	4	5	4	49	86
Sunrise 3	2,667	2,445	2,222(8)	220	227	21.8	22.0	57.8	55.4	2	2	2	2	54	93
Mean	**2,441**	**2,445**	**2,461**	**230**	**227**	**21.3**	**21.2**	**66.2**	**65.6**	**2.4**	**2.9**	**3.1**	**3.1**	**55**	**93**

[1]From 1= Very vigorous to 9= Very poor vigour
[2]From 1= Very early to 9= Very late
[3]N0=no fertlization, N1=fertilization with N60 P30 kg/ha
[4]Numbers in brackets indicate the ranking of the genotypes at each treatment
[5]DF=Days to flowering; DM=Days to maturity

Table 5. Seed yield (kg/ha) for sunflower on-farm variety trials across locations during the first season of 2007[1].

	Ocamon yang (Lira)	Adeko kwok (Lira)	Atik (Apac)	Atana (Apac)	Bar-Apwo (Lira)	Kasoka (Bukedea)			Nyero (Kumi)		
						N0[2]	N1[2]	Mean	N0[2]	N1[2]	Mean
DKF 68-22	1,200(8)	1,600(4)	1,000(9)	1,200(4)	2,667(1)	1,867	2,067	1,967(6)	2,200	1,677	1,934(3)
Alexandra	1,467(6)	800(9)	800(12)	666(10)	1,533(3)	1,800	2,000	1,900(7)	2,000	2,067	2,034(2)
Arena	1,667(3)	1,667(3)	1,600(5)	1,333(3)	1,533(3)	2,000	2,000	2,000(4)	2,067	1,667	1,867(5)
NKMY	267(15)	400(10)	1,133(7)	867(8)	867(11)	667	533	600(11)	733	1,000	867(10)
NKAR	733(11)	333(11)	1,000(9)	800(9)	600(13)	1,200	867	1,034(9)	1,600	1,533	1,567(7)
AGSUN 4672	400(14)	333(11)	-	333(12)	267(14)	533	400	467(12)	733	400	567(12)
AGSUN 5282	1,267(7)	1,267(5)	1,800(4)	1,200(4)	1,200(6)	2,467	2,200	2,334(1)	1,333	1,467	1,400(8)
AGSUN 5383	1,800(2)	1,267(5)	2,000(2)	1,467(2)	933(10)	2,467	800	2,200(2)	2,000	1,667	1,834(6)
AGSUN 5551	1,000(9)	1,200(7)	1,600(5)	1,133(6)	1,133(7)	2,600	1,467	2,000(4)	1,733	2,533	2,133(1)
AGSUN 8251	1,867(1)	2,600(1)	2,333(1)	1,533(1)	1,467(5)	2,533	2,000	2,167(3)	1,733	2,067	1,900(4)
AGSUN 8751	733(11)	200(13)	1,000(9)	467(11)	-	2,333	1,200	1,000(10)	933	467	700(11)
Sunrise	467(13)	1,000(8)	-	-	1,600(2)	800	-	-	-	-	-
Hysun 33	1,600(4)	-	-	-	1,133(7)	-	-	-	-	-	-
PAN 7351	1,000(9)	1,867(2)	2,000(2)	1,133(6)	867(11)	-	-	-	-	-	-
8998	1,533(5)	-	1,067(8)	200(13)	1,000(9)	1,333	1,200	1,267(8)	1,200	1,133	1,167(9)
Mean	**1,133**	**1,118**	**1,444**	**949**	**1,200**	**1,678**	**1,478**	**1,578**	**1,522**	**1,472**	**1,498**

[1]Numbers in brackets indicate the ranking of the genotypes at each treatment
[2]N0=no fertlization, N1=fertilization with N60 P30 kg/ha

During the first season of 2007 (Table 5), yield data were recorded and compared across locations in eastern and northern parts of Uganda. In Lira and Apac, which are located in northern Uganda, AGSUN 8251 had the highest yields in four locations while hybrid DKF68-22, Arena, and AGSUN 5383 were considered stable across locations. Kasoka (Bukedea) on-farm trial had the highest mean yield of 1,578 kg/ha followed by Nyero (Kumi district) with 1,497 kg/ha. As a result of this on-farm trial and other trials evaluated on government trial centers across locations, three hybrids were officially released in Uganda for commercial production on top of PAN 7351, which was earlier released in 2003. These new hybrids released are: DKF68-22, DK4040 and AGSUN 8251.

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REFERENCES:

Bua, A., and R. and Molo. 1985. A review of research on oil crops in Uganda. In: Proc. Second oil crops Network Workshop, Hydderabad, India.

Krizmanic, M, A. Mijic, I, Liovic, M. Bilandzic, and T. Duvnjak. 2006. Sunflower breeding at the Agricultural Institute Osijek. Helia 29(44): 153-158.

Laker-Ojok, R. 1994. The potential returns to oilseeds research in Uganda: The case of groundnuts and sesame. MSU International Development Working Paper No. 45. Dept. of Agric. Economics, Michigan State University.

VODP Progress Report. 2007. Impact Assessment Study. By: Bergen Consultant (U) Ltd.

Initial growth of sunflower in soils with high concentrations of boron and heavy metals

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ABSTRACT

Phytoremediation studies have been conducted in an area contaminated by heavy metals, located in Piracicaba - SP, Brazil. This area was contaminated accidentally by the addition of auto scrap shredding to the soil and was limed later to reduce heavy metal mobility in the environment. Previous characterization showed that it also presents high concentration of boron, which has limited the initial plant development of some species. As sunflower plants require a high boron supply and the literature describes its use in the phytoremediation of soils contaminated with heavy metals under some conditions, the aim of this work was to evaluate its potential for the remediation of this area. In the present study, the results of preliminary tests are presented, aiming at the evaluation of sunflower plant germination and its initial development when cultivated in the contaminated soil described. Two sunflower hybrids were sown in soils treated with different rates of boron and in the soil from the contaminated area in study. The results showed that sunflower plants had a normal initial development, even in the soil from the contaminated area. Therefore, sunflower is a promising crop and further studies will be developed to evaluate the sunflower efficiency in phytoextraction or phytostabilization of heavy metals in areas where boron contamination also occurs, as is the case in the study area.

Key words: boron – contamination – *Helianthus annuus* L. – phytoremediation – phytotoxicity.

INTRODUCTION

Boron is an important micronutrient, but when found in soils at high phytoavailable concentrations it can cause phytotoxicity. Many crops are very sensitive to boron toxicity, showing severe symptoms, such as yellowing of the leaf tips and stunted growth. High concentrations of B may occur naturally in the soil or in groundwater, or can be added to the soil from mining, fertilisers, or irrigation water (Nable et al., 1997). Another anthropogenic source of boron in soils is the use of wastes as fertilizers. Some industrial residues, such as from steelmaking, should be highlighted, once they frequently contain boron and heavy metals in their composition.

Nable et al. (1997) consider that B concentrations in soil higher than 5 mg dm^{-3} are toxic to the plants. However, this value could vary as a function of plant species, sampling time, soil characteristics, among others. Studies carried out in Brazil, testing different soils and species, showed that toxics levels of Boron vary from 1.8 to 8.3 mg kg^{-1} (Mariano et al., 2000; Fageria, 2000; Lima et al., 2007).

In Piracicaba city, located in São Paulo state, Brazil, there is an area which was contaminated accidentally by the addition of auto scrap shredding to the soil and was limed later to reduce heavy metal mobility in the environment. The environmental protection agency of the state isolated the area due to its high heavy metal concentration, and allowed researchers to run remediation studies using the soil from this area. The soil presents the following concentrations of heavy metals (mg kg^{-1}): 8 of Cd; 268 of Pb; 160 of Cu; 103 of Cr; 47 of Ni and 2454 of Zn. So, we are concentrating our efforts in the phytoremediation of Zn and Pb. However, previous studies showed that the high concentrations of available boron found (4 to 14 mg kg^{-1}) were limiting plant development and/or causing the death of some plant species.

Sunflower plants (*Helianthus annuus* L.), when compared with other species, require a large supply of boron. That is why this species is frequently used as an indicator plant for boron deficiency (Schuster and Stephenson, 1940). In addition, sunflower is able to absorb heavy metals selectively (Tan, 2000), presenting potential to be used to phytoremediate (phytoextract and/or phytostabilize) contaminated areas.

Based on the following statements: (i) sunflower plants require large boron supply; (ii) the major factor that limits initial plant development in the contaminated area was the high concentration of boron

in the soil; (iii) sunflower plants present potential to phytoremediate areas contaminated with heavy metals; we decided to evaluate the initial development of two sunflower hybrids cultivated in a test soil with increasing rates of boron and in the soil from the contaminated area in Piracicaba city.

MATERIALS AND METHODS

Two hybrids of sunflower, Helio 250 and Helio 358, were sown in pots containing 500 g of soil, in a greenhouse located in Embrapa Environment Unit, Jaguariúna city, SP, Brazil. The soil samples used corresponded to subsurface soil samples (B horizon) of a typical oxisol and surface soil samples (A horizon) of the area contaminated (CA) with heavy metals and boron, located in Piracicaba (Cambisol). The experimental design was completely randomized with three replicates, and the treatments were arranged in a 2x6 factorial design, that is, two sunflower hybrids and six boron rates.

The evaluated variables were plant height, dry matter, shoot boron concentration and soil boron concentration (extracted by hot water). The data were submitted to variance analysis by the SISVAR software and the means of the treatments were compared by Tukey test (5%).

The experiment was performed based on the following treatments, corresponding to B rates added to the oxisol, for each hybrid evaluated: Control – Co (oxisol, no fertilization); Boron 0 – B0 (oxisol + mineral fertilization, no boron added); Boron 2 – B2 (oxisol + mineral fertilization + 2 kg ha^{-1} of B); Boron 4 – B4 (oxisol + mineral fertilization + 4 kg ha^{-1} of B); Boron 8 – B8 (oxisol + mineral fertilization + 8 kg ha^{-1} of B) and Contaminated Area – AC (soil from contaminated area, no fertilization). Boron was added in the form of boric acid.

After filling the pots with soil, lime was added to the oxisol treatments, in order to raise the base saturation to 70%, as indicated by Ambrosano et al. (1996). The soil from the contaminated area presented a pH of 7.4, so it was not necessary to lime it. Then, all the pots were incubated for fifteen days, and soil humidity was maintained at 70% of the soil water retention capacity.

The mineral fertilization consisted of 63 mg dm^{-3} of N (NH_4NO_3), 150 mg dm^{-3} of P ($Na_2HPO_4.2H_2O$), 120 mg dm^{-3} of K (KCl), 30 mg dm^{-3} of S ($MgSO_4.7H_2O$), 1 mg dm^{-3} of Cu ($CuSO_4$), 5 mg dm^{-3} of Zn ($ZnSO_4.7H_2O$) and 5 mg dm^{-3} of Mn ($MnCl_2.4H_2O$).

After the incubation period, mineral fertilization and boron addition were performed according to each treatment and ten seeds were sown per pot. During germination and the initial development of the sunflower plants, soil humidity was also maintained at 70% of the soil water retention capacity.

Twenty five days after sowing, plant shoots were harvest, washed, and dried (60 °C). Dry matter was quantified, and the samples were ground (2 mm) and analyzed for boron concentration (US-EPA, SW-846, method 3050B, with determination by ICP-AES). Soil samples from each pot were collected, dried (60 °C), ground (2 mm) and homogenized to be analyzed for concentration of boron extracted by hot water (Berger and Truog, 1939).

RESULTS AND DISCUSSION

For the evaluated variables (plant height, dry matter, shoots B concentration and soil B concentration), only dry matter production was statistically different when considering the sunflower hybrids. Helio 358 was more efficient (24.02 g per pot) than Helio 250 (20.09 g per pot) in dry matter production. For the other variables, differences were only observed for boron rates factor. Plants cultivated in the soil from the contaminated area presented the highest dry mass production in the study. Plant height was not different for the treatments containing increasing B rates, except for treatments with no boron added, which were lower than the others (Fig. 1).

Fertilizer recommendation of Boron in sunflower cultivation for the São Paulo State is 1 kg ha^{-1} of B when the soil presents 0 to 0.20 mg dm^{-3} of B extracted with hot water and 0.5 kg ha^{-1} of B when the soil presents 0.21 to 0.60 mg dm^{-3} (Ambrosano et al., 1996). The original concentration of boron in the oxisol soil used in the experiment was 0.30 mg dm^{-3}. The rates of B added to the soil (2, 4 and 8 kg ha^{-1}) were deliberately higher than the recommendation, since the aim was to evaluate sunflower tolerance to the excess of boron in the soil. Despite this, the available Boron concentration in the soil was not as high as expected, even for the highest rate added (B8), which was 1.91 mg kg^{-1} (Fig. 2). Boron availability depends on different attributes of the soil, such as pH, organic matter content, parent material, mineralogy (Gupta, 1993); and, consequently, it depends on its adsorption on soil colloids (Goldberg, 1993). Therefore, the low concentration of available boron observed could be the result of high adsorption of this element on the oxisol studied.

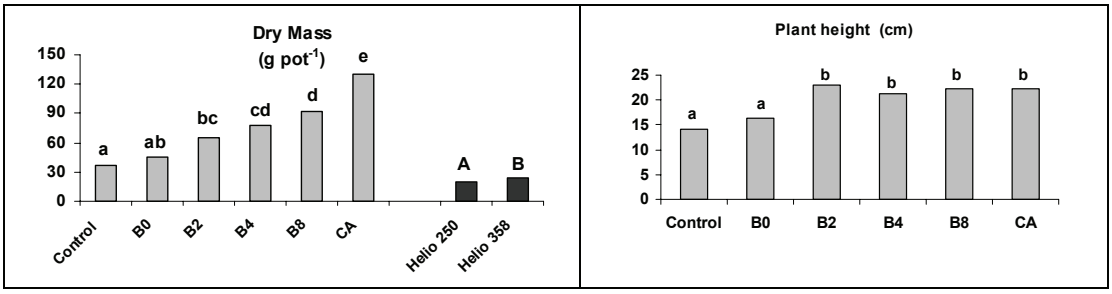

Fig. 1. Dry matter production and plant height of sunflower hybrids cultivated in soils treated with different boron rates[1].

[1]Control (oxisol with no fertilization); B0 (oxisol + mineral fertilization, no boron added); B2 (oxisol + mineral fertilization + 2 kg ha^{-1} of B), B4 (oxisol + mineral fertilization + 4 kg ha^{-1} of B), B8 (oxisol + mineral fertilization + 8 kg ha^{-1} of B); CA (soil from the contaminated area, no fertilization). Within each figure, values followed by the same letter (lower case: boron treatments; upper case: sunflower hybrids) are not statistically different (Tukey, 5%).

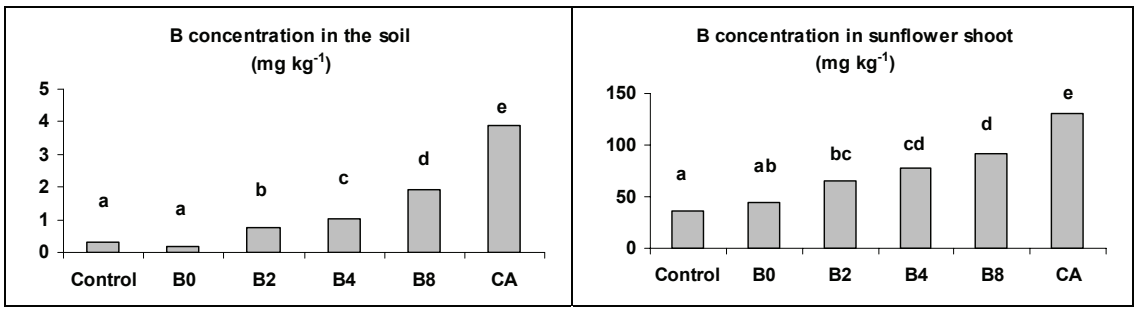

Fig. 2. Soil boron extracted with hot water and Boron concentration in the shoots of sunflower hybrids cultivated in soils with different boron rates[1].

[1]Control (oxisol with no fertilization); B0 (oxisol + mineral fertilization, no boron added); B2 (oxisol + mineral fertilization + 2 kg ha^{-1} of B), B4 (oxisol + mineral fertilization + 4 kg ha^{-1} of B), B8 (oxisol + mineral fertilization + 8 kg ha^{-1} of B); CA (soil from the contaminated area, no fertilization). Values followed by the same letter are not statistically different (Tukey, 5%).

In the treatment with soil from the contaminated area, available boron concentration was 3.90 mg kg^{-1} (Fig. 2). Although this concentration could be considered high, it was expected to be even higher, since other determinations performed with soil samples from the same area found boron concentrations extracted with hot water up to 14.87 mg kg^{-1} (Gonçalves et al., 2007a, b). This result reflects the high heterogeneity of the soil from the contaminated area.

Boron concentrations in the leaves from 15 to 20 mg kg^{-1} are considered adequate for plant nutrition (Malavolta, 2006). Specifically for sunflower, Sfredo et al. (1984) suggested that the suitable boron level in the leaves should be 40 mg kg^{-1}, based on studies carried out in the south region of Brazil (Paraná state). In the present study, boron levels in the shoots varied from 36 to 130 mg kg^{-1} (Fig. 2). Even for the plants cultivated in the soils that received the highest amount of boron, no toxicity symptoms were observed, neither was there any initial development reduction. This indicates that sunflower plants have a potential for being cultivated in soils contaminated with Boron, which is frequently found in soils contaminated with heavy metals.

It can be concluded that sunflower plants present a normal initial development when cultivated in soils that receive high amounts of boron. Therefore, sunflower is promising and should be tested as a phytoextractant and/or phytostabilizer of heavy metals in areas where boron contamination also occurs, as is the case of the study area.

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REFERENCES

Ambrosano, J.A., R.T. Tanaka, H.A.A. Mascareñas, B. Van Raij, J.A. Quaggio, and H. Cantarella. 1996. Leguminosas e Oleaginosas. p. 198. In: Raij B. van, Cantarella, H. Quaggio, J.A., Furlani, A.M.G. Recomendações de adubação e calagem para o Estado de São Paulo (Boletim 100). 2ed. IAC, Campinas/Sp, Brazil.

Berger, K.C., and K. Truog. 1939. Boron determination in soils and plants using the quinalizarin reaction. Ind. Eng. Chem. 11:540-545.

Fageria, N.K. 2000. Níveis adequados e tóxicos de boro na produção de arroz, feijão, milho, soja e trigo em solo de cerrado**.** R. Bras. Eng. Agr. Amb. 4:57-62.

Goldbach, H.E., Q. Yu, R. Wingender, M. Schulz, M. Wimmer, P. Findeklee, and R. Baluska. 2001. Rapid response reactions of roots to boron deprivation. J. Plant Nut. Soil Sci. 164:173-181.

Goldberg, S. 1993. Chemistry and Mineralogy of Boron in soils. p.3-44. In: GUPTA, U.C. (Ed.). Boron and its role in crop production. CRC Press, Boca Raton, FL, USA.

Gonçalves, F.A., A.R. Coscione, and R.S. Berton. 2007a. Efeito no cultivo de milho em solo contaminado com resíduo de sucata automobilística. CD-Rom. In: Proc. XXXI Cong. Bras. Ciên. Solo, Gramado/RS, Brazil.

Gonçalves, F.A., A.R. Coscione, and R.S. Berton. 2007b. Efeito no cultivo de milho em solo contaminado com resíduo de sucata automobilística. CD-Rom. In: Proc. XXXI Cong. Bras. Ciên. Solo, Gramado/RS, Brazil.

Gupta, U.C. 1993. Boron and its Role in Crop Production. CRC Press, Boca Raton, FL, USA.

Lima, J.C.P.S., C.W.A. Nascimento, J.G.C. Lima, and M.A. Lira Jr. 2007. Critical and toxic boron levels in corn plants and soils of Pernambuco, Brazil. Rev. Bras. Ciênc. Solo 31:73-79.

Malavolta, E. 2006. Manual de nutrição mineral de plantas. Ceres, São Paulo, Brazil.

Mariano, E.D., V. Faquin, A.E.F. Neto, A.T. Andrade, and I.O. Mariano.2000. Níveis críticos de boro em solos de várzea para o cultivo do feijoeiro. Pesq. Agropec. Bras. 35:1637-1644.

Nable, R.O., G.S. Bañuelos, and J.G. Paull. 1997. Boron toxicity. Plant Soil 193:181-198.

Sfredo, G.J., R.J. Campo, and J.R. Sarruge. 1984. Girassol: nutrição mineral e adubação (Circular Técnica 8). EMBRAPA-CNPSo: Londrina, PR, Brazil.

Schuster, C.E., and R.E. Stephenson, R.E. 1940. Sunflower as an indicator plant of boron deficiency in soil. J. Am. Soc. Agron. 32:607-621.

Impact des facteurs limitants du rendement du Tournesol (*Helianthus annuus* L.) en conditions réelles d'utilisation par les agriculteurs, en Midi-Pyrénées – Etude de cas

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is largely cropped in France, particularly in Midi-Pyrénées (second producing area) and it is naturally adapted to South-west agro-climate conditions. The yield depends on plant/environment interactions in its growth cycle. The main limiting factors are drought stress and cryptogrammic diseases. The aim of this work was i) to identify which factors influence the sunflower yield limitations, and ii) to evaluate the importance of cryptogrammic diseases and deep tillage in these limitations. This study presents the results of an initial experiment carried out in 2007. Two types of tillage were compared: control and deep tillage. The results showed that *Phoma macdonaldii* was the main factor influencing yield, its components, and morphological data. Significant differences on tillage were observed in morphological data stem length. Moreover, connections between organs linked to stress were highlighted. The slight difference between the two types of tillage on yield can be attributed to three main facts, i) neither tillage treatment was discriminating enough, ii) the presence of *Phoma macdonaldii*, iii) the absence of drought conditions. Similar studies will be carried out from 2008 to 2010 in different agro-ecosystems, with the aim of establishing the importance of physical soil constraints on the limitation of sunflower yield in Midi-Pyrénées (France).

Key words: compaction – fatty acids – France – *Phoma macdonaldii* – tillage – yield.

RESUME

Le Tournesol (*Helianthus annuus* L.) est une culture largement cultivée en Midi-Pyrénées (seconde région productrice de France) et naturellement adaptée à ses conditions agro-climatiques. Le rendement est la résultante d'interactions tout au long du cycle cultural. De nombreux facteurs limitants existent, au premier rang desquels figurent les maladies cryptogamiques et le stress hydrique. Les objectifs de ce travail sont i) de définir quels facteurs agissent sur la limitation du rendement du tournesol et ii) dans quelles mesures la présence de maladies et le type de travail du sol y jouent un rôle. Ce travail présente les résultats d'une étude de cas préliminaire réalisée au cours de la saison 2007, en suivi de parcelles d'expérimentations, selon les pratiques agricoles usuelles. Sur cet essai étaient comparés deux types de travail du sol, un témoin, un travaillé en profondeur. Nos résultats montrent que le premier facteur impactant sur le rendement, ses composantes, et les données morphologiques recueillies, est *Phoma macdonaldii*. Des différences significatives entre les deux types de travail du sol ont été observées sur une donnée morphologique, la longueur de la tige. Par ailleurs des relations liées au stress entre différents organes (aériens et souterrains), ont aussi été mises en évidence. La faible différence entre les deux types de travail du sol sur le critère rendement peut être imputée à plusieurs faits: i) les deux traitements n'étaient pas suffisamment discriminants, ii) la présence de *Phoma macdonaldii*, iii) l'absence de conditions sèches. Des études similaires seront conduites de 2008 à 2010, dans différents agro-écosystèmes, dans le but d'établir l'importance des contraintes physique du sol sur la limitation du rendement en Midi Pyrénées.

Mots clés: acides gras - *Phoma macdonaldii* - rendement - tassement - travail du sol - tournesol.

INTRODUCTION

La culture du tournesol (*Helianthus annuus* L.) présente des atouts agronomiques et environnementaux dans les régions à faible disponibilité en eau comme le Sud-ouest de la France, tel que la tolérance aux stress thermiques et hydriques (Merrien et Milan, 1992). Le rendement obtenu est le résultat d'interactions entre la plante et son milieu tout au long du cycle cultural ; de nombreux facteurs limitants sont signalés au premier rang desquels figurent les maladies cryptogamiques et le stress hydrique (Alignan, 2006; Merrien et Milan, 1992). En France, le rendement moyen oscille entre 20 et 25 quintaux hectare, alors que

le potentiel des meilleures variétés avoisine 45 quintaux hectare dans les milieux les plus favorables (CETIOM, 2006). Ceci rend le tournesol peu compétitif vis-à-vis des autres grandes cultures, et ne s'explique pas par un défaut de progrès génétique (Vear et al., 2003). Le Phoma (*Phoma macdonaldii*) classé en 2004 seconde maladie plus importante après le mildiou (Alignan, 2006), est un facteur déterminant de la limitation du rendement du tournesol. De la même façon, le tassement sous-superficielle du sol et donc de la résistance qu'offre le sol à la pénétration des racines agit négativement dans l'élaboration du rendement pour les espèces à système pivotant (Andrade et al., 1993; Montagu et al., 2001; Diaz-Zorita, 2004; Sadras et al., 2005).

L'évolution des pratiques culturales depuis les vingt dernières années a engendré des impacts majeurs sur les sols cultivés (Le Bissonais et al., 2002). L'agrandissement des parcelles, la spécialisation des cultures, l'évolution des techniques culturales, entrainent une diminution de la qualité de la structure des sols et la baisse de la teneur en matière organique (Girard et al., 2005). Ces contraintes pourraient être liées au phénomène naturel de tassement (Andrade et al*.,* 1993; Lampurlanès et Cantero-Martinez, 2003; Sadras et al., 2005). Les réductions du rendement attribuables aux évolutions du tassement des sols ont été décrites pour différentes cultures, dans différents types de sols, et dans différentes régions productrices à travers le monde (Diaz-Zorita, 2004; Tennant et Hall, 2001). Chez le tournesol, de fortes conditions de tassement sur des sols à texture fine réduisent l'expansion foliaire, la biomasse aérienne et le développement des racines (Andrade et al., 1993; Diaz-Zorita, 2004). La différence entre les rendements réels et potentiels pourrait être en partie expliquée par une plus faible efficience d'absorption hydrominérale, consécutive à une réduction du volume de sol exploré par les racines (Connor et al., 1992; Andrade et al., 1993; Diaz-Zorita, 2004; Goodman et Ennos, 1999). Ceci ayant pour conséquence la réduction de la quantité d'eau absorbée et de fait la diminution de l'absorption des éléments minéraux (N, P, K) et des oligoéléments (B) (CETIOM, 1983; Colomb et al., 1995).

Les objectifs de ce travail sont i) de définir quels facteurs agissent sur la limitation du rendement du tournesol, et ii) dans quelles mesures la présence de maladies et le type de travail du sol y jouent un rôle.

MATÉRIELS ET MÉTHODES

Le cultivar MELODY (Syngenta SEEDS SAS, Semences NK) a été suivi en parcelle d'expérimentation (dispositif split plot, quatre répétitions, parcelles de 12 rangs sur 10 mètres) au cours de la saison 2007, en conditions réelles d'utilisation agricole sur l'exploitation de l'E.I. Purpan: Ferme de Lamothe, (43°30'11.75''N ; 1°14'54.53''E). Le semis a eu lieu le 30 avril 2007 (semoir pneumatique, écartement 0.6m, 52700 plantes/hectare), en sol Limoneux Sablo-argileux (A: 22.4; L: 47.5; S: 27.3; pH: 6.2). Un covercrop a d'abord été passé sur l'ensemble de la parcelle, suivi d'un passage de décompacteur (profondeur: 0.5m, écartement: 0.6m). Un second passage de décompacteur a été passé perpendiculairement sur la zone correspondant au second traitement du futur essai. Un passage simple constituait le traitement un (T1), deux passages perpendiculaires le traitement deux (T2). La phénologie a été surveillée tout au long de la saison sur trois plantes consécutives dans chaque parcelle expérimentale. L'évolution des surfaces foliaires a été notée à cinq reprises à partir du stade 3.1, jusqu'au stade 5.4 (Hutley Bull, 1995). Pour chaque notation, l'Indice Foliaire (IF) a été estimé à partir de la mesure de la largeur des feuilles (Scheiner et Lavado, 1999). La floraison est intervenue le 17 juillet 2007, stade 4.3. La présence de *Phoma macdonaldii* a été remarquée dès le 24 juillet 2007, des notations de diamètre et de tâche de Phoma au collet ont été réalisées conformément au Tableau 1.

Tableau 1. Echelle de notation inspirée par l'échelle de notes «G2» sur Phoma (*Phoma lingam*) du colza (*Brassica napus*) (CETIOM, 2004).

Notation	Taille de la tache
1	Moins de ¼ de la circonférence
2	Entre ¼ et ½ de la circonférence
3	Entre ½ et ¾ de la circonférence
4	Entre ¾ et toute de la circonférence
5	Tâche encerclante

La parcelle a été récoltée le 11 septembre 2007. Les données de rendement ont été obtenues à partir de prélèvements de capitules réalisés dans chaque parcelle sur huit mètres consécutifs. Du fait de la contamination par *Phoma macdonaldii*, nous avons effectué une double récolte sur chaque répétition, la première étant qualifiée fortement touchée (note Phoma = 5): M1; la seconde de «saine»: M2. Afin

d'obtenir des données physiologiques et morphologiques précises, nous avons extrait dans chaque parcelle et pour chaque traitement, trois plantes entières successives. Les différents organes des plantes entières extraites ont été nettoyés, séparés et caractérisés. Le diamètre des capitules de tournesol a été mesuré avant égrenage. Les feuilles ont été mises en étuve (72h à 45°C), les tiges et les racines séchées à l'air. L'ensemble de l'appareil aérien et souterrain a été pesé et mesuré (diamètre et longueur). Le Poids de Mille Grains (PMG) a été obtenu. Les données de nombre et de poids des grains par capitule, des poids, longueurs et volumes spécifiques, du peuplement hectare par micro parcelle, ont été obtenues par recoupage des données précédentes. Par ailleurs le calcul des données de longueur et volume spécifiques de la tige et de la racine, a été emprunté au manuel de STICS (Brisson, 2002). Les données de qualité d'huile ont été obtenues par spectroscopie proche infrarouge (Ayerdi Gotor et al., 2007). En termes d'analyses statistiques, des modèles linéaires généraux et des régressions linéaires multiples ont été réalisées pour l'ensemble des données recueillies.

RÉSULTATS ET DISCUSSION

Durant la saison 2007 aucun symptôme de stress hydrique n'a été observé sur les plantes. Le rendement de l'essai était égale à 37.5 quintaux / hectares (peuplement récolte égal à 47,083 plantes/hectare).

Dans les conditions expérimentales, l'appareil foliaire présente une relation directe avec les composantes de rendements, mais aussi avec la partie aérienne de la plante (Merrien et Milan, 1992) (Tableau 2). Conformément aux observations de Sadras et al. (1993), le poids de l'appareil foliaire augmente à mesure que la longueur de la tige diminue (Fig. 1). De plus l'augmentation de la biomasse des feuilles présente une corrélation positive avec l'augmentation de la biomasse souterraine (appareil racinaire) (Fig 1.). Ces deux systèmes, le système racinaire et le système aérien (tige et feuilles), ont un impact direct sur les composantes de rendement. En effet, le PMG augmente en parallèle du poids de la tige, et dans une moindre mesure avec le diamètre de la racine (Fig. 1). Le type de travail du sol a eu un impact sur la longueur de tige, qui s'accroit avec un passage supplémentaire de décompacteur, (Tableau 2).

Tableau 2. Mesures morphologiques réalisées sur plantes entières prélevées au champ: Analyses de variance, moyenne des traitements[1]. Travail du sol: T; Présence de Phoma: M.

Mesures morphologiques	Moyenne T1	Moyenne T2	Moyenne M1	Moyenne M2	Moyenne des Traitements
Nombre de feuille	16.1	16.3	15.3 a**	17 b**	16.2 ± 0.45
Nombre de grains par capitule	1,153.9	1,234	1,080 a**	1,307.9 b**	1,193.9 ± 65.4
Longueur spécifique des racines	1.3	1.4	1.5 a**	1.2 b**	1.3 ± 0.1
Longueur tige	147.7 a**	153 b**	149	15.8	150.4 ± 1.5
Poids des grains par capitule	43.1	48.1	36.1 a***	55.1 b***	45.6 ± 3.4
Poids spécifique de la racine	29.2	23.4	19.2 a**	33.5 b**	26.3 ± 4.2
Poids de Mille Grains	36.1	38.1	32.6 a***	41.7 b***	37.1 ± 1.4
Volume de la racine	19.8	18.6	16.7 a*	21.7 b*	19.2 ± 1.7
Volume spécifique de la racine	0.8	0.9	0.9 a**	0.8 b**	0.9 ± 0.1
Biomasse feuille	17	16.6	14 a**	19.6 b**	16.8 ± 1.7
Biomasse Tige	50.5	52.8	45.7 a**	57.9 b**	51.6 ± 3.1
Longueur spécifique de la tige	3.2	3.1	3.5 a**	2.8 b**	3.1 ± 0.2

[1]a, b: groupes homogènes selon le Test de Student; *: Probabilité significatives à 0.05; **: Probabilité significatives à 0.01; ***: Probabilité significatives à 0.001.

Les conditions climatiques de la saison 2007 ont été particulièrement favorable au développement de *Phoma macdonaldii* (PROLEA, 2005; CETIOM, 2006). Des symptômes ont été observés après le stade 4.3, bien que le tournesol soit sensible depuis le stade cotylédon (Alignan, 2006). D'après le CETIOM (PROLEA, 2005), le taux de pieds secs tendrait à augmenter avec la surface foliaire. Une perte du poids de la biomasse foliaire de l'ordre de 16% a été observée, traduisant non pas une baisse du nombre de feuilles (non significatif), mais à un dessèchement précoce de ces dernières dû à la maladie (Tableau 2). De plus les infections issues de la tige (causant notamment les tâches encerclantes) sont plus agressives que celles issues des feuilles (PROLEA, 2005), ainsi nous avons pu constater une perte de 11% de la masse de la tige (Tableau 2). Les résultats présentés dans le Tableau 2 montrent que l'infection impacte négativement les organes directement liés aux fonctions de nutrition (racine, feuille) et de réserve (tige, capitule), ce qui limite la synthèse de ces même réserves ou leur acheminement vers la graine (Abou Al Fadil, 2006; Darvishzadeh, 2007). Les données dont nous disposons, nous amènent à penser que cette relation est probablement expliquée par ces deux hypothèses de manières successive ou simultanément.

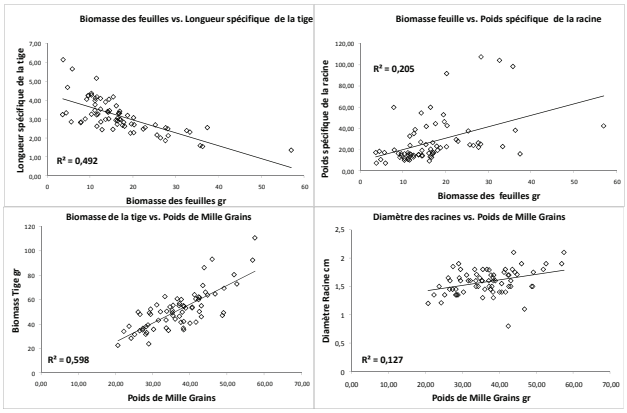

Fig. 1 Relations liées aux stress entre organes. Les corrélations sont significatives (régressions linéaires, P<0,001) entre les données morphologiques.

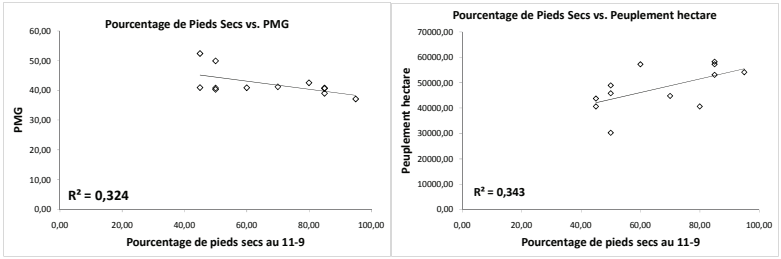

Fig. 2. Relation entre le pourcentage de pied sec (note Phoma = 5) par parcelle au 11septembre 2007, le PMG (régression linéaire, P=0.053) et le peuplement hectare (régression linéaire, P=0.045).

Les contaminations par le sol causent entre autre les nécroses au niveau du collet (zone de jonction entre la tige et la racine), celles-ci se traduisent alors par des nécroses encerclant la tige empêchant le transfert des assimilas (Alignan, 2006). Ainsi nous avons pu constater une perte de 27% du poids spécifique de la racine, de 13% du volume de celle-ci (Tableau 2). L'ensemble des symptômes, se traduit par une sénescence précoce de la plante ayant pour conséquence directe une perte de rendement. Le pourcentage de pieds secs par parcelle réalisé le 11 septembre 2007, nous montre que le PMG décroit à mesure que le taux augmente (Fig. 2). Dans les conditions expérimentales, le Phoma a pour conséquence une diminution de 12% du PMG. Nous avons aussi pu observer une diminution significative du nombre de grains par capitule de 10%. Ceci ayant pour conséquence une diminution significative de 49% du poids des grains par capitule, et plus globalement de 25% de perte de rendement sur l'ensemble de la parcelle expérimentale (Tableau 2 et 3). Ceci est en relation avec les observations réalisées par Abou Al Fadil (2006) et Darvishzadeh (2007). La diminution significative du nombre de grains par capitule nous amène à penser que l'infection se serait produite à partir du stade correspondant à la mise en place du nombre de grains par capitule et ce jusqu'à maturité (Alignan, 2006). De plus, le fait que nous ayons observé une augmentation du pourcentage de pieds secs avec le nombre de plantes à l'hectare, nous amène à émettre l'hypothèse de la présence d'une contamination secondaire (Fig. 2).

Tableau 3. Mesures et analyses réalisées sur des capitules prélevés sur huit mètres consécutifs: Analyses de variance, moyenne des traitements[1]. Travail du sol: T; Présence de Phoma: M.

Mesures au champ	Moyenne T1	Moyenne T2	Moyenne M1	Moyenne M2	Moyenne Des Traitements
Rendement	36.2	39.9	28.6 a**	47.5 b**	38.1 ± 4.2
PMG	42	42.4	36.4 a***	48.1 b***	42.2 ± 2.1
Poids spécifique	42.5	42.7	41.2 a***	43.9 b***	22.6 ± 0.4
Protéines	15.6	14.9	15.9 a*	14.6 b*	15.3 ± 0.5
Acide Palmitique	6.8 a*	6.9 b*	6.9 a***	6.7 b***	6.8 ± 0.04
Acide Stéarique	2.8	2.8	3 a*	2.6 b*	2.8 ± 0.1
Acide Oléique	23.1 a*	21.3 b*	20.8 a***	23.7 b***	22.2 ± 0.6
Acide Linoléique	68 a*	68.9 b*	70.1 a **	67.8 b**	68.5 ± 0.6

[1]a, b:groupes homogènes selon le Test de Student ; *: Probabilité significatives à 0.05; **: Probabilité significatives à 0.01; ***: Probabilité significatives à 0.001.

Dans le contexte expérimental les teneurs en acides palmitique, stéarique et linoléique sont plus élevées en présence de stress induit par le Phoma (M1), seule la teneur en acide oléique tend à diminuer dans le traitement M2. Cette diminution peut s'expliquer par la sénescence précoce de la plante (CETIOM, 1996). En effet, une plante de tournesol soumise à un stress dû à la maladie souffrira de prématurité, et de ce fait sera récoltée à surmaturité, lorsque ses congénères seront arrivées à leur maturité physiologique (CETIOM, 1996). Dans ce cas, Baldini et al. (2002) ont observé que la surmaturité entrainait une baisse de la teneur en acide oléique dans les graines récoltées. Les protéines étant les premières composantes à s'accumuler dans la graine de tournesol (Roche, 2005), elles souffriront moins du phénomène de prématuration. De la même manière, les acides palmitiques, stéarique, étant des précurseurs de l'acide oléique (Lagravère, 1999), ils auront tendance à moins souffrir de la sénescence précoce de la plante causée par le champignon, conformément à nos observations (Tableau 3). Le fait que la durée d'activité de la feuille est directement liée à la lipidogenèse, tend à confirmer ce fait (Merrien et Milan, 1992). Nous avons observé des différences sur la teneur des différents acides gras selon le type de travail du sol, bien que la teneur en huile ne soit pas en elle-même significative (Tableau 3). Nos résultats nous montrent une amélioration significative des teneurs en acide palmitique, stéarique et linoléique sur le traitement T2 (passage supplémentaire de décompacteur). Seule la teneur en acide oléique tendrait à s'améliorer sur un sol moins travaillé, et à décroitre sur T2. Les variations de la teneur en acide gras saturés et insaturés dues aux conditions environnementales restent encore peu connues et controversées. Flagella et al. (2006), ont mis en évidence que la teneur en acide oléique était positivement influencée par l'irrigation, et la teneur en acide linoléique négativement impactée par celle ci. Cependant, Baldini et al. (2002) ont observés un effet positif d'un léger stress hydrique, sur la teneur en acide oléique ; et de fait un effet négatif sur la teneur en acide linoléique. L'augmentation de la teneur en acide oléique sur T2, semblerait concordante avec les résultats de Baldini et al. (2002). Cependant en l'absence de stress hydrique réel, ces résultats seront à mettre en relation avec les résultats de nos travaux de 2008 à 2010.

Le rendement est le résultat d'une série d'interactions entre la plante et son milieu tout au long de son cycle. Il dépend dans les conditions de l'expérimentation, de la bonne capacité de la plante de tournesol à s'adapter à son milieu, à absorber l'eau et les nutriments nécessaires, à intercepter suffisamment de rayonnement solaire et à résister aux attaques de ravageurs et aux maladies. Si toutes ces conditions sont réunies, la plante sera à même d'optimiser son nombre de grains par capitule et son remplissage. Contrairement aux travaux de Diaz-Zorita (2004), une réduction du rendement liée au travail du sol n'a pas été observée. Les données précédentes nous amènent à penser que bien que le travail du sol a un effet sur la morphologie de la plante de tournesol, il ne se traduit pas directement sur les données de rendement au champ. Parallèlement dans les conditions expérimentales, nous avons constaté une baisse du rendement lié au Phoma. Cette relation directe s'établirait sur une de ses composantes principales, le PMG et sur le nombre de grains par capitule. Il existerait donc une relation entre le degré d'infection de la plante et la baisse du nombre et remplissage de ces grains. D'un point de vue strictement morphologique (dans l'attente de données physiologiques), l'expression du stress causé par le Phoma (hormis les symptômes caractéristiques) semble difficile à distinguer des symptômes de stress observés par Andrade (1993) et Diaz-Zorita (2004). La présence de cette maladie pourrait expliquer le fait qu'aucune différence significative n'ait été observée entre les deux types de travail du sol sur le critère rendement. Ceci pourrait aussi s'expliquer par le fait que ces deux traitements n'étaient pas suffisamment discriminants dans les conditions expérimentales. L'absence de contraintes liées à la sécheresse durant la saison 2007 peut aussi être une des raisons de l'absence de différence significative entre les deux types de travaux du sol sur la quasi-totalité des critères étudiés.

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REFERENCES

Abou Al Fadil, T. 2006. Déterminisme de la tolérance du tournesol à *Phoma macdonaldii* au collet et sur racines: approches génétiques et histologiques. Thèse doctorat, Institut National Polytechnique de Toulouse, 196 p.

Alignan, M. 2006. Phoma du tournesol: déterminisme de la tolérance de l'hôte à la maladie. Thèse de doctorat, Institut National Polytechnique de Toulouse, 297 p.

Andrade, A., D. Wolfe, and E. Fereres. 1993. Leaf expansion, photosynthesis and water relations of sunflower plants grown on compacted soil. Plant Soil 149:175-184.
Ayerdi Gotor, A., E. Farkas, M. Berger, F. Labalette, S. Centis, J. Dayde, and A. Calmon. 2007. Determination of tocopherols and phystosterols in sunflower seeds by NIR spectrometry. Eur. J. Lipid Sci. Technol. 109:525-530.
Baldini, M., R. Giovanardi, S. Tahmasebi-Enferadi, and G.P. Vannozzi. 2002. Effects of water regime on Fatty Acid accumulation and final Fatty Acid composition in the oil of standard and high oleic sunflower hybrids. Italian J. Agron. 6:119-126.
Brisson, N. 2002. STICS, Notice concepts et formalismes. INRA-Logiciel déposé à l'Agence pour la protection des programmes n°99 17002600, version 6.0.
CETIOM. 1983. Physiologie de la formation du rendement chez le tournesol. Informations techniques, n° 83.
CETIOM. 1996. Guide Pratique, Les accidents du tournesol, édition CETIOM, p. 112.
CETIOM. 2004. Guide de l'expérimentateur colza, édition CETIOM, 2004, p. 206.
CETIOM. 2006. Les techniques culturales, le contexte économiques, édition CETIOM. p.26.
Colomb, B., A. Bouniols, and C. Delpech. 1995. Effect of various phosphorus availabilities on radiation-use efficiency in sunflower biomass until anthesis. J. Plant Nutr. 18:1649-1658.
Connor, D.J., and V.O. Sadras. 1992. Physiology of yield expression in sunflower. Field Crops Res. 30:333-389.
Darvishzadeh, R. 2007. Déterminisme Génétique de la résistance du Tournesol au Phoma. Thèse de doctorat, Institut National Polytechnique de Toulouse, 201 p.
Díaz-Zorita, M. 2004. Efecto de la compactación subsuperficial de un hapludol Típico sobre la producción de girasol (*Helianthus annuus* L.). Ciencia del Suelo 22:40-43.
Flagella, Z, R. Di Caterina, M. Monteleone, L. Giuzo, M. Pompa, E. Tarantino, and T. Rotunno. 2006. Potentials for sunflower cultivation for fuel production in southern Italy. Helia 29:81-88.
Girard, M., C. Walter, J.R. Rémy, J. Berthelin, and J.L. Morel. 2005. Sols et Environnement, édition DUNOD, Paris. ch. 24:538-553.
Goodman, A.M., and A. Ennos. 1999. The effect of soil bulk density on the morphology and anchorage mechanics of the root systems of sunflower and maize. Ann. Bot. 83:293-302.
Hutley Bull, P.D. 1995. Sunflower growth stages, A summary of Sunflower Growth Stages for UK Use. Adapted and translated from CETIOM.
Lagravère, T. 1999. Determinisme Agro-physiologique de l'expression de la très haute teneur en acide oléique dans l'huile de Tournesol. Thèse de doctorat, Institut National Polytéchnique de Toulouse: Toulouse. 145 p.
Lampurlanès, J., and C. Cantero-Martinez. 2003. Soil bulk density and penetration resistance under different tillage and crop management systems and their relationship with barley root growth. Agron. J. 95:526-536.
Le Bissonnais, Y., J. Thorette, C. Bardet, and J. Daroussin. 2002. L'érosion hydrique des sols en France. INRA-IFEN. p. 106.
Merrien, A., and M.J. Milan. 1992. Physiologie du tournesol, édition CETIOM. Paris, 65 p.
Montagu, K., J. Conroy, and J. Atwell. 2001. The position of localized soil compaction determines root subsequent shoot growth responses. J. Exp. Bot. 52:2127-2133.
PROLEA. 2005. Neutraliser le Phomopsis et le Phoma du tournesol. Oléoscope, n° 80.
Roche, J. 2005. Composition de la graine de tournesol (*Helianthus annuus* L.) sous l'effet conjugué des contraintes agri-environnementales et des potentiels variétaux. Thèse de doctorat, Institut National Polythéchnique de Toulouse, 305 p.
Sadras, V.O., D.J. Connor, and D.M. Whitfield. 1993. Yield, yield components and source-sink relationships in water-stressed sunflower. Field Crops Res. 31:27-39.
Sadras, V.O., G.J. O'Leary, and D.K. Roget. 2005. Crop responses to compacted soil: capture and efficiency in the use of water and radiation. Field Crops Res. 91:131-148.
Scheiner, J.D., and R.S. Lavado. 1999. Soil water content, absorption of nutrient elements, and responses to fertilization of sunflower: a case study. J. Plant Nutr. 22:369-377.
Tennant, D., and D. Hall. 2001. Improving water use of annual crops and pastures - limitations and opportunities in western Australia. Aust. J. Agric. Res. 52:171-182.
Vear, F., H. Bony, G. Joubert, D. Tourvieille, I. Pauchet, and X. Pinochet. 2003. 30 year of sunflower breeding in France. Oléaginaux Corps Gras Lipides 10:66-73.

Planting date effect on yield and yield components of sunflower in Miyaneh region

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ABSTRACT

An experiment was conducted at the research field of Islamic Azad University, Miyaneh, Iran, during the agricultural year 2005-2006, to study the effect of planting date on yield and yield components of sunflower varieties and hybrids. A factorial analysis, on the basis of a randomized complete block design with three replicates, was used. Factor A (planting date) consisted of three planting dates: April 9, April 30 and May 20 and factor B (variety) were the cultivars Sour, CMS26xR103, Azargol, and Armavirsky. Measured traits during study were plant height, head diameter, 1000 seed weight, oil percentage, oil yield, total biomass and seed yield. The results revealed that the effect of planting date on number of days to emergence, plant height, head diameter and oil yield were not significant, but the effect of planting dates on 1000 seed weight, total biomass and oil yield were significant. In contrast, the results showed that the effect of variety on all measured traits was significant. The interaction of planting date as well as variety on measured traits was significant, except for days to emergence, head diameter and oil yield. Second planting date (April 20) and Armavirsky cultivar were determined, respectively, as an appropriate planting date and variety.

Key words: biomass – *Helianthus annuus* L. – hybrids – yield – yield components.

INTRODUCTION

Oil crops are very important in human diet. In the last decade, Iranian yearly oil production was 85,000 t but its consumption was 992,000 t, so many oil products has to be imported every year (Anonymous, 2006). National oil production program emphasizes on applying optimum management methods besides genetic resources capacity. One of the most important decisions in plant cultivation is determining the best planting date for every cultivar. Attention is being paid to sunflower hybrids with uniform establishment, easier cultivation practices, higher yield, and higher tolerance to diseases. For these reasons, open pollinated cultivars are being substituted by hybrids. Research on both types of cultivars has been important. Miyaneh region, in North West of Iran, is a favorable area for sunflower cultivation. Its special geographical situation makes it possible to use different cultivars and planting dates and the introduction of new promising varieties. Plant yield is determined by the sun radiation received via canopy, so that a decrease in radiation received causes a decrease on seed and oil yields. Growers have moved sunflower cultivation from hot to temperate and cool climates. Delay in sowing increased the hull/achene ratio and decreased kernel oil percentage and total oil content (Alyari, 2000).

Khodabande (1989) showed that, at Zaria and Mehr, the best planting dates for combatting peregrine sparrows were between 1st September to 10th October. Thompson and Unger (1986) studied ten planting dates between 25th March and 1st August and showed that only planting dates until 19 July led to an increase in the head diameter and after that date head diameter decreased.

Goksoy et al. (2000) conducted a two year experiment on SUNBRED-265, H-1 and Vinimac 8931 cultivars in three densities (30, 47.5 and 95 thousand plant/ha) and three planting dates from March to April in upland dry farming. They showed that 95,000 plant/ha in mid March led to a higher thousand kernel weight and oil yield. Parmar and Kharwave (1992) showed that among four planting dates, 22 February, 4, 14, and 24 March, the planting in 14 March produced the highest yield. Alessi et al. (1977) showed that the best planting date for North Dakota was middle to end May and earlier or later planting dates led to a decrease in seed and oil yields.

MATERIALS AND METHODS

An experiment was conducted at the research field of Islamic Azad University, Miyaneh in the agricultural year 2005-2006 following a randomized complete block design with three replicates. The station, located on 25º, 37’ E and 43º, 47’ N. on Marton and Umburge divisions, has semi arid and semi warm conditions and dry summers and cold and wet winters. Average annual temperature is 13ºC, with a

minimum of 6.2ºC and a maximum of 19.8 ºC. Average precipitation at this location is 306 mm, with a minimum of 168 mm and a maximum of 500 mm. Frozen temperatures have been reported for 110 days. Soil texture is clayloam with 7.5 pH. Factor A (planting date) consisted of three levels: April 9, April 30 and May 20 and factor B (variety) consisted of four cultivars, Sour, CMS26xR103, Azargol, Armavirsky. After soil preparation, planting was done by hand and fertilization was done according to a previous soil analysis. Each plot was 5 x 2.4 m. Plant distances within row were of 25 cm and distance between rows was 60 cm. Weeds were controlled on time.

Measured traits were number of days to emergence, plant height, head diameter, 1000 seed weight, oil percentage, biomass, seed yield and oil yield.

RESULTS AND DISCUSSION

Results showed that there were significant differences among cultivars in days from planting to emergence (Table 1). It would seem that this period was affected by seed potential, soil nutrients, temperature and humidity more than by the planting date (Sindagi and Virupakshappa, 1990). Armavirsky emerged 4.78 days earlier than other cultivars (Table 2).

Table 1. Anova table of measured traits

SV[1]	df	Mean squares						
		Days to emergence	Plant height	Head diameter	1000 seed weight	Seed Yield	Oil Yield	Biomass
Rep	2	18.36**	72.33	33.58*	15.1**	249988	2800782	96.82
A	2	2.69	67.25	10.08	8.42**	2338233**	594281	1019**
B	3	19.58**	54.35	29.26*	27.67**	10803285**	3654338*	3912**
AxB	6	0.694	569*	17.12	0.29*	1394063**	546051	519**
e	22	1.028	224	10.92	0.1	497147	1199359	3.3
cv %		14.9	9.79	9.15	2.29	12.17	15.53	10.11

[1]Sources of variation (SV): A= Planting date B= Cultivars; df= degrees of freedom
*, ** significant at 5% and 1% respectively.

Table 2. Mean comparisons of measured traits[1]

Treatments	Days to emergence	Plant height (cm)	Head diameter (cm)	1000 seed weight (g)	Seed yield (kg/ha)	Oil yield (kg/ha)	Biomass (kg/ha)
Planting date							
Apr 19	7.333 a	160.42 a	22.42 a	43.25 b	3,816 ab	1,755 b	11,818 a
Apr 30	6.667 a	152.67 a	23.33 a	46.50 b	4,376 a	2,100 a	11,873 a
May 20	6.417 a	145.42 a	24.25 a	41.25 c	3,685 b	1,615 b	11,548 a
Cultivars							
SOUR	8.233 a	138.33 c	23.22 b	37.33 d	3,140 b	1,336 b	10,007 b
CMS26xR103	7.444 ab	128.33 c	18.89 c	41.00 c	2,744 bc	1,280 b	10,334 b
Azargol	6.778 b	162.22 b	24.11 ab	46.67 b	4,158 ab	2,065 a	11,433 b
Armavirsky	4.778 a	182.78 a	27.11 a	49.67 a	5,228 a	2,439 a	12,868 a

[1]Means with same letter show no difference at Duncan 5%

Plant height also differed among cultivars (Table 2). Armavirsky, with 182.3 cm and CMS26xR103 with 128 cm, showed the highest and the lowest height, respectively among cultivars. This difference was significant but differences between Sour and the others were not significant (Table 2). Plant height is a trait controlled genetically but environment can affect it. This was shown also by Ayin (1998), Hatami (1995), and Meinke et al. (1993).

Head diameter showed significant differences among cultivars (Table 2). Armavirsky and CMS26xR103 showed the highest and the lowest diameter, respectively. Head diameter has a great effect on yield, but there is an optimum value that maximizes seed yield. A larger head has more flowers that produce seeds. Cultivated sunflower cultivars have only one head and high yielding cultivars have a

bigger head (Alyari, 2000). Multiheaded plants may result from low density, early planting, soil higher N, and alternative drought stress. Garside (1998) showed that one of the Armavirsky traits with respect to other cultivars was its bigger head and more fertile flowers (Table 2).

Interaction of planting date x cultivar was significant for 1000 seed weight (Table 1). The 30th May planting date produced the highest value (48.67 g), and 21st May produced the lowest value (41.25 g) (Table 2). For cultivars, Armavirsky showed the highest value for 1000seed weight (48.67 g) and Sour with 37.33g the lowest. The high temperatures during the seed filling period in May plantings probably increased respiration and led to disturbance in seed filling resulting in an increase in the proportion of lighter seeds or hull. Goksoy et al. (2000) showed also that delay in planting led to decreasing 1000 seed weight.

Interaction of planting date x cultivar was also significant for yield (Table 1). Second planting date produced the highest yield but did not have a significant difference with respect to the first one (Table 2). Armavirsky produced 5,228 kg/ha and CMS26xR103 2,744 kg/ha, which were the highest and lowest seed yields observed (Table 2). Majid and Schneider (1987) showed that hybrid cultivars reaction to changing the planting date was lesser and Garside (1998), in a 4 planting dates (15, 30 March, 15 and 30 April) experiment and 4 cultivars, showed that effect of planting date on yield was significant.

The highest seed and oil yields were produced by Armavirsky on the second planting date. The reason for the decrease in seed yield in the later planting date was the decrease in 1000 seed weight. Thompson and Unger (1986) reported that yield is the most sensitive attribute to planting date changes. In their experiments, the 1st May planting produced the highest yield in all three years. Most reports showed that yield was higher at early and normal planting dates (Goksoy et al., 2000; Sindagi and Virupakshappa, 1990). Some reports also showed that no consideration of suitable planting date led to decreasing yields (Parmar and Kharwave, 1992).

Interaction of planting date x cultivar was significant on biomass (Table 1). Armavirsky, with 12,826 kg/ha and Sour, with 10,007 kg/ha produced the highest and the lowest biomass yield (Table 2). Planting date was also significant for this trait.

Effects of cultivar on oil yield were significant (Table 1). Armavirsky, with 2,439 kg/ha and CMS26xR103, with 1280 kg/ha, produced the highest and the lowest oil yield. The reason for the changes in oil yield in different cultivars is based on genetic potential, growth and developmental attributes and environmental conditions (Alyari, 2000). Factors such as higher biomass, head diameter and 1000 seed weight probably led to higher yield in Armavirsky. The higher temperatures during the seed filling period, especially 25 ºC in the flowering period, decreased oil yield. Miller et al. (1984) showed that the delay in planting from early May to early June decreased oil yield because the seed filling period coincided with hot days and short season. Alessi et al. (1997) reported that delayed planting resulted in a shortened growth period and, consequently the 1000 seed weight and the oil yield decreased.

REFERENCES

Alessi, J., J.F. Power, and, D.C. Zimmerman. 1977. Sunflower yield and water use as influenced by planting date, population and row spacing. Agron. J. 693:465-469.

Alyari, H. 2000. Oil seed physiology and cultivation. Amidi Publ. 225 p.

Anonymous. 2006. Agricultural information. Agriculture Ministery. Islamic Republic of Iran.

Ayin, A. 1998. Plant density and cultivation pattern on growth and yield of sunflower in Djiroft region. Tabiat Modarres University. 119 p.

Garside, A.L. 1998. Sowing time effects on the development, Yield and oil of sunflower in semi-arid tropical Australia. Aust. J. Exp. Agric. Anim. Husb. 24:110-119.

Goksoy, A., Z. Turan, and E. Acikgoz. 1998. Effect of planting date and plant population on seed and oil yields and plant characteristics in sunflower. Helia 21(28):107-115.

Hatami, A. 1995. Planting date effect on qualitative and quantitative attributes of sunflower cultivars in Karadj. Tabiat Modarres University. 115 p.

Khodabande, A. 1994. Determination of sunflower cultivars best planting date for Khuzestan North region. Seed Plant J. 10:13-23.

Majid, H.R., and, A.A. Schneiter. 1987. Yield and Quality of Semi dwarf and standard height sunflower hybrids grown of five plant populations. Agron. J. 79:681-684.

Meinke, B., D. Hummer, and, A. Chapman. 1993. A sunflower simulation model II. Simulating production risks in a variable sub-tropical environment. Agron. J. 85:735-742.

Miller, B.C., E.S. Oplinger, R. Rand, J. Peters, and G. Weis. 1982. Effect of planting date and plant population on sunflower performance. Agron. J. 76:511-515.

Parmar, P. S. and, P.C. Kharwave. 1992. Effects of sowing data on growth. And yield of sun flower (*H. annuus*) varieties at two levels. Indian. J. Agr. Sci. 62:343-344.

Sindagi, S.S., and, K. Virupakshappa. 1990. Sunflower. Indian council of Agricultural Research, Krishi Anusandhan Bhavan, New Delhi.

Thompson, F., and, K. Unger. 1986. Growth and development of irrigated sunflower in Texas. Agron. J. 78:507-515.

Some aspects of sunflower crop management in Romania

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ABSTRACT

The paper presents some research results regarding the technological management of sunflower, with the following conclusions: (i) sunflower should not be sown in the same field more than once every 6 years, avoiding the alternance with soybeans, due to the potential attack of *Sclerotinia*; (ii) the possibility of reducing soil tillage for fuel saving, without a yield decrease; (iii) the establishment of optimal sowing time in connection with soil temperature; (iv) optimal plant density varying between 40–50 thousands plants/ha. Although sunflower is a tolerant crop to dryness, the association between water deficit and high temperatures during the growing season caused very important yield losses and decreased the seed oil content.

Key words: plant density – Romania – *Sclerotinia* – soil tillage – sowing time – sunflower management.

INTRODUCTION

Sunflower is the main oleaginous crop in Romania, cultivated on an area that doubled in the 1990's, attaining 1 million hectares. The favorable market price sustained by the edible oil demands and by biofuel production, maintains the growth tendency of cultivated area. But this trend is limited by the crop rotation necessity and, also, by an increase in the rapeseed area enlargement.

Aiming to elaborate a crop management adapted to the agrobiological requirements of sunflower, a large number of experiments have been carried out, and their results have contributed to establishing some technological norms.

Research on the previous crop effect, the soil tillage, the sowing time, plant density, fertilizer application, the weeds and diseases control have been made in different countries, as well as in Romania (Blanchet et al., 1987; Sin et Ioniță, 1990; Vannozzi et al., 1990; Vrânceanu, 2000; Bonari et al., 1992; Sarno et al., 1992).

This paper presents some technological aspects resulting from the experiments carried out.

MATERIALS AND METHODS

This research work was carried out in the National Agricultural Research and Development Institute – Fundulea, under rainfed conditions, during a long period, on chernozem soil with a good fertility: organic matter (humus) - 2.5%; N – 0.18%; P_2O_5 – 28 ppm; K – 98 ppm; TON content – 30%; pH – 6.5. The annual mean rainfall is 560 mm, ununiformly distributed during the year and the annual mean temperature is 10.5 °C.

The experiments have included 2 – 6 years rotations and monoculture, soil tillage methods, different sowing treatments with dates and densities (3–6 plants/m^2). The size of an experimental plot varied between 100–500 m^2, and the number of replications between 3–5.

The experiment treatments are presented here, together with the research results.

RESULTS AND DISCUSSION

Crop rotation

The experiments aimed to establish the influence of previous crops and the minimum number of years for sunflower return in the same place, in connection with crop production and disease attacks.

The data presented in Table 1 emphasize the following aspects:

- the highest crop yield was obtained in the 6 years of rotation, demonstrating the necessity of sunflower growing in the same place not for a shorter time than 6 years;
- the 4 - year rotation favored the *Sclerotinia sclerotiorum* attack. Even the soybean in a crop rotation with sunflower increased the attack of *Sclerotinia*;

- the greatest *Sclerotinia* attack and the lowest yields were obtained in the case of sunflower cultivated after soybean and sunflower.

Table 1. Effect of the crop rotation on sunflower yield and *Sclerotinia* attack (4-year average)

Crop rotation	Yield q/ha	Difference q/ha	Difference %	*Sclerotinia* attack - %
Sunflower-Wheat-Sugar beet-Maize-Maize-Wheat (6-years rotation)	32.0	-	-	3.0
Sunflower-Wheat-Sugar beet-Maize (4-years rotation)	30.0	-2.0	7	12.1
Sunflower-Soybean-Wheat-Maize (4-years rotation)	27.9	-4.1	13	16.5
Sunflower-Soybean (2-years rotation)	23.0	-9.0	28	26.0
Sunflower-Sunflower	22.8	-9.2	29	23.4
LSD 5%		1.9		

Soil tillage

The research regarding soil tillage has taken into consideration soil fertility conservation and fuel consumption reduction. The experiment results showed that sunflower reacts weakly to soil tillage methods and to the loosening depth, the crop yield differences being non-significant (Table 2).

The lowest yield was obtained in the case of no-till soil.

The advantage of reduced soil tillage was represented by fuel saving.

Table 2. Relationship between tillage method, fuel consumption and sunflower yield (4-years average)

Soil tillage	Yield q/ha	Difference q/ha	Fuel consumption, %	
			Ground tillage	Total soil tillage+sowing
Plowing 20 cm	22.4	-	100	100
Plowing 30 cm	23.0	+0.6	130	120
Chiseling	21.9	-0,5	57	69
Paraplowing	21.6	-0.8	75	83
Disking	22.0	-0.4	20	41
No-till	20.3	-2.1	-	14
LSD 5%		2.2		

The fuel consumption by soil plowing at 20 cm depth (100%) diminished by 86% in the no-till and by 17–50% using tillage methods with chisel, paraplow and disk, instead of plow, without a yield reduction.

Sowing

This concerns the experiments including different sowing dates and plant densities:

The data presented in Fig. 1 show the yield variation in three different years, depending on planting date, connecting with the soil temperature at sowing depth.

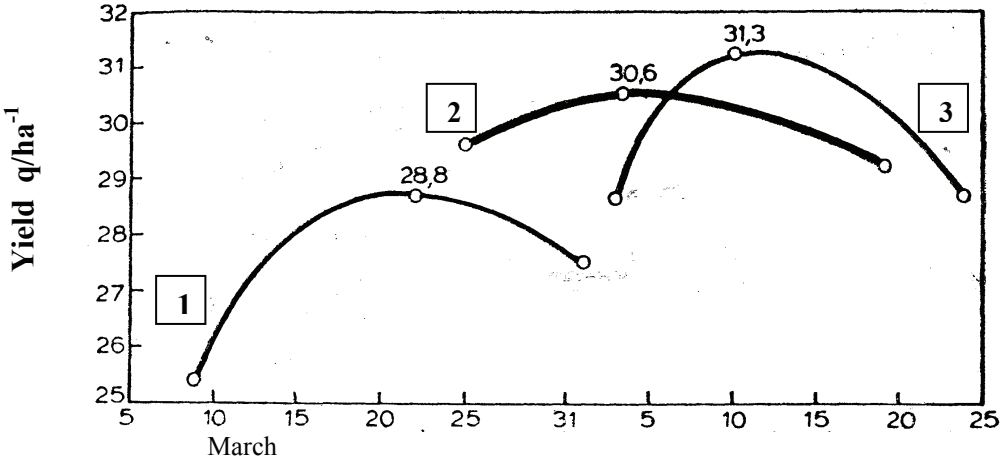
The highest yield was obtained when the soil temperature reached 7 °C. This relation was observed in early spring (1) and, also, in late spring (3), the optimal sowing data being marked by the occurrence of the respective soil temperatures. To sow earlier or later than this moment causes yield losses.

The plant density has been studied taking into account a variation from 30 to 60 thousand plants/ha.

The average results obtained for late hybrids are presented in Table 3.

The data pointed out a relation between the sowing density and the harvested plants, resulting in a decrease of 9.0–12.7%, with a growth tendency as the density increased.

The difference between both densities could be diminished by a better control of diseases and pests and by more careful cultivations, which could reduce the plant number.



1	2.7	6.9	8.1					
2	2.9	3.6	5.3	6.9	8.2	8.5	8.2	
				5.0	7.5	8.2	7.2	3

Soil temperature, in three different years (1, 2, 3)

Fig. 1. The effect of sowing time on sunflower yield.

Table 3. The effect of plant population on sunflower yield (10- year average)

Sowing density, 10^3 seeds/ha	Harvested plants/ha		Yield		Difference q/ha
	10^3	%	q/ha	%	
30	27.3	90.0	27.3	100	-
40	35.4	88.5	29.6	108	2.3
50	43.8	87.6	30.0	109	2.6
60	52.4	87.3	29.0	106	1.7
LSD 5%			2.0		

The sunflower yield varied non-significantly at a population of between 40 and 60 thousand plants/ha. The weak reaction to the variation in plant density is explained by a high capacity for compensation of crop yield components.

Relationship between water supply and sunflower yield

An analysis of rainfall regime during the years 2006 (wet year) and 2007 (with droughts) shows a net differentiation regarding plant growth, yield formation and its quality (Table 4).

Table 4. Relationship between climate conditions (rainfall amount and air temperature), yield level and seed oil in 2006 and 2007

	Wet year 2006	Dry year 2007	Difference
Rainfall, mm			
- Oct.2005-March 2006	301		
- Oct.2006-March 2007		122	179
- April-August	313	190	123
Mean temperature, °C			
- June	20.9	24.1	3.2
- July	22.9	26.9	4.0
- August	23.0	24.1	1.1
Seed yield, kg/ha	2400	550	1850
Seed oil content, %	50.8	42.5	8.3
Oil production, kg/ha	1219	234	985

The rainfall during October – March period and April – August growing period greatly varied with obvious differences between the two years of 179 mm and 123 mm, respectively.

The drought conditions of 2007 were stressed by the association of humidity shortage and high temperature, which was higher by 3 – 4 ^{0}C in 2007, as compared with the previous year.

This phenomenon negatively influenced the plant growth and yield formation, leading to a difference of 1850 kg/ha (77%).

The drought also reduced the seed oil content (8.3%), so that the oil production in 2007 was lower by 985 kg/ha, representing 19% from the oil production obtained in 2006.

CONCLUSIONS

Sunflower must be included in a 6 - year rotation, avoiding its alternance with soybean crop, due to the potential attack of *Sclerotinia*.

The soil tillage method has a weak influence on sunflower yield, offering the possibility to apply a reduced tillage, that ensures a way for fuel saving.

The optimal time for sunflower sowing is indicated by achieving the temperature of 7 °C, at sowing depth.

The optimal plant density for late hybrids is of 40 – 50 thousand plants/ha.

Although the sunflower is a tolerant crop to dryness, the association of water deficit with high temperatures during the growing season caused yield losses of up to 77% and decreased the seed oil content.

REFERENCES

Blanchet, R., N. Gelfi, and J. Puech. 1987. Alimentation azoté, surface foliare et formation du rendement du tournesol. Agrochimica 3:233-244.

Bonari, E., G.P. Vannozzi, A. Benvenuti, and M. Baldini. 1992. Modern aspects of sunflower cultivation techniques. p. 3-51. In: Proc. 13th Int. Sunflower Conf., Pisa, Italy.

Sarno, R., C. Leto, R. Cibella, and A. Carrubba. 1992. Effects of different sowing times on sunflower. p. 390-409. In: Proc. 13th Int. Sunflower Conf., Pisa, Italy.

Sin, Gh., and Şt. Ioniţă. 1990. Research regarding the influence of crop rotation, soil tillage and sowing time on sunflower. Int. Symposium Crop Technologies, Institute Dobrudja, Bulgaria.

Vannozzi, G.P., E. Solera, and M. Baldini. 1990. Sunflower yield characteristics as affected by weed control, plant density, nitrogen level and sowing time. Helia 13:73-86.

Vrânceanu, Al. V. 2000. Floarea Soarelui hibridă. Ceres, Bucharest, Romania.

Screening and drying conditions for early harvested sunflower

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ABSTRACT

Screening tests were held to examine the method to remove admixture from the sunflower grain before drying process. Moisture contents of samples were 34.4% and 20.0%. Screening sizes examined were 1.0-5.0 mm. According to the results obtained, it is difficult to fix a certain screen gap size to remove most admixtures because size of grain varies from year to year and grain and admixture have almost the same size. However, using the 2.0 mm gap screen, a certain ratio (26-75%) of admixture, which is smaller than the grain, can be removed, and this is good for drying process in order to reduce machine trouble and extra energy to dry the admixture. The drying conditions and their effects on quality of sunflower, especially on oil quality, were also analysed for POV (peroxide value), AV (acid value) and color of oil. The sunflower plant material was cv Harurinzo (Pioneer:63M80) and the initial moisture contents were of 15.8% w.b. and 31.5%. Each sample was dried in an oven with 45°C, 55°C , 65°C for 24 hours and a circulating dryer (capacity: 1 t) for 11 hours. Samples were expressed with a small expeller. The oil samples were analysed for POV and AV. POV of sunflower oil ranged from 1.9 meq/kg for 45°C drying with 15.8% initial moisture content to 6.8 meq/kg for 65°C with 31.5% initial moisture content. According to the results obtained, it is concluded that in order to avoid the degradation of oil quality, the initial moisture content should be low enough, and, if the harvested sunflower has a high moisture content, drying it at a lower temperature is better to maintain the quality of the oil.

Key words: acid value – drying – early harvesting – peroxide value – screening

INTRODUCTION

Recently, sunflower and rapeseed cultivated areas have increased rapidly in Japan because of the growing expectations of bio-fuels made from oil from those crops. But there are some problems in producing sunflower in Japan. One major reason is that farmers do not have enough experience of growing, harvesting and drying of sunflower.

The high admixture rate is a problem. In drying process much energy is lost in drying grain with so many admixtures. Moreover, with respect to the oil expressing process, a high admixture rate reduces the yield of the oil expressed and sometimes causes machine troubles. The admixture rate in a harvested crop occasionally reaches 20%. The high admixture rate is the result of harvesting sunflower under high moisture conditions, especially with high moisture in the stem and receptacle, with a harvester designed for rice or wheat.

In Japan, because of the high humidity in the climate, the drying process is inevitable. But farmers do not know the appropriate drying condition for sunflower, because of their lack of enough experience, and sometimes they degrade the quality of the sunflower grain through drying process. Therefore, it would be necessary to clarify the effect of the drying conditions on the quality of sunflower grain and its oil and to fix the drying conditions for this crop.

The size of the screen gap for removing the admixture from sunflower before its drying process was examined. Also, the drying conditions for keeping the oil quality high were examined with POV (peroxide value) and AV (acid value).

MATERIALS AND METHODS

Screening test

Experiments were held in 2006 and 2007. In 2006, the sample was harvested with 34.4% moisture content in Hikawa city, Shimane pref., Japan. The sample was sorted with the size grader before drying. The screen gaps examined were 2.0, 2.4, 2.8, 3.2, 3.6, and 4.0 mm. In 2007, the sample was harvested with 21.0% moisture content. The sample was sorted with the size grader before drying and the screen gaps examined were 2.0, 3.0, 4.0, and 5.0 mm. In both cases, the admixture was separated after drying with a winnower for experimental use and its weight was measured.

Material and initial moisture contents
The sunflower cultivar Harurinzo (Pioneer:63M80) was grown with usual cultivation practices from June to October of 2006 in the south of Ibaraki Prefecture, Japan. Samples were harvested twice. The moisture contents of each sampling were "*I*: 15.8%" and "*II*: 31.5%". Moisture contents were measured with the 10 g (grain) -105°C-24 hours method.

Drying settings
To fix the best drying temperature for each moisture contents samples were dried with the air of "*A*: unheated", "*B*: 45°C", "*C*: 55°C" and "*D*: 65°C." A circulating dryer (*E*) was used because this dryer is very popular with Japanese rice farmers and it is useful if it can be utilized for drying of sunflower.

Drying settings were shown in Table 1. For *A* the ventilation dryer, Issingo Kaneko Agricultural machinery co., Ltd, has 6.6 m^2 mesh deck and air flow upward by blower (0.75kW) without burner burning. The drying oven was Espec Convection Oven LC-123. The circulating dryer was Iseki GA100 (Capacity: 400-1200 kg of wheat).

Each sample from *A* to *D* was about 2 kg and packed in 30cm x 40cm plastic mesh bags. Sample *E* was dealt as bulk. After drying, each sample was preserved in 10°C refrigerator.

Table 1. Drying Settings

	Dryer	Air Temperature (°C)	Drying time (hr.)
A	Ventilation dryer	Unheated	24
B	Drying oven	45	24
C	Drying oven	55	24
D	Drying oven	65	24
E	Drying oven	Unheated-55 (changing)	11

Expression of oil sample
A small expeller (San-Seiki S100-200, Capacity: 3.5 kg/h) was used. Moisture contents of samples were 5-6% when expelled. Yield of oil was 20-30% of the input grain weight.

Evaluation of oil quality
Expelled oil samples were examined by POV (peroxide value), AV (acid value) and the color of oil. POV and AV are used as indices for oil as food constituent. The measurement of POV and AV were outsourced to Japan Institute of Oil, Fats and Other Foods Inspection Foundation and analysed using Standard Methods for the Analysis of Fats, Oils and Related Materials (Japan Oil Chemists' Society).

RESULTS AND DISCUSSION

Screening test
Results of the screening test are shown in Fig. 1 and Fig. 2. In 2006 (Fig. 1), the admixture rate was 14.4% of whole weight. The admixture classified into 0-2.0 mm was 10.8%, others were lower than 1.0% of whole sample. 75% of the total admixture was classified into 0-2.0 mm. For the grain, 55.7% of whole sample, 65.1% of grain, was classified into more than 4.0 mm class.

In 2007, (Fig. 2) the admixture rate was 20.4% of whole weight. The admixture classified into 0-2.0 mm was 5.4%, 2.0-3.0 mm 4.2%, 3.0-4.0 mm 7.1%, 4.0-5.0 mm 3.0% and 5.0 mm- 0.7%. 26.5% of the admixture was classified into 0-2.0 mm. For the grain 37.0% of whole sample, 45.5% of grain was classified into more than 3.0-4.0mm class. And 36.2% of whole sample, 45.4% of grain was classified into more than 4.0-5.0 mm class.

From the results it is difficult to fix a certain size of screen gap to remove most of the admixture from mixture of grain and admixture because the grain size is different every year due to environmental conditions. However, screening with 2.0 mm screen removes 26-75% of the admixture. This seems to be of use as a rough screening before drying process because it will reduce the energy used for drying and the risk of machine trouble from the dryer even if the screening process after drying is inevitable.

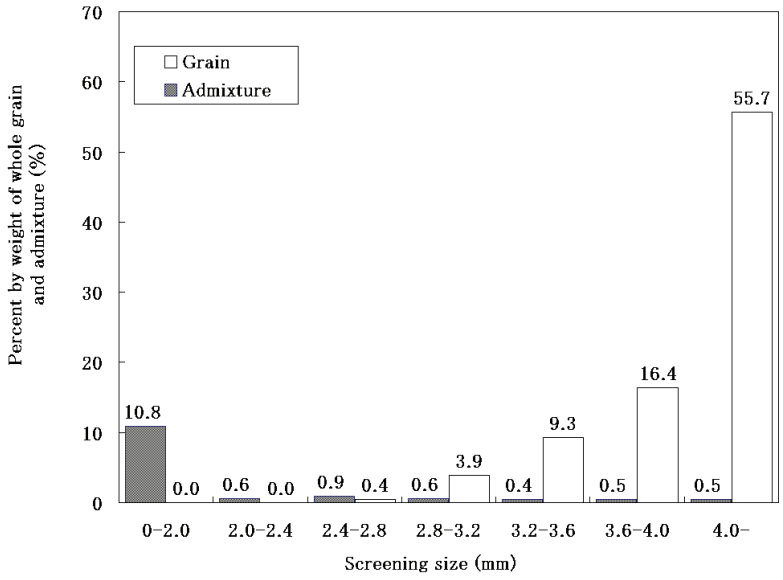

Fig. 1. Results of admixture screening test in 2006

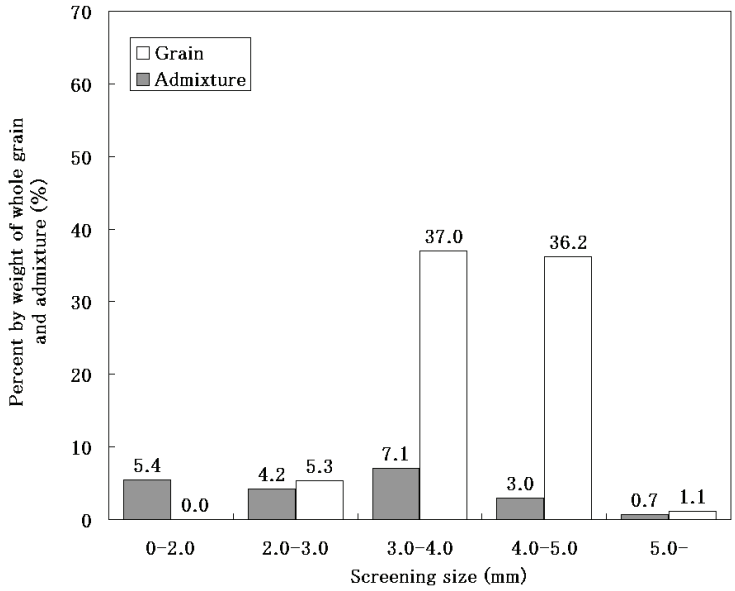

Fig. 2. Results of admixture screening test in 2007

Drying conditions

Fig. 3 shows POV of sunflower dried under each setting. When the initial moisture content was 15.8% (*I*), *D* setting had the highest POV followed by *C, B* and *A* which was the lowest. When the initial moisture content was 31.5% (*II*), *E* setting had the highest POV followed by *D, C, B* and *A*, which was the lowest.

The higher drying temperature resulted in a higher POV. That tendency was stronger for higher initial moisture content, 31.5% (*II*) than for 15.8% (*I*). The results also showed the effect of the initial moisture content on POV. POV of *II* (31.5%) was higher than that of *I* (15.8%) in all drying conditions from *A* to *D*.

It is concluded that to avoid the degradation of oil, firstly it is important to harvest enough dried grain below 16%. Secondly, if the harvested grain has a high moisture content of around 30% it should be dried with unheated or low temperature air.

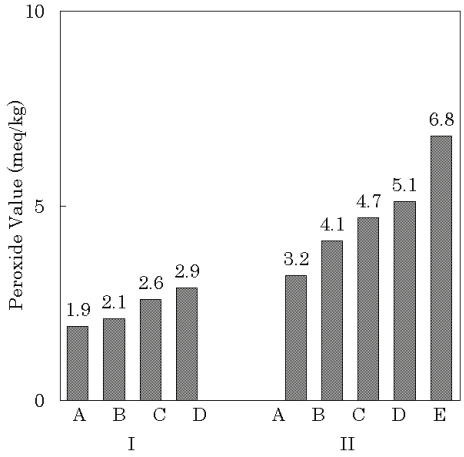

Fig. 3. POV of sunflower under each drying condition

The AV of sunflower oil of each sample varied from 0.3 to 0.7 and did not show any effects from the drying condition. Average of the AV of *I* was 0.3 and *II* 0.48. AV seems to have a proportional relationship with the initial moisture content.

The oil with an AV of over 1.0 was classified as being unsuitable for food, based on Japanese Standard. The AV of sunflower oil fulfilled the standard. The drying conditions did not have much effect on the deterioration of the AV. This implied that the higher initial moisture content of the grain caused a higher AV.

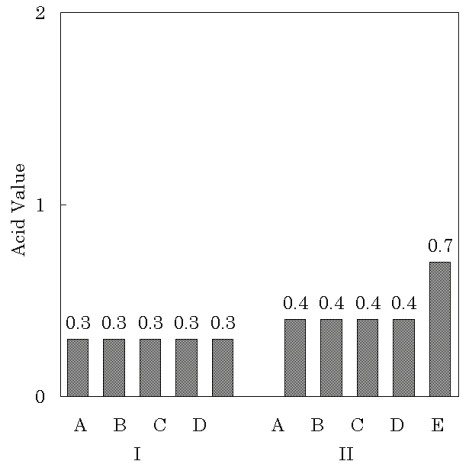

Fig. 4. AV of sunflower under each drying condition

CONCLUSIONS

The size of the screen gap in removing the admixture from sunflower before its drying process was examined. Also, the drying conditions for keeping the oil quality high were examined with POV (peroxide value) and AV (acid value). The conclusions of this study are follows:

1) From the results it was seen to be difficult to fix a certain size of screen gap to remove most of the admixture from mixture of grain and admixture because the grain size is different every year due to environmental conditions. However, screening with 2.0 mm screen removed 26-75% of the admixture. This would seem useful as a rough screening before drying process because it reduces drying energy and the risk of machine trouble from the dryer even if the screening process after drying is inevitable.

2) To avoid the degradation of oil, firstly it is important to harvest enough dried grain below 16%, and, secondly, if the harvested grain has a high moisture content of around 30% it should be dried with unheated or low temperature air.

3) The drying conditions did not have much effect on the deterioration of the AV. This implied that the higher initial moisture content of the grain gave a higher AV.

Physiological maturity in sunflower. Correspondence between the quantitative and the visual definition

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ABSTRACT

The identification of physiological maturity (PM) in sunflower (*Helianthus annuus* L.) by visual methods is highly subjective. In order to find an indirect method for objectively defining PM, this study was conducted to correlate, in two sunflower hybrids, Macón and MG60, quantitative color parameters in the receptacle (Hue and Chroma in the *HSB* color space and *L**, *a** and *b** in the *CIE* color space) with physiological markers such as fruit dry weight (FDW) and fruit water content (FWC). Fruits from each cultivar were sampled at 2-day intervals from first anthesis until harvest maturity (HM). Fruit dry weight and color changes of the receptacle base, using digital images, were followed over time until HM. Fruits attained their maximum dry weight when the capitulum color turned from dark green to pale green in MG60 and when it turned from dark green to buttery-yellow in Macón. The color parameters L*, a*, b*, Hue and Chroma were tested against the fruit dry weight, and several good correlations were found, but from a crop management point of view the Hue (r_{MG60}= 0.876; $r_{Macón}$= 0.794) appeared to be a valid color parameter to define visual PM.

Keywords: color correlation – color parameters – *Helianthus annuus* – physiological maturity – sunflower.

INTRODUCTION

Physiological maturity (PM; Schneiter and Miller, 1981), is an important reproductive stage of the sunflower crop. At PM fruit dry weight (FDW) has reached its maximum value with a water content (FWC; d.w.b.) of about 38% (Rondanini, 2007). In the decimal notation by Schneiter and Miller (1981), the most frequently used scale to define the developmental stages of sunflower, PM, also defined as phenostage R9, is externally observed when the phyllaries become brown and brittle and the receptacle base turns buttery yellow.

The time elapsed to attain PM varies according to genotypes and environmental conditions such as nitrogen and soil water availability, temperature and photoperiod (Connor and Hall, 1997). The same genotype can differ from between 7 and 10 days to reach PM in response to changes in the variables mentioned (Kaya et al., 2004). Therefore, although the scale by Schneiter and Miller (1981) is a useful tool to study many sunflower genotypes, it fails for others. In fact, in some "stay green" (SG) genotypes the base of the receptacle at PM is green or yellowish green; only the phyllaries can become slightly brown (Cukadar-Olmedo and Miller, 1997).

Changes in color of the sunflower receptacle when approaching PM are recorded at naked eye. This is why the method is highly subjective. The aim of this work was to determine the correspondence among chromaticity of the receptacle base, by analyzing digital images of the receptacle development from first anthesis (FA) until harvest maturity (HM), the phenostage scale developed by Schneiter and Miller (1981) and the evolution of FDW and FWC.

MATERIALS AND METHODS

Two short season sunflower hybrids: Macón (Syngenta, Argentina) and MG-60 (Dow-Agrosciences, Argentina) were used in the study. Plants were grown at the Chacra Experimental de Barrow (INTA-MAA, Tres Arroyos, Argentina; Lat. S. 38º20'; Long. W. 60º13') following conventional cultural practices.

Qualitative determination of phenological stages was made using the scale by Schneiter and Miller (1981). At FA (phenostage R5.1; Schneiter and Miller, 1981) twelve plants of each hybrid were selected and labeled. FDW and FWC (d.w.b.) were measured in 6 plants of each hybrid by taking samples of fruits from the capitulum's rim at 3-day intervals from FA to HM.

A biphasic fit of FDW vs. time (days from FA) was performed using the model: **y** = a+b.X (for X<c); **y** = b.c (for X>c), where **c** corresponds to the unknown break point of the two linear functions, this being the maximum grain weight of the fruit F(t), where PM is attained.

Simultaneously with fruit sampling, photographs of the receptacle base were taken from 8:00 a.m to 9:00 a.m. to the remaining 6 plants of each hybrid using a digital camera. A color reference scale was included in each image. Digital images were corrected for light intensity changes and analyzed to determine the parameters *L**, *a** and *b** within the CIE *L*a*b** color space, (CIE, 1986, 2001), using Photoshop CS2 software (Adobe Systems Inc.; San José, CA, USA).

*L**, *a** and *b** values were furthermore converted into the *HSB* color space (Adobe Systems Inc., 2000; MacEvoy, 2005), defining the parameters Hue (the attribute of color by means of which it is perceived to be red, yellow, green, blue, etc. Pure white, black, and gray possess no Hue) and Chroma (also called "saturation" and indicating the amount by which a color differs from gray, white or black, from neutral to fully saturated color). The values run from 0%, which is no color saturation, to 100%, which is the fullest saturation of a given Hue, using the algorithms:

$$\text{Hue} = h^* = \tan^{-1}(b^*/a^*), \text{ [when } a^*>0 \text{ y } b^*\geq 0]; \text{ Hue} = h^* = 180 + \tan^{-1}(b^*/a^*) \text{ [when } a^*<0]$$

$$\text{Chroma} = C^* = [a^{*2} + b^{*2}]^{1/2}$$

RESULTS AND DISCUSSION

Maximum FDW significantly differed ($p<0.01$) between genotypes, with 0.043 g/fruit, 31 days after FA in MG60 (Fig. 1A) and 0.045 g/fruit, 28 days after FA in Macón (Fig. 1B). Maximum FDW for both hybrids was attained with a FWC of 38.6% in MG60 (Fig. 1A) and 39.2% in Macón (Fig. 1B). These values showed no significant differences ($P<0.05$). However, Macón showed a higher average FWC (Fig. 1B), possibly as a consequence of green mass retention at PM.

The magnitudes of the *L*a** and *b** at the time of PM were: *L**: 68.3 (MG60) and 73.6 (Macón); *a**: -4.2 (MG60) and -6.2 (Macón); *b**: 48.4 (MG60) and 52.3 (Macón) (Fig 2. A and B, respectively).

The *L** magnitude showed important fluctuations (Fig. 2A-B) in response to variations in daily luminosity when digital images were taken. This probably masked the real magnitude of luminosity as maturity advanced (Shewfelt et al., 1988). However, it was observed that *L** magnitude decreases with capitulum maturity in response to opacity and darkening of the capitulum's tissue (Fig. 2 A-B).

A significant correlation between the FDW and colorimetric parameters *a** (0.752; 0.638), *b** (0.771; 0.670), Hue (0.876; 0.794) and Chroma (0.669; 0.593) for MG60 and Macón, respectively, were observed. Nevertheless, it was found that both Hue and Chroma were the best color parameters to be considered when working in a relationship between their changes with time of capitulum maturation and FDW.

In early developmental stages the presence of a high concentration of chlorophyll in the receptacle tissues is significantly related to the green color observed. So, as maturity advances, chlorophyll degradation, (Sexton and Woolhouse, 1985) and the predominance of xanthophylls and other carotenoid pigments (Sinecker et al., 2002) are the reason for the variation in color turning from green to yellow.

Magnitudes of *a** and *b** moved over time from minus *a** (green component; HunterLab., 2001) to plus *a** (yellow-red component; HunterLab., 2001) (Fig. 2A-B). The parameter *b** (yellow-blue component; HunterLab., 2001) always had positive values.

For both hybrids, results showed that the magnitude of *b** tends to increase up to the moment of the maximum value of FDW and then decreases (Fig 2 A-B) following the diminution of FWC, in response to plant senescence (Fig. 1A-B). The *a** value increases as capitulum maturity advances (Fig 2 A-B), allowing the *b** component (yellow) to stand out. Yellowing of the receptacle was characterized, as expected, by a constant increase in the value of *a** (less green) and a maximum magnitude of *b** (more yellow) (Fig. 2A-B).

Hue values decreased from 122.8 to 74.6 in MG60 (Fig. 1A) and from 115.4 to 71.1 in Macón (Fig. 1B). Chroma increased until 28 days after anthesis in MG60 (Fig. 1A) and in Macón (Fig. 1B), when both hybrids attained their maximum FDW. From that moment on Chroma magnitude started decreasing.

The maximum Chroma (maximum color saturation) in MG60 was attained 2 days before PM (Chroma=59; Fig. 1A); the Hue at that time was 98 showing a buttery yellow capitulum base and brown phyllaries. The maximum Chroma in Macón was attained at PM (Chroma=62; Fig. 1B) with a yellowish receptacle base and the phylllaries still green. Also, in this hybrid with a higher retention of green tissue, PM was attained 12 days before phenostage R9 was observed (Fig. 1B).

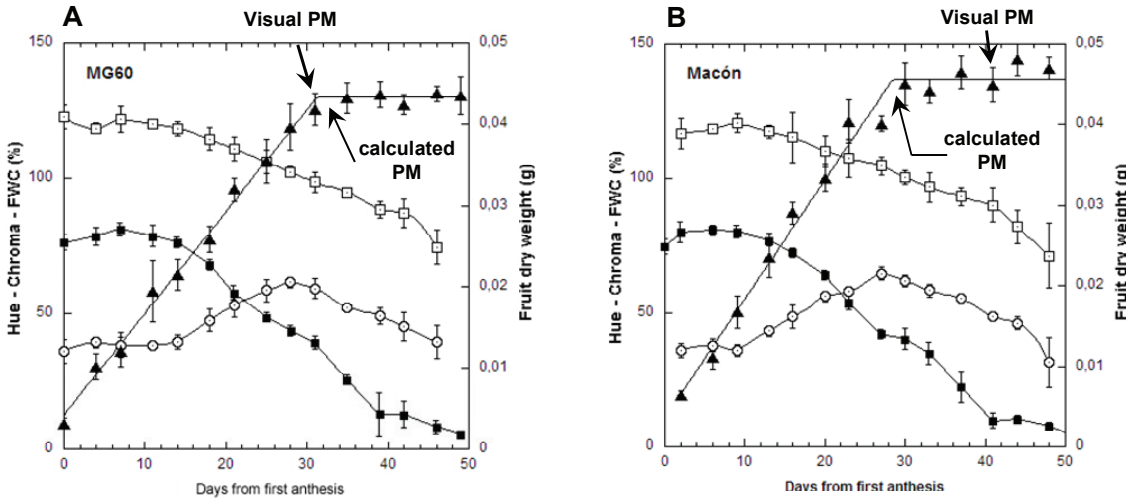

Figure 1: Evolution from first anthesis until harvest maturity of fruit dry weight (FDW), fruit water content (FWC) and the color parameters Hue Chroma in MG60 (A) and Macón (B). The visual PM (R9; Schneiter and Miller, 1981) and maximum FDW (calculated PM) in MG60 (A) was atta 31 days after anthesis. The maximum FDW in Macón (B) was attained 28 days after anthesis, while the visual PM was approximately 12 days l Then only in the genotype MG60 (B), the maximum FDW coupled the visual PM according to the morphological characteristics defined by Schn and Miller (1981). (□) Hue; (○): chroma; (▲): fruit dry weight (FDW); (■): Fruit water content (FWC). Vertical bars: ± 1SE.

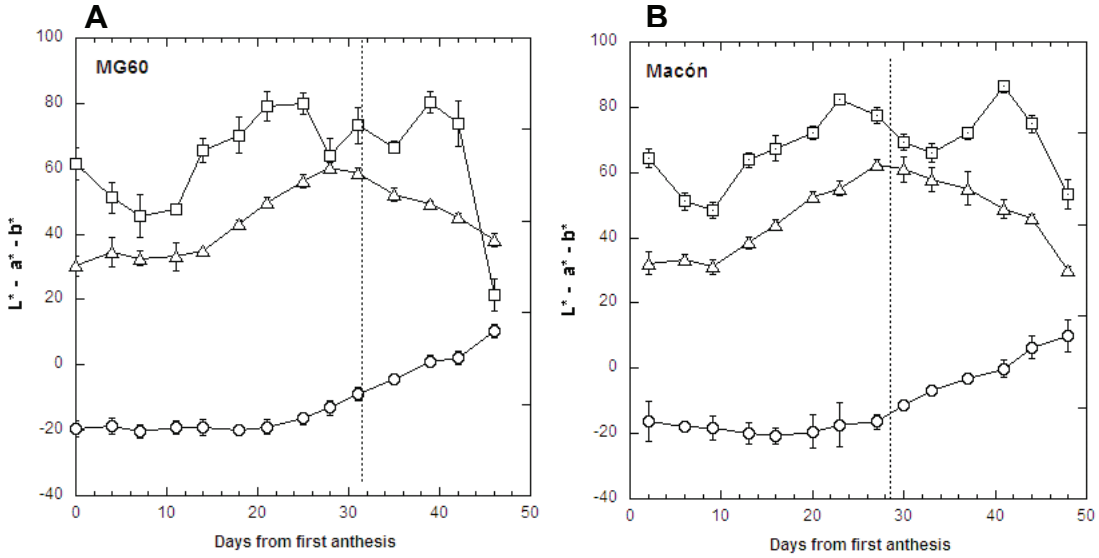

Fig. 2. Changes in the CIE *L*a*b** parameters in the base of capitula of the sunflower hybrids MG60 (A) and Macón (B) from first anthesis until HM. The vertical dashed line indicates the time when the maximum FDW, and hence PM was attained. (□) *L**; (○): *a**; (△): *b**. Vertical bars: ± 1SD.

The hybrid MG60 attained visual PM (R9; Schneiter and Miller, 1981) 31 days after first anthesis (Fig. 1A) while Macón, attained visual PM 40 days after FA (Fig. 1B). In MG60 visual PM (Hue= 98) and measured PM were reached at the same time (Fig. 1A). In Macón the maximum FDW was attained 12 days earlier than visual PM (Fig. 1B) indicating that fruits reached their maximum dry weight when the receptacle base was still green with a Hue of 103.

The linear variations in Hue, between 10 and 40 days after FA in both hybrids (Fig. 1A-B), showed the direct relationship between the receptacle color change and the advance of fruit maturity. The Hue is then best associated with the attainment of the visual PM, corresponding to phenostage R9, this value being nearly similar for both hybrids: Hue Macón=103; Hue MG60=98 (Fig. 1A-B). Therefore, the Hue of the receptacle base could be a useful parameter to express differences or similitudes between sunflower genotypes in the attainment of PM.

This work demonstrates that visual scales, which are generally widely subjective, are not always appropriate for determining maturity stages of crop plants, particularly sunflower. The brown phyllaries as a qualitative concept of PM cannot be applied to all genotypes. Using quantitative color parameters in genotypes grouped by maturity length and/or green mass retention could be a more precise approach to determine the correspondence between the measured PMs and their visual morphological characteristics.

ACKNOWLEDGEMENTS

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REFERENCES

Adobe Systems Inc. 2000. Color Models. The Munsell Color System. (http://web.archive.org/web/20030813092028/www.adobe.com/support/techguides/color/colormodels/munsell.html) [URL verified 18/12/2007].

CIE. 1986. Colorimetry, 2nd ed.; Publication CIE No. 15, 2; Central Bureau of the Commission Internationale de L'Eclairage 27-A-1030,Viena.

CIE. 2001. Publication CIE No. 142. Improvement to industrial colour difference evaluation.Central Bureau of the Commission Internationale de L'Eclairage, Viena.

Connor D.J. and A.J. Hall. 1997. Physiology. p. 113-182. In*:* Sunflower Science and Technology. J. Carter (Ed.), American Society of Agronom, Madison, WI, USA.

Cukadar-Olmedo, B. and J.F. Miller.1997. Inheritance of the stay green trait in sunflower. Crop Sci. 37: 150-153.

HunterLab. 2001. Basic Principles for Color Measurement and Perception. (http://www.hunterlab.com/pdf/color-s.pdf) [URL verified 18/12/2007].

Kaya, Y., D. Baltensperger, L. Nelson, and J. F. Miller. 2004. Determining Physiological Maturity in Sunflower. Trakia Univ. J. Sci. 5:1-10.

MacEvoy, B. 2005. Modern Color Models. In: Watercolors. (http://www.handprint.com/HP/WCL/color7.html) [URL verified 18/12/2007]

Rondanini, D.P., R. Savin, and A.J. Hall. 2007. Estimation of physiological maturity in sunflower as a function of fruit water concentration. Eur. J. Agron. 26: 295-309.

Schneiter, A.A. and J.F. Miller. 1981. Description of sunflower growth stages. Crop Sci. 21:901-903.

Sexton, T. and H.W. Woolhouse. 1985. Senescence and abscission. p. 468-497. In: M.B. Wilkins (ed.), Advanced Plant Physiology, Longman.

Shewfelt, R., C.N. Thai, and J.W. Davis. 1988. Prediction of changes in color of tomatoes during ripening at constant temperatures. J. Food. Sci. 53:1433-1437.

Sinecker, P., M.S. Gomes, J.A.G. Arêas, and U.M.L. Marques. 2002. Relationship between color (instrumental and visual) and chlorophyll contents in soybean seeds during ripening. J. Agric. Food Chem. 50:3961-3966.

Influence of desiccation on germination and field emergence of sunflower

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ABSTRACT

In this paper we present results on the influence of desiccation on laboratory germination and field emergence of sunflower hybrid Favorit and its parental lines. As desiccants we used Reglone forte [diquat] (3 l/ha) and Harvade 25F [dimethipin] (2 l/ha), with water usage of 500 l/ha. Desiccation was done on August 20, 2006, and, after 20 days, the genotypes were harvested. In each trial variant (combination of genotype and treatment) laboratory germination was performed on four subsequent dates with 2-month intervals. With relation to the control, the Harvade 25F variant had a statistically significant higher germination of 4.38%, while the Reglone forte variant had a statistically significant higher germination in relation to both, control and Harvade 25F, of 7.32% and 2.95%, respectively. Regarding field emergence, differences among the treatments were small, statistically non-significant. Like in the laboratory germination test, Reglone forte treatment had the highest field emergence (78.55%) which was higher than the control and Harvade 25F in 2.94% and 2.86%, respectively.

Key words: desiccation – field emergence – Harvade 25F – laboratory germination – Reglone forte – sunflower.

INTRODUCTION

Chemical desiccation before harvesting is a very useful agro-technical measure, which is applied on different crops like cotton, rice, potato, alfalfa, soybean, oil rape and sunflower. Desiccation of sunflower is performed at technological maturity of plants, when seeds contain 24-50% of moisture (Degtyarenko, 1976; Palmer and Sanderson, 1976; Kosovac and Sudimac, 1980; Tombu, 1988; Miklič et al., 2001; Johnson et al., 2004; Radić, 2006). At that time the process of seed forming and filling has been completed, the seeds begin to lose their moisture and plants are still green.

Desiccation remarkably decreases moisture of seeds, leaves, heads and stalks, accelerates maturation and enables earlier harvest. Losses of seeds in combining as well as drying expenses, bird damage and presence of weeds on fields are reduced. It is possible to prepare the field earlier for the next crop and there are no harmful consequences for oil and byproducts quality (Hill et al., 1974; Kosovac and Sudimac, 1980). Desiccation has an especially positive influence in years with heavy rainfalls during the maturation period of sunflower when attacks of fungal diseases are very intensive.

Besides its positive influence on grain yield, desiccation also improves seed quality (Dembinski et al., 1974; Palmer and Sanderson, 1976; Miklič et al., 2004; Đukić et al., 2006). The objective of this research was to test desiccation influence on germination and field emergence of the sunflower hybrid Favorit and its parental lines by treating them with the desiccants Reglone forte (total herbicide) and Harvade 25F (growth regulator).

MATERIALS AND METHODS

Research was conducted in the experimental field and laboratory of the Agricultural Institute Osijek. Field trial was sown on April 22, 2006, with inter row space of 70 cm, in-row space of 23.5 cm, giving a plant population of around 61000 plants/ha. Main plot had surface of 14 m^2 (4 rows by 5 m length).

Genotypes in the research were the sunflower hybrid Favorit and its parental lines, developed at the Agricultural Institute Osijek. Treatments in the research were Reglone forte [diquat] (3 l/ha) and Harvade 25F [dimethipin] (2 l/ha), with water usage of 500 l/ha, and non-desiccated control treatment. Desiccation was done on August 20, 2006, and, after 20 days, the genotypes were harvested. In each trial variant (combination of genotype and treatment) laboratory germination was performed four times (October 23, 2006; December 21, 2006; February 22, 2007 and April 23, 2007). Seed vigor was calculated as the percentage of seeds that germinated after four days. Seed germination was calculated as the percentage of

seeds that germinated after 10 days (Official Gazette, 4/2005). For field emergence determination, seeds were sown in the field on April 13, 2007, and counting of emerged seedlings was carried out on May 11, 2007. The experimental data obtained were processed by SAS for Windows (SAS, 2003) software.

RESULTS

Seed samples of sunflower hybrid and parental lines were taken at the same time, before desiccation. Seed moisture of hybrid was 24%, female line 22.8%, and pollinator line 34.4%. The latter had distinctly the highest moisture. Twenty days after desiccation, the genotypes were harvested. Table 1 presents seed moisture of analyzed treatments and genotypes. As we expected, the highest seed moisture was found in the control (8.48%), then Harvade 25F, and a statistically significant lower moisture content than the control (1.28%) was found with the Reglone forte treatment. Among the genotypes, statistically significant differences were also found. The pollinator line had a statistically significant higher moisture content in relation to the hybrid (0.40%) and the female line (0.55%).

Table 1. Sunflower seed moisture at harvest of tested variants and genotypes

		Seed moisture (%)
Treatment	Control	8.48
	Reglone forte	7.20
	Harvade 25F	8.21
	LSD 0.05	0.36
Genotype	Favorit	7.88
	Female line	7.73
	Pollinator line	8.28
	LSD 0.05	0.36

Laboratory germination was estimated in the seed laboratory of the Agricultural Institute Osijek on four subsequent dates at 2-month intervals (Table 2). In the first count on October 23, 2006, seed vigor was very low (29.89%) as well as germination (53.83%). This could be explained by distinctive seed dormancy. In the second and third count, seed vigor and germination had almost the same values. In the fourth, final count on April 23, 2007, seed vigor was 91.39%, and germination 92.39%, respectively, which was statistically significantly greater ($P<0.05$) than in the previous count.

Table 2. Seed vigor and germination in subsequent germination tests

Germination test	Seed vigor (%)	Germination (%)
October 23, 2006	29.89	53.83
December 21, 2006	87.18	89.37
February 22, 2007	87.27	89.58
April 23, 2007	91.39	92.39
LSD 0.05	2.40	2.40

Among the treatments evaluated, we found statistically significant differences for seed vigor and germination (Table 3). In relation to control, the Harvade 25F variant had a statistically significant higher germination of 4.38%, while Reglone forte showed a statistically significant higher germination in relation to control and Harvade 25F of 7.32% and 2.95%, respectively. Among the genotypes, hybrid Favorit and female parental line did not exhibit any statistically significant differences in seed vigor and

germination, but the pollinator line had distinctly lower seed vigor and germination in relation to both hybrid Favorit and female parental line of almost 10%.

Table 3. Seed vigor and germination of tested variants and genotypes.

		Seed vigor (%)	Germination (%)
Treatment	Control	70.77	77.39
	Reglone forte	77.52	84.71
	Harvade 25F	73.51	81.77
	LSD 0.05	2.08	2.08
Genotype	Favorit	77.36	84.03
	Female line	77.05	84.74
	Pollinator line	67.39	75.10
	LSD 0.05	2.08	2.08

Among the treatments, differences in field emergence were small, statistically non-significant (Table 4). Again, Reglone forte treatment, as in the laboratory germination test, had the highest field emergence (78.55 %), which was higher than control and Harvade 25F for 2.94% and 2.86%, respectively.

Table 4. Field emergence of tested treatments and genotypes

		Field emergence (%)
Treatment	Control	75.61
	Reglone forte	78.55
	Harvade 25F	75.69
	LSD 0.05	ns
Genotype	Favorit	82.39
	Female line	77.45
	Pollinator line	70.02
	LSD 0.05	6.02

Among the genotypes, the highest field emergence was shown by hybrid Favorit (82.39%), which had a field emergence significantly higher (12.38%) than pollinator line and non-significantly higher (4.94%) in relation to female line. Also, the female line had statistically significant higher field emergence (7.43%) than the pollinator line.

DISCUSSION

Desiccation is a very important agro-technical measure, which has a positive influence on grain yield and seed quality in seed production. According to the research of Miklič et al. (2006), the highest germination occurs when moisture in harvest is below 32%, and in most cases between 22-23%. Desiccation accelerates moisture reduction in seed and plant parts, enabling earlier sunflower harvesting. After application of Reglone forte, moisture decreased at harvest level for 5-10 days (Dembinski et al., 1974; Kosovac and Sudimac, 1980), and with Harvade 25F application for 3-4 weeks (Ames and Walz, 1988).

In this research, when the seeds were harvested 20 days after the desiccant treatments seed moisture was distinctly reduced by 26.12% in the pollinator line, 16.12% in the hybrid, and 15.07% in the female line, enabling a considerably earlier harvest.

With the aim of estimating seed quality, after harvesting, laboratory germination of analyzed sunflower seed variants was tested. In the first count, seed vigor and germination were very low, which can be attributed to seed dormancy. Also, there was a large difference between seed vigor and

germination rate (23.94%). In further counts, seed vigor and germination increased, and the differences between them greatly declined.

Tested desiccants have shown different responses regarding seed vigor and germination of analyzed genotypes. Reglone forte showed statistically significant higher germination in relation to the control. These results are in accordance with those of Dembinski et al. (1974), Palmer and Sanderson (1976) and Miklič et al. (2004).

Harvade 25F is a growth regulator which shows good results in years when there have been many precipitations during maturation (Ames and Walz, 1988; Lebedev et al., 1997). Because this was not the case in this research, the results given with Harvade 25F were as expected. Germination of variants treated by Harvade 25F was significantly higher in relation to the control, but lower in relation to Reglone forte. Among the genotypes tested, almost the same values of seed vigor and germination were shown by the hybrid Favorit and female line, while for the pollinator line differed from both of them. In the field emergence test, Reglone forte had the highest statistically non-significant field emergence, while Harvade 25F and the control had almost the same behaviour.

On the basis of these results, a chemical desiccation had a favorable influence on seed maturity acceleration. Desiccant Reglone forte showed higher seed vigor and germination in laboratory germination and field emergence tests, hence its recommendation for use in seed production.

REFERENCES

Ames, R.B., and A.W. Walz. 1988. The use of dimethipin as a plant maturity regulator on sunflowers in the USA. p. 236-239. In: Proc. 12th Int. Sunflower Conf., Novi Sad, Yugoslavia.

Degtyarenko, V.A. 1976. Preharvest desiccation of sunflower. p. 174-178. In: Proc.7th Int. Sunflower Conf., Krasnodar, USSR.

Dembinski, F., CZ. Musnicki, and T. Ponikiewska. 1974. Sunflower desiccation before combine harvesting and its effect on quality and quantity of grain yields and nutritional value of oil meal. p. 597-602. In: Proc. 6th Int. Sunflower Conf., Bucharest, Romania.

Đukić, N., V. Miklič, D. Stefanović, and G. Malidža. 2006. Desikacija u proizvodnji suncokreta. Biljni lekar 34(4-5): 19-425.

Hill, J., B.A.G. Knight, and J.M.E. Ogilvy. 1974. The significance of a new harvest technology in the intensive production of sunflower. p. 589-596. In: Proc. 6th Int. Sunflower Conf., Bucharest, Romania.

Johnson, B.L., T.D. Larson, and R.A. Henson. 2004. Desiccation of stay-green and conventional sunflower. p. 213-217. In: Proc. 16th Int. Sunflower Conf., Fargo, USA.

Kosovac, Z., and V. Sudimac. 1980. Testing the ground for regular desiccation of sunflower before harvesting. p. 357-361. In: Proc. 9th Int. Sunflower Conf., Torremolinos, Spain.

Lebedev, V.B., D.A. Yusupov, and L.M. Kudimova. 1997. The efficacy of Harvade. Zashchita i karantin rastenii 7: 17.

Miklič, V., N. Dušanić, J. Crnobarac, and J. Joksimović. 2001. Effect of time of desiccation on oil content in different sunflower hybrids. Helia 24(34): 95-103.

Miklič, V., J. Crnobarac, N. Dušanić, and J. Joksimović. 2004. Effect of time of desiccation on some quality parameters of sunflower seed. p. 219-224. In: Proc. 16th Int. Sunflower Conf., Fargo, USA.

Miklič, V., J. Crnobarac, J. Joksimović, N. Dušanić, D. Vasić, and S. Jocić. 2006. Effect of harvest date on seed viability of different sunflower genotypes. Helia 29(44):127-134.

Ministry of Agriculture, Forestry and Water Management. 2005. Pravilnik o temeljnim zahtjevima kakvoće, načinu ispitivanja, pakiranju i deklariranju sjemena poljoprivrednog bilja. Official Gazette 4/2005.

Palmer, J.R., and J. F. Sanderson. 1976. Canadian experience with the pre-harvest desiccation of sunflower with Reglone. p. 167-173. In: Proc. 7th Int. Sunflower Conf., Krasnodar, USSR.

Radić, V. 2006. Effect of maturation period on seed quality; optimum time for desiccation in sunflower (*Helianthus annuus* L.) genotypes. Helia 29(44):145-152.

SAS Institute Inc. (2003). SAS for Windows (r) 9.1. Cary, NC, USA.

Tombu, B. 1988. Five years of development with Harvade 25F on sunflowers in France. p. 240. In: Proc. of 12th Int. Sunflower Conf., Novi Sad, Yugoslavia.

Physiological traits for quantification of drought tolerance in sunflower

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ABSTRACT

Five Romanian sunflower hybrids were grown in the greenhouse under two watering regimes for each genotype: control variant – in which plants were maintained at 70% from TSWC (total soil water capacity)] and *stress treatment* in which sunflower seedlings were irrigated no more than 40% from TSWC. The results showed that water stress induced the decrease of leaf area, shoot size, chlorophyll content and yield. Some differences between the tested sunflower hybrids were recorded.

Key words: biomass – chlorophyll content – drought – leaf area – sunflower.

INTRODUCTION

Drought is probably the most important factor limiting crop yields worldwide, and in Romania, too. Because of its complexity, drought tolerance is probably the most difficult trait to improve through conventional plant breeding. The challenge is even greater for developing drought tolerant cultivars for Romanian environment where the occurrence, timing and severity of drought may fluctuate from year to year.

In Romania, NARDI at Fundulea has devoted considerable effort during the past ten years to improve drought tolerance in wheat, maize and sunflower. Extensive research has been conducted in the area of breeding, agronomy, and most recently, physiology.

The physiology work has focused on morpho-physiological traits induced by drought and associated with drought tolerance of plants, and the elaboration of screening methods for rapidly measuring of drought tolerance using plants in an early stage of vegetation.

Sunflower is a well adapted to drought crop, essentially because of its powerful water uptake due to its efficient root system (Belhassen, 1995).

The present paper reports the responses of five Romanian sunflower genotypes to water stress. The aim was to identify morpho-physiological traits that could be used as screening criteria in a breeding programme for drought tolerance, and which could be rapidly measured using plants in an early stage of vegetation.

MATERIALS AND METHODS

Seeds of five sunflower hybrids: Alex, Favorit, Justin, Romina and Splendor were germinated and then planted at a depth of 3–4 cm in PVC tubes (35 cm long and 11 cm diameter) and in Mitcherlich pots filled with a soil-sand mixture (1:1). The plants were grown in a greenhouse up to the four leaf stage for the experiment from PVC tubes, and up to harvest maturity for another experiment.

In both experiments each genotype was tested in five replicates and two watering regimes: control variant – [in which plants were maintained at 70% from TSWC (total soil water capacity)] and stress treatment (where sunflower seedlings were irrigated no more than 40% from TSWC).

The biomass of the above and below-ground parts was measured after drying them to the constant weight.

The chlorophyll concentration was assessed using a SPAD-502 chlorophyll meter (Minolta, Japan).

Leaf area was calculated with the formula: L x l x 0.66 where: L = leaf length; l = leaf width and 0.66 = correction coefficient for sunflower. The root volume was measured by water displacement from a filled beaker.

RESULTS AND DISCUSSION

Under water stress conditions the reduction in leaf area and height of plants was recorded. Leaf area was insignificantly reduced in sunflower seedlings grown for one week under drought conditions (from 0.4% for Romina up to 15% for Justin hybrid) and significantly reduced in all sunflower genotypes grown for two weeks under drought conditions (up to 50%). It is obvious that young plants are a little more sensitive than mature

ones when water stress acts for two weeks (Table 1). This response could be considered as a usual reaction of sunflower plants in order to reduce water use.

In all sunflower hybrids, the effect of drought treatment consisted of a significant decrease in height of plants, less in hybrid Alex and more in hybrid Justin (Table 2).

Table 1. The effect of water stress on leaf area of sunflower seedlings

Hybrids	Relative reduction of leaf area due to water stress (%)			
	Seedlings (stressed one week)	Seedlings (stressed two weeks)	Plants (stressed one week after flowering)	Plants (stressed two weeks after flowering)
Alex	8.5	55.2	23.8	48.0
Favorit	0.8	52.6	24.3	48.7
Justin	15	42.4	24.1	44.7
Romina	0.4	47.8	25.5	30.9
Splendor	4.3	50	24.2	36.3

Table 2. The effect of water stress on shoot size of sunflower seedlings

Hybrids	Variants	Height of plants	
		mm	%
Alex	Control	621	100
	Water stress (2 weeks)	457	73.6
Favorit	Control	641	100
	Water stress (2 weeks)	459	71.6
Justin	Control	600	100
	Water stress (2 weeks)	385	64.2
Romina	Control	659	100
	Water stress (2 weeks)	450	68.3
Splendor	Control	571	100
	Water stress (2 weeks)	413	72.3

The significant positive correlation between leaf area and plant height under water stress condition is obvious (r = 0.953***, Fig. 1).

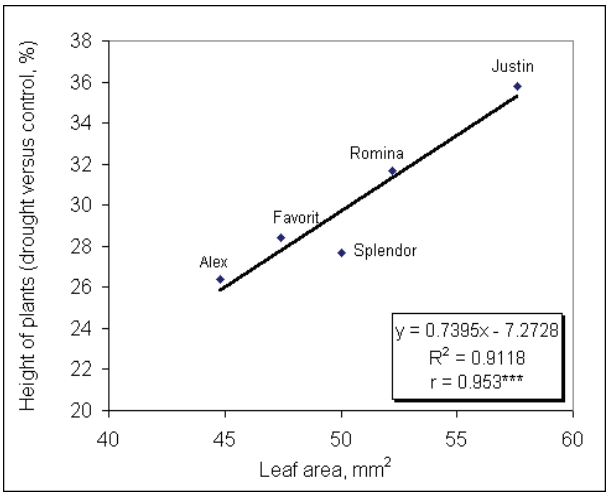

Fig. 1. Relationship between leaf area and height of plants.

The chlorophyll content is considered one of the most important indicators of vegetation stage and its degradation is normally considered a measure of drought resistance (Beard, 1973; Kim et al., 1989). The total chlorophyll content (expressed as SPAD units) was reduced under drought conditions, except for Favorit and Justin which presented the same SPAD units after first water stress period (Fig. 2).

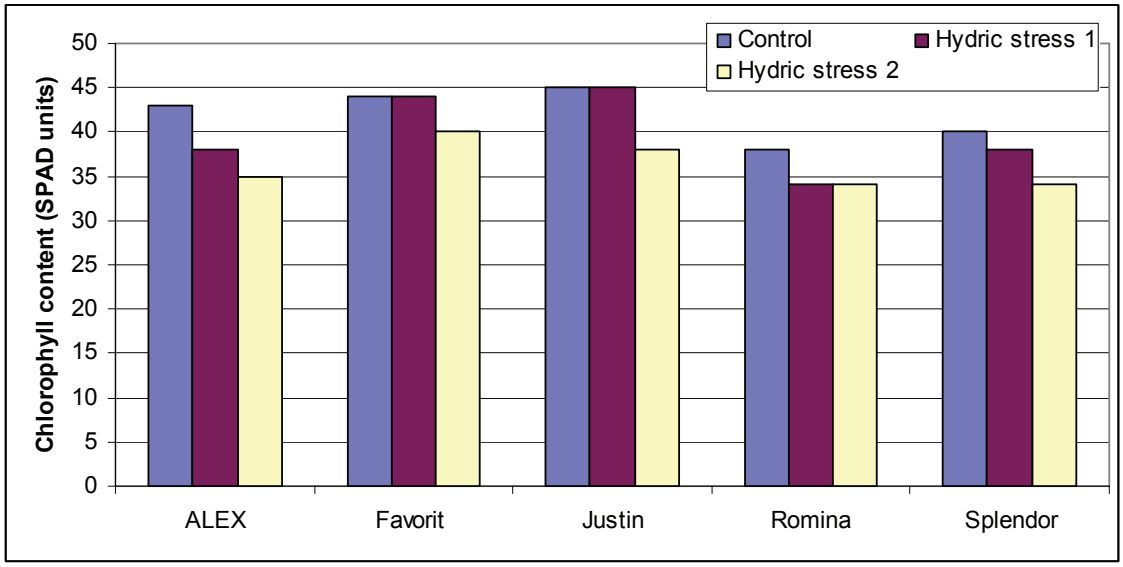

Fig. 2. Effect of water stress on chlorophyll content in sunflower leaves

Dry matter production of shoots, leaves and roots was significantly reduced under water stress conditions for all tested genotypes. Besides the genetic variability of tested sunflower hybrids, differences were recorded between the analyzed organs, too. It is obvious that leaves and shoots were more influenced by water stress than roots.

Thus, dry matter accumulation in roots of Favorit hybrid under drought was higher than under the control. Also, the values of the rest of the tested sunflower hybrids were up to 70%. Under water stress conditions, the root/shoot ratio increased. The increase in the Favorit hybrid was obvious (72%) and from 23 to 45.8% in Romina and Justin, respectively. These results show that the total root mass increases with drought stress (Table 3).

Table 3. The effect of water stress on biomass accumulation in seedling sunflower hybrids

Hybrids	Experimental variants	Biomass accumulation (g dry matter)						Roots/shoot	
		Leaves		Shoots		Roots			
		g	%	g	%	g	%	ratio	%
Alex	Control	2.87	100	4.37	100	2.35	100	0.32	100
	Water stress (2 weeks)	1.48	51.5	1.93	44.1	1.42	60.42	0.42	131.2
Favorit	Control	3.24	100	4.60	100	1.52	100	0.19	100
	Water stress (2 weeks)	1.86	57.4	2.87	62.2	1.58	103.9	0.33	173.6
Justin	Control	2.72	100	4.97	100	1.86	100	0.24	100
	Water stress (2 weeks)	1.70	62.5	2.91	58.5	1.60	86.02	0.35	145.8
Romina	Control	3.22	100	5.29	100	1.83	100	0.22	100
	Water stress (2 weeks)	2.22	68.9	2.5	47.2	1.29	70.49	0.27	123
Splendor	Control	3.12	100	4.88	100	1.87	100	0.23	100
	Water stress (2 weeks)	1.81	58.0	3.51	71.9	1.60	85.56	0.30	130.4

The shoot/root mass ratios consistently decrease under drought stress, which is a universal expression of adaptation (Blum, 1988). The increase in root/shoot ratio is mentioned in literature (Sharp and Davies, 1985; Sharp and Boyer, 1986). Previous reports underlined the genetic diversity of hybrid sunflower roots and the influence of soil environmental conditions on the rooting system (Terbea et al., 1995; Petcu et al., 1997; Agüera et al., 1997).

Our results show that during the first days of water stress the nutritive reserves of sunflower seedlings were conducted towards developing the roots, in order to facilitate deep soil moisture extraction. This happened in detriment of shoot development, and, in this case, a drift occurred in the main sink to survive. Concerning the root/shoot ratio, the response of mature plants to water stress is different from seedling response as the sink is different.

The root/shoot ratio of mature plants decreased under drought stress in Favorit and Splendor but increased in Alex, Justin and Romina hybrid. So, some differences between the tested genotypes in response of drought were noticed (Table 4).

Table 4. The effect of water stress on biomass accumulation in mature sunflower plants.

Hybrids	Experimental variants	Biomass accumulation (g dry matter)						Roots/shoot	
		Shoots		Leaves		Roots			
		g	%	g	%	g	%	ratio	%
Alex	Control	36.8	100	20.4	100	10.8	100	0.19	100
	Water stress (2 weeks)	21.6	58.7	18.6	91.18	9.4	87.1	0.23	123.8
Favorit	Control	36.8	100	38	100	21.2	100	0.28	100
	Water stress (2 weeks)	35.6	96.7	21.4	56.32	6.4	30.1	0.11	39.6
Justin	Control	34.4	100	29.2	100	9.4	100	0.15	100
	Water stress (2 weeks)	17.4	50.5	18	61.64	6.6	70.2	0.19	126.1
Romina	Control	28.8	100	18.8	100	7.6	100	0.16	100
	Water stress (2 weeks)	15.6	54.1	18.2	96.81	7.2	94.7	0.21	133.4
Splendor	Control	29.4	100	31.2	100	22.4	100	0.37	100
	Water stress (2 weeks)	21.4	72.7	23.4	75.00	10.4	46.4	0.23	62.8

It is well known that water stress has a profound effect on sunflower yield (Muriel and Downes, 1974; Talha and Osman, 1975). In our experience, the yield was affected by water stress with the low status treatment yielding 10-13% less than the control (Fig. 3). The highest productive hybrids under drought conditions were Favorit and Justin but Romina presented a high stability in yield level, both when plants were stressed for one week and for two weeks, too.

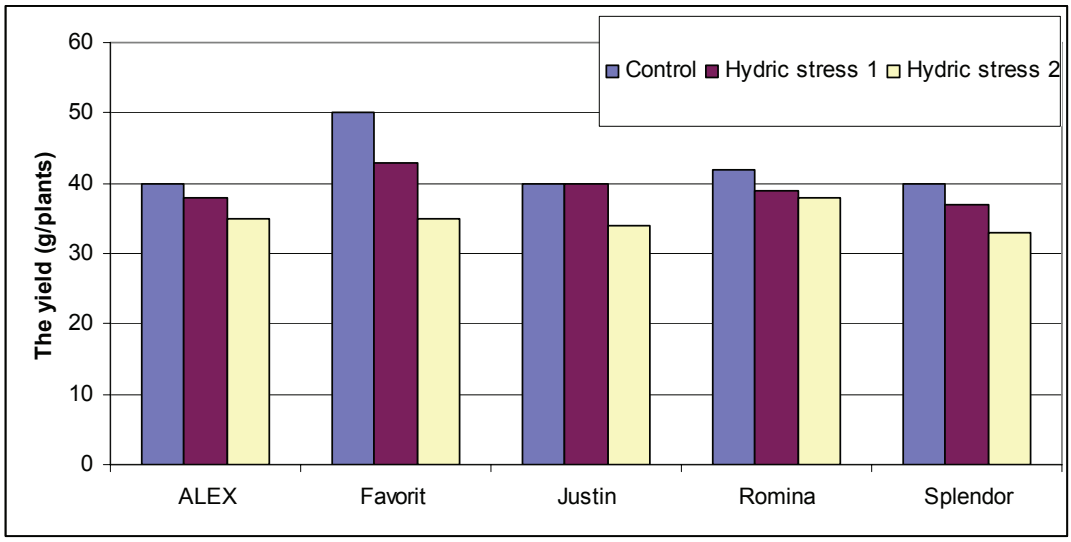

Fig. 3. The effect of water stress on yield of the tested sunflower hybrids

This suggests that although in Justin hybrid most of the nutritive reserve was conducted towards root development, its yield is not influenced.

CONCLUSIONS

The reduction in leaf area, shoot size and biomass accumulation of sunflower seedlings under water stress conditions determined the increase in root/shoot ratio. This suggests that for young plants the main sink was survival. In a late stage of vegetation, the root/shoot ratio decreased under drought stress in some hybrids but increased in other hybrids, this suggesting that for mature plants the main sink was the yield.

REFERENCES

Agüera, F., F.J. Villalobos, and F. Orgaz. 1997. Evaluation of sunflower (*Helianthus annuus*, L) genotypes differing in early vigor using a simulation model. Eur. J. Agron. 7:109–118.

Beard, J.B. 1973. Turfgrass: Science and culture. Prentice-Hall, Englewood Cliffs, NJ, USA.

Belhassen, E. 1995. An example of interdisciplinary drought tolerance study. Looking for physiological and molecular markers of low cuticular transpiration. In: Proc. Int. Congr. Integrated Studies on Drought Tolerance of Higher Plants, Interdrought 95 Montpellier, France.

Blum, A. 1988. Breeding for stress environments. CRC Press, Boca Raton, FL, USA.

Kim, K.S., J.B. Beard, and S.I. Sifers. 1989. Drought resistance comparisons among major warm-season turfgrasses. USGA Green Section Record.

Muriel, J.L., and R.W. Downes. 1974. Effect of periods of moisture stress during various phase of growth of sunflower in the greenhouse, p. 127-132. Proc.6th Int. Sunflower Conf., Bucharest, Romania.

Petcu, E., M. Terbea, A.V. Vrânceanu, D. Craiciu, and R.G. Zglimbea. 1997. Relationship between free proline content and drought tolerance in some Romanian sunflower genotypes. p. 437-443. In: Proc. Int. Congress Drought and Plant Production, Belgrade, Serbia.

Sharp, R.E., and J.S. Boyer. 1986. Photosynthesis at low water potentials in sunflower: lack of photosinhibitory effects. Plant Physiol. 82:90–95.

Sharp, R.E., and W.J. Davies. 1985. Rooth growth and water uptake by maize plants in draying soil. J. Exp.Bot. 36:1441-1456.

Talha, M., and F. Osman. 1975. Effect of soil water stress on water economy and oil composition in sunflower (*Helianthus annuus* L.). J. Agric. Sci. 84:49–56.

Terbea, M., A.V. Vrânceanu, E. Petcu, and G. Micut. 1995. Physiological response of sunflower drought stresses plants. p. 9-10. In: Proc. Int. Congr. Integrated Studies on Drought Tolerance of Higher Plants, France.

El vuelco en el cultivo de girasol: características anatómicas y mecánicas del sistema radical

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ABSTRACT

The objectives of this study were to identify the anatomical, morphological and mechanical properties of the root system of sunflower plants related to their tolerance to root lodging, and to evaluate the effects of increases in crop population density on these properties. An experiment was carried out using crops of two genotypes of susceptibility to lodging (CF29: tolerant and Zenit: sensitive) sown to achieve densities of 5.6, 10 and 16 plants.m^{-2}. At the R6 (end of anthesis) developmental stage plants were artificially lodged and the following response variables assessed: total root biomass in the root plate (hemisphere of roots and soil formed when lodging occurs) and separated by layers (0–5 and > 5 cm depth), root number (classified in three categories [0–1 mm; 1.1–2 mm; >2 mm diameters]), axial tension required to provoke root failure, and the number and cell wall thickness of vascular bundles in root cross-sections. The CF29 genotype had 1.35 fold greater root biomass than Zenit, mainly located in the first layer of soil (0–5 depth). This higher biomass of CF29 reflected a higher root number than Zenit for all three categories of root diameter. Roots of CF29 exhibited higher axial tension failure thresholds than those of Zenit, and these thresholds increased more sharply with root diameter than for Zenit. In addition, CF29 roots had vascular bundles with thicker cell walls (30% greater) with respect to Zenit. In summary, the better anchorage of CF29 with respect to Zenit arises from several mutually reinforcing characteristics at both root-plate and individual root axis morphology and histology levels.

Key words: cell wall thickness – crop population density – root axial tensile strength – root biomass – root lodging – root number

RESUMEN

Este trabajo tuvo como objetivos identificar las propiedades morfo–anatómicas y mecánicas del sistema radical de plantas de girasol que le confieren tolerancia al vuelco, y evaluar los efectos del aumento en la densidad poblacional del cultivo sobre dichas propiedades. Se realizó un experimento utilizando dos genotipos de comportamiento contrastante al vuelco (CF29: tolerante y Zenit: susceptible) sembrados a densidades crecientes (5.6, 10 y 16 plantas.m^{-2}). En la etapa R6 (fin de antesis), las plantas fueron volcadas artificialmente y se midieron las siguientes variables: biomasa radical total y por estrato (0–5 y > 5 cm prof.) en el plato de raíces (hemiesfera de suelo y raíces formado cuando la planta vuelca), número de raíces (en tres categorías diamétricas [0–1 mm; 1.1–2 mm; >2 mm]), tensión axial requerida para la ruptura radical, número de los haces vasculares en la estela radical y espesor de las paredes celulares. Los resultados mostraron que CF29 tuvo 1.35 veces más biomasa radical en el plato que Zenit, concentrada en los primeros centímetros de suelo. La mayor biomasa radical de CF29 estuvo asociada con un mayor número de raíces con respecto a Zenit en las tres clases diamétricas exploradas. Además, las raíces de CF29 fueron más resistentes a la tensión axial que las de Zenit, diferencias que se incrementaron con diámetros radicales crecientes. La mayor resistencia de las raíces de CF29 estuvo relacionada a haces vasculares con paredes secundarias más gruesas (30% mayor respecto a Zenit). Resumiendo, el mejor sistema de anclaje de CF29 frente a Zenit tuvo su origen en varias características, expresada a los niveles de sistema radical total y de la morfo-histología de ejes radicales individuales, que se reforzaban mutuamente.

INTRODUCCIÓN

El fenómeno de vuelco de las plantas es un factor abiótico importante que limita la producción en el cultivo de girasol, y en éste, como en otros cultivos (*Triticum aestivum*, *Hordeum vulgare* y *Avena sativa*), provoca no solo reducciones significativas del rendimiento en grano, sino también de su calidad (Kelbert et al., 2004). Para Argentina, se ha estimado que un 10% del área se vuelca cada año, causando pérdidas estimadas en 40 millones de U$D por año (Bragachini et al., 2001). La probabilidad de ocurrencia de vuelco depende tanto de cuestiones ambientales (p.ej., velocidad del viento, resistencia al

cizallamiento del suelo mojado), como intrínsecos de la planta (p.ej., rigidez del tallo, propiedades del sistema radical en las inmediaciones de la base del tallo) (Pinthus, 1973; Berry et al., 2000; Cleugh et al., 1998). El vuelco se asocia principalmente con la lluvia (Baker et al., 1998) que debilita el sistema de anclaje de la planta, combinada con la fuerza que ejerce el viento y que actúa sobre la parte aérea de de la planta, dando como resultado un momento de palanca en la base del tallo que excede el momento de quiebre de las raíces (Berry et al., 2004).

Recientemente se ha demostrado que el cultivo de girasol tiene el potencial de incrementar su rendimiento ante aumentos considerables en la densidad poblacional del cultivo por arriba de las densidades comerciales usuales en Argentina (López Pereira et al., 2004). Sin embargo la implementación de esta práctica se ve limitada por la mayor probabilidad de ocurrencia de vuelco. Por otro lado, es sabido que existe una amplia variabilidad en la tolerancia a este fenómeno entre distintos genotipos, habiéndose evaluado al momento dos genotipos de comportamiento contrastante: CF29 (tolerante) y Zenit (susceptible) (Sposaro et al., 2008). Analizar los efectos que tiene el aumento de la densidad poblacional sobre las características del sistema radical de las plantas en genotipos de tolerancia contrastante al vuelco, resulta un paso importante para aumentar el conocimiento de las bases de este proceso. Los objetivos de este trabajo fueron: (i) identificar las propiedades morfo–anatómicas y mecánicas del sistema radical asociadas con la tolerancia al vuelco en dos genotipos de comportamiento contrastante y (ii) evaluar los efectos de la densidad poblacional del cultivo sobre dichas propiedades.

MATERIALES Y MÉTODOS

*Genotipos y diseño experimental:*Se utilizaron dos genotipos de comportamiento contrastante frente al vuelco: uno tolerante (en adelante CF29) y uno susceptible (en adelante Zenit). Los genotipos fueron sembrados a densidades poblacionales crecientes de 5.6, 10 y 16 plantas.m^{-2} en el campo experimental de la Facultad de Agronomía–UBA sobre un suelo argiudol típico. El experimento consistió en un arreglo factorial con "genotipo" (2 niveles: CF29 y Zenit) y "densidad poblacional del cultivo" (3 niveles: 5.6, 10 y 16 pl.m^{-2}) como factores principales en un diseño en bloques aleatorizados (DBCA) con tres repeticiones. Las plantas crecieron durante todo su ciclo bien provistas de agua a través de un sistema de riego por goteo. Se aplicó fertilización en dos momentos del ciclo con 60 kg N.ha^{-1}. Se realizaron aplicaciones con insecticidas y fungicidas para mantener el cultivo libre de enfermedades y plagas.

Variables de respuesta y análisis estadístico: Las plantas fueron volcadas de forma artificial en fin de antesis (R6, Schneiter y Miller, 1981) utilizando la metodología descripta en Sposaro et al. (2008). Brevemente, la técnica involucra aplicar fuerzas crecientes a los tallos de plantas que crecen en subparcelas cuyo suelo ha sido previamente llevado a capacidad de campo hasta producir el vuelco de las mismas. La hemiesfera (o plato) de raíces (masa de suelo y raíces formada cuando se vuelca una planta) así obtenida fue dividida en dos estratos de acuerdo a su profundidad (0–5 cm y >5 cm), luego de lavadas las raíces, éstas se clasificaron en tres clases diamétricas (0–1 mm; 1.1–2 mm; >2 mm). Se obtuvo la biomasa de las raíces de cada estrato a través de su secado en estufa hasta peso constante (72 hs a 80°C) y se contó el número de raíces en cada una de las categorías previamente definidas. Para determinar la tensión axial requerida para la ruptura de los ejes radicales individuales (vivos) se adaptó la metodología descripta por Striker et al. (2006). Las raíces se sometieron a tensiones axiales crecientes causadas por el desplazamiento del pistón de un minicilindro neumático conectado a un circuito de aire presurizado con un regulador de flujo. Dicho sistema estaba conectado a un transductor de presión (ADZ Nagano S–010bar) y a un datalogger para registrar la tensión axial que provocó la ruptura de las raíces. La fuerza de ruptura se midió en raíces de diferentes diámetros en los rangos de las tres categorías definidas con el propósito de estudiar la posible existencia de un efecto del tamaño de las raíces sobre esta variable. Se realizaron cortes transversales de muestras de ejes radicales, tiñendo los mismos con una tinción doble (Safranina y Fast Green) para distinguir las paredes secundarias. El número de haces vasculares y el grosor de sus paredes se determinó mediante microscopía óptica y digitalización de imágenes, utilizando el software ImageTool 3.0 para Windows (University of San Antonio, Texas).

Los datos de biomasa por estrato y número de raíces por categoría se analizaron a través de ANOVAs de dos vías, con "genotipo" y "densidad poblacional de plantas" como factores principales. La relación entre la tensión axial de ruptura de las raíces y el diámetro radical se evaluó por medio de análisis de correlación de Pearson (Steel y Torrie, 1988). La significancia de las diferencias entre genotipos para dicha relación se evaluó con un test de pendientes para las ecuaciones ajustadas. Los datos correspondientes al número de haces vasculares y al grosor de sus paredes se analizaron a través de test de Student. La homogeneidad de varianzas y normalidad de los datos se verificó para todo el conjunto de

datos. Los análisis se realizaron con el software estadístico InfoStat versión 2007 (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina). Los resultados son presentados cómo valores promedios ± el error estándar.

RESULTADOS Y DISCUSIÓN

Estudios previos han demostrado que se requiere una fuerza significativamente mayor para volcar en forma artificial las plantas del genotipo tolerante CF29 que para volcar las del genotipo susceptible Zenit (67.3 vs. 27.4 N m, respectivamente, ver Sposaro et al., 2008). En el presente estudio se halló que el diámetro del plato de raíces fue significativamente mayor en CF29 que en Zenit (23.7 y 22.5 cm respectivamente). El mayor diámetro del plato de raíces de CF29 se relacionó con una mayor biomasa radical total que en Zenit, para las tres densidades evaluadas (genotipo= $p<0.0001$, genotipo × densidad poblacional: $p=0.53$, Fig. 1). Esto coincide con la idea de que en plantas de girasol una mayor biomasa radical se asocia a una mayor eficiencia en el anclaje de la planta (Ennos, 1993). Para CF29, la biomasa radical total en el plato fue similar para las tres densidades poblacionales ($p=0.20$) mientras que para Zenit se detectó un efecto negativo de la densidad poblacional sobre dicha variable ($p<0.01$, Fig. 1). Por lo tanto, Zenit no solo presentó una biomasa de raíces en el plato equivalente al 42% de la registrada para CF29, sino que el aumento de la densidad poblacional debilitó aún más su sistema de anclaje.

En ambos genotipos la mayor biomasa de raíces se concentró en el primer estrato (0–5 cm de profundidad, Fig. 1) sin detectarse diferencias en la proporción del total de raíces presentes en ese estrato entre las densidades poblacionales: 78% CF29 y 70% Zenit ($p=0.88$, genotipo × densidad poblacional: $p=0.99$). Estudios previos en girasol (Ennos, 1989) han demostrado que la biomasa de raíces en los primeros centímetros de suelo, es la más importante para determinar un óptimo anclaje de la planta. Por otro lado, en el estrato más profundo (> 5 cm) el aumento de la densidad poblacional de plantas determinó una disminución en la biomasa radical en ambos genotipos (densidad poblacional = $p<0.0001$, genotipo × densidad poblacional: $p=0.18$, Fig. 1). De esta manera, ante el aumento de la densidad poblacional ambos genotipos parecieran haber priorizado el mantenimiento de la biomasa radical en el estrato superior del suelo en detrimento de la producción de raíces en el estrato inferior, lo que redundaría en un mejor anclaje de las plantas (Ennos, 1989).

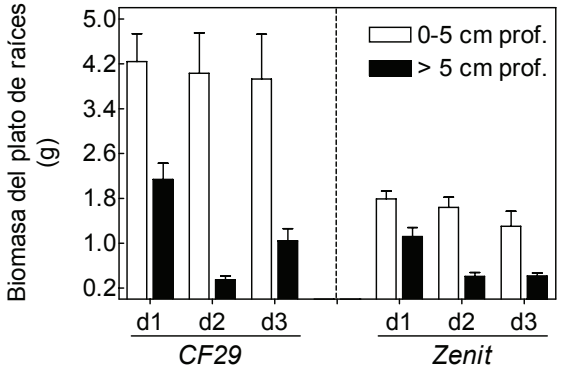


Fig. 1. Biomasa de raíces en el plato (g.planta^{-1}) para los dos genotipos en los dos estratos del plato para tres densidades poblacionales del cultivo (d1: 5.6; d2: 10; y d3: 16 pl.m^{-2}).

Fig. 1. Root plate biomass (g.plant^{-1}) for both genotypes in the two layers of the root plate for three crop population densities (d1: 5.6; d2: 10; y d3: 16 pl.m^{-2}).

El número total de raíces en el plato fue mayor en el genotipo CF29 que en Zenit ($p<0.05$), siendo este atributo uno de los que podría explicar la mayor tolerancia al vuelco que tiene CF29 respecto a Zenit. Al agrupar las raíces por su diámetro, se observó en ambos genotipos que las raíces más finas (0–1mm) fueron significativamente más abundantes que el resto de las categorías diamétricas ($p<0.0001$, Fig. 2, notar cambio de escalas entre el primer panel y los restantes). Otros trabajos han demostrado la importancia de las raíces finas en el conferimiento de la resistencia al vuelco de las plantas (Ennos, 1989; Reubens et al., 2007). El aumento de la densidad poblacional del cultivo produjo un incremento significativo en el número de raíces finas en el plato (0–1mm) sólo para Zenit ($p=0.009$, Fig. 2). Esta podría ser una respuesta del genotipo frente al incremento de la densidad poblacional, dado que una

mayor cantidad de raíces finas proveería una mayor fijación de la planta al suelo (Wu et al., 1988; Wu, 1995) en situaciones adversas donde el riesgo de vuelco se incrementa (Sposaro et al., 2008).

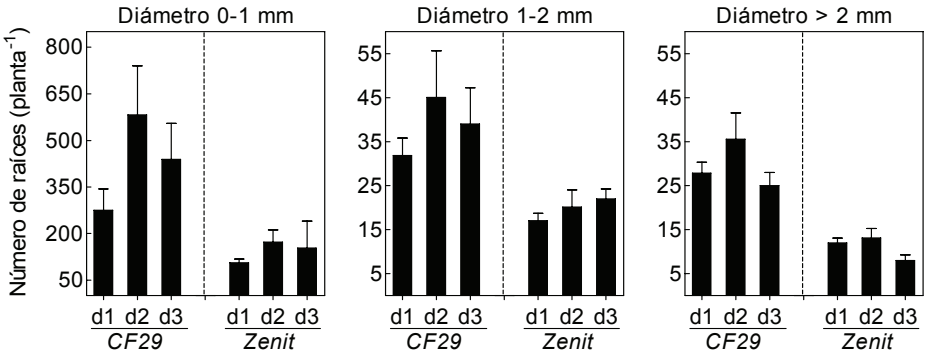


Fig. 2. Número de raíces por plato agrupadas por categorías diamétricas, para los genotipos CF29 y Zenit en tres densidades poblacionales de cultivo (d1: 5.6; d2: 10 y d3: 16 pl.m^{-2}). Notar el cambio de escala entre el primer panel y los restantes.

Fig. 2. Root number per root plate grouped by size category, for genotypes CF29 and Zenit grown at three crop population densities (d1: 5.6; d2: 10; y d3: 16 pl.m^{-2}). Note the change on scale between the left and remaining panels.

La fuerza de tensión axial requerida para provocar la ruptura de las raíces (como sucede durante el vuelco de la planta), fue mayor en CF29 que en Zenit (p=0.003, Fig. 3). Esta propiedad mecánica de las raíces no fue afectada por el aumento de la densidad poblacional de plantas (genotipo × densidad poblacional: p=0.16), por lo tanto, las mediciones hechas para las distintas densidades poblacionales fueron agrupadas, y el análisis se enfocó en las diferencias entre genotipos. En ambos genotipos la fuerza de tensión axial para provocar la ruptura de las raíces se correlacionó positivamente con el diámetro de las mismas (Fig. 3). CF29 presentó una pendiente mayor que Zenit para esta relación (test de pendientes: p< 0.0001, Fig. 3), indicando una mayor resistencia mecánica de sus raíces. El efecto del tamaño de las raíces ("*size effect*") sobre sus propiedades mecánicas ha sido recientemente estudiado en especies leñosas por Genet et al. (2005) y Bischetti et al. (2005). Estos autores demostraron la existencia de una correlación positiva entre el diámetro y la fuerza de ruptura de las raíces. Nuestros datos son consistentes con esos resultados y nuestro trabajo es el primero, para una especie herbácea anual de interés económico, en informar acerca de los efectos del tamaño de las raíces sobre sus propiedades mecánicas.

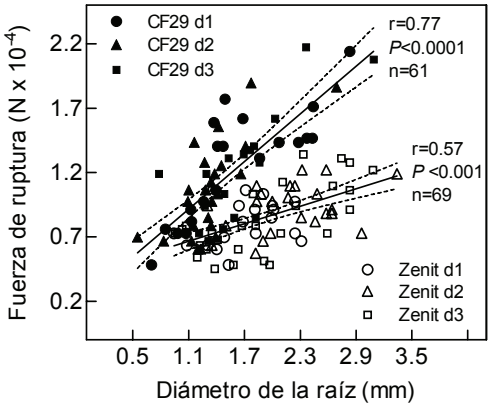
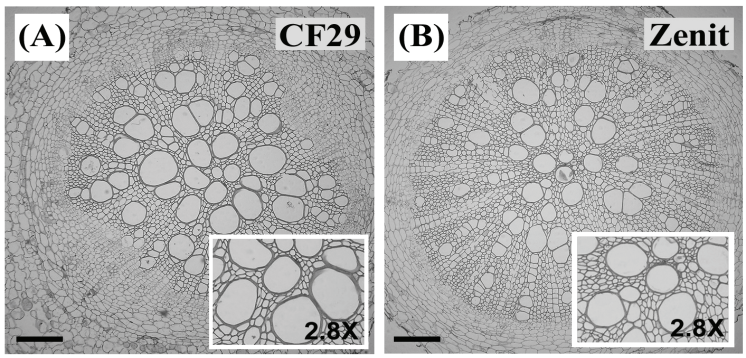


Fig. 3. Relación entre fuerza de ruptura (N) de las raíces y el diámetro para los genotipos CF29 y Zenit sembrados en tres densidades poblacionales de cultivo (d1: 5.6; d2: 10 y d3: 16 pl.m^{-2}). Las ecuaciones para cada genotipo son y= 0.61x + 0.24 para CF29, y= 0.23x + 0.41 para Zenit. Las líneas punteadas indican el intervalo de confianza del 95% para las ecuaciones ajustadas.

Fig. 3. Root failure threshold (N)/root diameter relationship for genotypes CF29 and Zenit, grown at three crop population densities (d1: 5.6; d2: 10; y d3: 16 pl.m^{-2}). Equations for each genotype are y= 0.61x + 0.24 for CF29, y= 0.23x + 0.41 for Zenit. Dashed lines indicate the 95% confidence intervals for the fitted equations.

El análisis anatómico mostró que los genotipos CF29 y Zenit no difirieron en la proporción de estela en la sección transversal de raíz (datos no mostrados), ni en el número de haces vasculares presentes en la sección (Fig. 4). Sin embargo, en el genotipo CF29, cuyas raíces tuvieron una mayor resistencia a la tensión axial, el grosor de la pared de los haces vasculares fue un 30% mayor al encontrado en el genotipo Zenit (Fig. 4c). Trabajos previos realizados sobre cultivos de trigo, cebada y arroz, han demostrado que tanto el número de haces vasculares como el grosor de sus paredes son parámetros altamente correlacionados con la tolerancia a las fuerzas de tensión axial, como las generadas durante el fenómeno de vuelco (Pinthus, 1973; Chatuverdi et al., 1995 citado por Oladokun y Ennos, 2006). Se sabe que el contenido de celulosa y lignina están positivamente correlacionados con la fuerza de tensión axial de las raíces (Hathaway y Penny, 1975; Kokubo et al., 1989; Genet et al., 2005). Por lo tanto, raíces del mismo diámetro y con similar número de haces vasculares, pero con mayor proporción de lignina y celulosa en sus paredes secundarias, brindan una mayor resistencia mecánica a la raíz. En este sentido, la mayor proporción de pared secundaria constitutiva de CF29 sería responsable, al menos en parte, de la mayor resistencia de sus raíces ante fuerzas de tensión axial.



(C)	CF29	Zenit
Nro de haces vasculares	115±14	112±10 NS
Grosor de pared (µm)	8.9±0.4	6.8±0.3 *

NS diferencia no significativa (P>0.05)

* diferencia significativa (P<0.01) basada en test de Student.

Figura 4. Cortes transversales de raíces de diámetro similar de los genotipos CF29 (A) y Zenit (B). La barra de escala es equivalente a 150 µm. En el cuadro (C) se muestra el número de haces vasculares y el grosor de la pared de dichos haces para cada genotipo. En la parte inferior derecha de (A) y (B) se muestran en detalle los haces vasculares para cada genotipo.

Figure 4. Transverse sections of roots of similar diameter of genotypes CF29 (A) and Zenit (B). Scale bar represents 150 µm. In the table (C) the number and cell wall thickness of vascular bundles are shown for each genotype. Inset photos (A) and (B) shown magnified (2.8X) sections of the stele centered on vascular bundles.

En síntesis, se detectaron diferencias importantes tanto a nivel de sistema radical de la planta como a nivel de raíz individual entre los genotipos que explicarían su tolerancia diferencial al vuelco. A nivel de planta el genotipo tolerante CF29 tuvo una mayor biomasa total en el plato de raíces que el susceptible Zenit. Dicha biomasa estuvo especialmente concentrada en los primeros centímetros del suelo, estrato donde se define gran parte del anclaje de las plantas. A su vez, la mayor biomasa radical registrada para CF29 estuvo asociada con un mayor número de raíces que en Zenit para todos los rangos diamétricos explorados. A nivel de raíz individual, CF29 tuvo raíces más resistentes a las fuerzas de tensión axial que las del genotipo Zenit, detectándose un mayor efecto positivo del diámetro de las raíces sobre dicha variable a favor de CF29. A su vez, la mayor resistencia de las raíces de CF29 estuvo relacionada con la presencia de haces vasculares con paredes secundarias considerablemente más gruesas que en Zenit. De esta manera, estas características diferenciales a nivel anatómico, morfológico y de biomasa radical entre CF29 y Zenit definirían en conjunto un sistema de anclaje más eficiente en el primer genotipo que reducen las probabilidades de ocurrencia de vuelco de sus plantas.

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REFERENCIAS

Baker, C.J., P.M. Berry, J.H. Spink, R. Sylvester-Bradley, R.K. Scott, and R.W. Clare. 1998. A method for the assessment of the risk of wheat lodging. J. Theor. Biol. 194:587–603.

Berry, P.M., J.M. Griffin, R. Sylvester-Bradley, R.K. Scott, J.H. Spink, C.J. Baker, and R.W. Clare. 2000. Controlling plant form through husbandry to minimize lodging in wheat. Field Crops Res. 67:59–81.

Berry, P.M., M. Sterling, J.H. Spink, C.J. Baker, R. Sylvester-Bradley, S.J. Mooney, A.R. Tams, and A.R. Ennos. 2004. Understanding and reducing lodging in cereals. Adv. Agron. 84:217–271.

Bischetti, G.B., E.A. Chiaradia, T. Simonato, B. Speziali, B. Vitali, P. Vullo, and A. Zocco. 2005. Root strength and root area ratio of forest species in Lombardy (Northem Italy). Plant Soil 278:11-22.

Bragachini, M., A. von Martini, and A. Mendez. 2001. Pérdidas de cosecha. Evaluación y tolerancias en cosecha de Soja, Maíz, Girasol y Trigo. Proyecto agricultura de precisión. INTA E.E.A. Manfredi. Córdoba, Argentina.

Cleugh, H.A., J.M. Millar, and M. Böhm. 1998. Direct mechanical effects of winds on crops. Agroforest Syst. 41:85-112.

Ennos, A.R. 1989. The mechanics of anchorage in seedlings of sunflowers *Helianthus annuus*. New Phytol. 113:185-192.

Ennos, A.R. 1993. The scaling of root anchorage. J. Theor. Biol. 161:61-75.

Genet, M., A. Stokes, F. Salin, S.B. Mickovski, T. Fourçaud, J.F. Dumail, and R. Van Beek. 2005. The influence of cellulose content on tensile strength in tree roots. Plant Soil 278:1-9.

Hathaway, R.L., and D. Penny. 1975. Root strength in some *Populus* and *Salix* clones. New Zeal. J. Bot. 13:333-343.

Kelbert, A.J., D. Spaner, K.G. Briggs, and J.R. King. 2004. The association of culm anatomy with lodging susceptibility in modern spring wheat genotypes. Euphytica 136:211–221.

Kokubo, A., S. Kuraishi, and N. Sakurai. 1989. Culm strength of Barley. Correlation among maximum bending stress, cell wall dimensions, and cellulose content. Plant Phys. 91:876-882.

López Pereira, M., N. Trápani, G. Piñeiro, and A.J. Hall. 2004. Responses of sunflower to stand structure and crop population density: effects on leaf area and yield. In: 16th International Sunflower Conference, Fargo North Dakota.

Oladokun, M.A.O., and A.R. Ennos. 2006. Structural development and stability of rice *Oryza sativa* L. var. Nerica 1. J. Exp. Bot. 57:3123-3130.

Pinthus, M.J. 1973. Lodging in wheat, barley, and oats: The phenomenon, its causes, and preventive measures. Adv. Agron. 25:209-263.

Reubens, B., J. Poesen, F. Danjon, G. Geudens, and B. Muys. 2007. The role and coarse roots in shallow slope stability and soil erosion control with a focus on root system architecture: a review. Trees 21:385-402.

Schneiter, A.A., and J.F. Miller. 1981. Description of sunflower growth stages. Crop Sci. 21:901-903.

Sposaro, M.M., C. Chimenti, and A.J. Hall. 2008. Root lodging in sunflower. Variations in anchorage strength across genotypes, soil types, crop population densities and crop developmental stages. Field Crops Res. 106:179-186.

Steel, R.G.D., and J.H. Torrie. 1988. Bioestadística. Principios y procedimientos. McGraw–Hill, México.

Striker, G.G., P. Insausti, A.A. Grimoldi, and R.J.C. León. 2006. Root strength and trampling tolerance in the grass Paspalum dilatatum and the dicot Lotus *glaber* in flooded soil. Funct. Ecol. 20:4-10.

Wu, T.H. 1995. Slope stabilization. p. 221-264. In: Slope stabilization and erosion control, a bioengineering approach. E&FN Spon, London.

Wu, T.H., R.M. McOmber, R.T. Erb, and P.E. Beal. 1988. Study of soil-root interactions. J. Geotech Eng. 114:1351-1375.

Influence of drought stress on growth, protein expression and osmolyte accumulation in sunflower *Helianthus annuus* L. c.v. Peredovik

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ABSTRACT

Drought stress causes considerable yield losses in agriculture and receives high attention in current sunflower breeding programs. For this study, an *in vitro* system based on MS media (Murashige and Skoog, 1962) supplemented with polyethylene glycol 6000 (Korell, 1997) was established to examine sunflower (*Helianthus annuus* L.) seedlings under artificial drought stress conditions. The objective is to identify parameters applicable as markers for drought stress, which would allow breeders to screen their material efficiently for drought tolerance. For this purpose, different morphological and physiological parameters were analysed by comparing control plants with plants grown under drought stress (-0,6 MPa). The evaluation of growth reveals a significant growth deficit of drought stressed plants compared to control plants concerning hypocotyl length, development of cotyledons and primary leaves, whereas for root fresh weight in relation to shoot fresh weight no significant difference could be detected. Qualitative and quantitative changes in osmolyte accumulation were examined by HPLC and gas chromatography. Osmolyte analyses revealed an accumulation of glucose (25-30fold), inositol (20-30fold), proline (10-20fold), fructose (3-6fold) and sucrose (4-5fold) in extracts from leaves of drought-stressed plants. Changes in protein expression of drought-stressed versus control plants were detected in colloidal Coomassie-stained 2D-PAGE gels. In future studies, differentially expressed proteins will be identified by peptide mass fingerprinting and used to develop molecular markers for breeding programs.

Key words: drought stress – PEG – osmolyte – 2D-PAGE.

INTRODUCTION

Drought is a potential major constraint to crop production all over the world, and causes considerable yield losses in agriculture. Global warming, deforestation, and urbanisation will all increase the severity and frequency of drought in the future, leading to a possible decrease in global food production. Therefore, there is a great need for the development of stable food crops that produce high and stable yields also when drought occurs. Water stress in its broadest sense encompasses both drought and salt stress. Water stress results in stomatal closure and reduced transpiration rates, a decrease in water potential of plant tissue, decrease in photosynthesis and growth inhibition. The accumulation of abscisic acid (ABA) serves as a signal, initiating acclimation reactions such as accumulation of compatible compounds like proline, mannitol, and sorbitol, or the formation of scavenging compounds like ascorbate, gluthathione, α-tocopherol etc. The acclimation process employs processes of differential gene expression leading to new proteins and mRNAs (Yordanov et al., 2003). Obviously, water stress acclimation is a multi-gene acclimation process, in which many different physiological processes and many drought stress-inducible genes are involved. Functionally, these gene products can be distinguished into: osmolyte synthases (Chen and Murata, 2002), protection factors for macromolecules (chaperons, LEA/dehydrin-type genes), proteases, membrane proteins (aquaporins, transporters), detoxification enzymes (GST, SOD), and genes of regulatory proteins like transcription factors, protein kinases, protein phosphatases (Wang et al. 2003; Zhu, 2002). Although the alterations in all of these processes related with drought stress have been widely investigated in many model species and a few crop plant species, reports on sunflower are limited. Physiological and molecular responses have been described by Kane and Rieseberg (2007), Kavas et al. (2006), Bailly et al. (2004), Liu and Baird (2003; 2004), Cellier et al. (1998; 2000) and Poormohammad et al. (2007). The aim of our *in vitro* studies of drought tolerance of sunflower seedlings was to analyse physiological key processes after applying drought stress conditions by supplementation of MS media with polyethylene glycol (PEG) 6000. This evaluation should result in an appropriate test system for breeders to select for drought tolerant breeding material while saving time, space and costs.

MATERIALS AND METHODS

Sunflower c.v. Peredovik seedlings were grown in a liquid MS medium (Murashige and Skoog, 1962) using 0.75 l Weck glasses. The plant material was incubated in climate chamber at 21°C, 16/8 hours light/dark cycle at 150 µM photons m^{-2} s^{-1}. After three days of cultivation half of the seedlings were transferred to drought stress medium, achieved by supplementation of MS medium with PEG 6000 to an osmotic potential of -0.6 MPa (MS6), while the control plants were transferred onto fresh MS0 medium without PEG addition. Seven days later plant growth was characterized by measurement of the hypocotyl length, characterizing the development of cotelydons and primary leaves (using a relative scale from 0 to 6 representing area of leaves up to >4 cm^2) and determination of fresh weight of root and shoots. Primary leaves were frozen in liquid nitrogen and stored at -20°C till extraction of proteins and osmolytes.

Total homogenates of leaves were obtained by grinding plant material in Eppendorf tubes on an ice bath with HEPES buffer (10 mM, pH 7.6) containing protease inhibitors (10 mM PMSF, protease inhibitor cocktail, Sigma P9599 according product information). The soluble protein fraction was obtained after centrifugation of the crude extract at 38,000 g and 4°C. After determination of the protein concentration (Bradford, 1976), these protein extracts (400 µg each) were used for 2D-PAGE according to Fulda et al. (2000, 2006) followed by differential analysis of colloidal Coomassie-stained 2D gels from drought-stressed plants versus control plants using Delta2D software (Decodon).

Qualitative and quantitative determination of osmolytes was done by HPLC and gas chromatography (GC). In the case of HPLC analyses, soluble protein extracts containing 400 µg of protein were taken as raw material. The high molecular contaminants were precipitated with 80% ethanol (containing an internal standard: 100 µg sorbitol) overnight at 4°C followed by centrifugation (28,000 g). Supernatants were dried in a vacuum centrifuge. Dry residues were washed once with 80% ethanol and dissolved in A. bidest. HPLC analyses were performed according to Schoor et al. (1995) using the combination of reversed-phase and ion-moderated partition chromatography. To verify the results of HPLC analyses, additional samples were analyzed by gas chromatography using a Focus GC (Thermo Scientific) equipped with a TR-5MS column (30 m x 0.25 mm x 0.25 µm) and an AS 3000 autosampler. For GC-analyses, a separate ethanolic extraction of ground leaves was performed with 80% ethanol at 68°C for two hours at first, followed by a second extraction at 68°C overnight. The combined extracts were purified by centrifugation at 38,000g, 4°C and dried in a vacuum centrifuge. Trimethylsilyl-derivates of sugars were obtained by incubation with 65 µl pyridine/methoxylamine (20 mg/ml, 90 min at 30°C) and 35 µl N, O-Bis(trimethylsilyl)-trifluoroacetamide (Sigma, 60 min at 60°C). A set of the following standards was chosen for qualitative analysis of the osmolytes: trehalose, maltose, glucose, sucrose, inositol, fructose, glycerol, mannitol, sorbitol, glycine betaine, proline. As an internal standard for quantification sorbitol was used (see above).

RESULTS AND DISCUSSION

In five independent experiments we observed a clear growth inhibition of plants cultivated in drought stress MS6 medium in comparison to MS0 plants (Fig. 1). In each experiment, about 200 plants were evaluated for growth. The rating reveals a significant growth deficit of drought-stressed plants compared to control plants concerning hypocotyl length (Fig. 2a) as well as the development of cotyledons (Fig. 2b) and primary leaves (Fig. 2c).

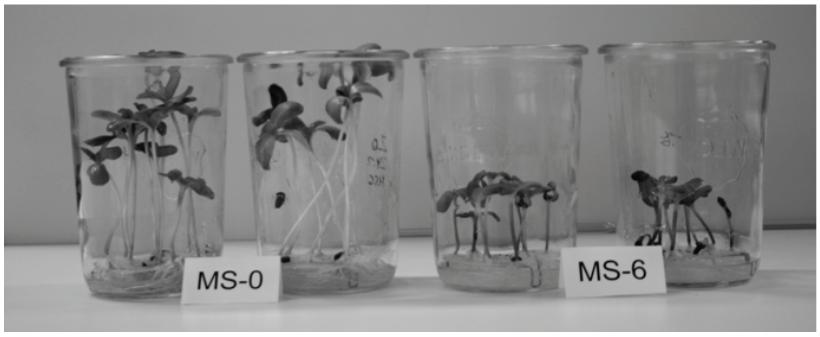

Fig. 1. Growth of 10-days-old *Helianthus annuus* L. c.v. Peredovik plants cultivated in control medium MS0 and drought stress medium MS6.

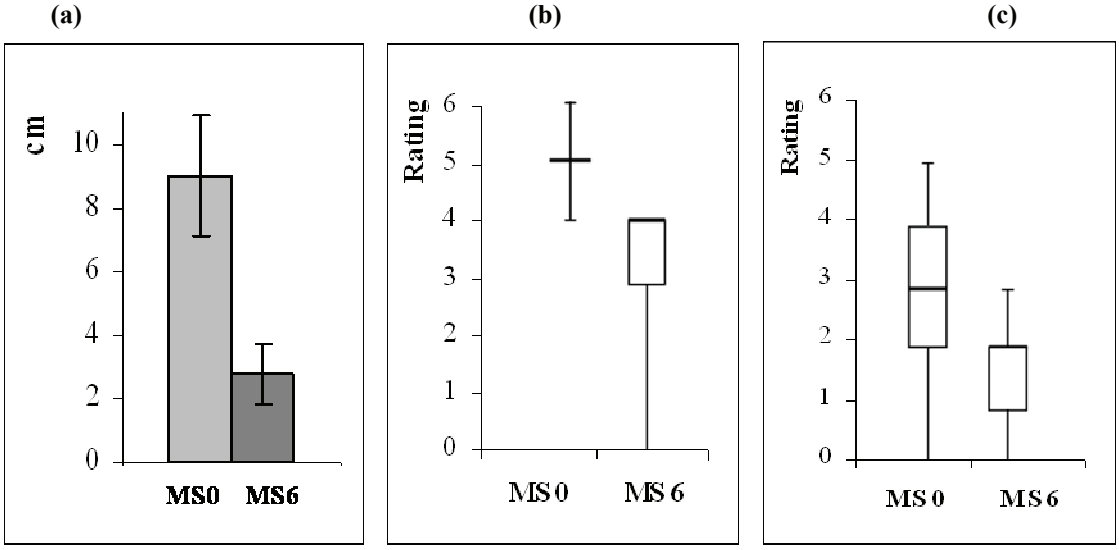

Fig. 2. Development of **(a)** hypocotyl length (cm), **(b**) cotyledons and **(c)** primary leaves of *Helianthus annuus* L. c.v. Peredovik grown in MS media with (MS6) and without (MS0) drought stress. Means and standard deviation (a) or medians (b, c) are given (n for MS0=87, n for MS6=98).

These observations agree with data published by Yordanov et al. (2003). Obviously, drought stress affects growth at the whole plant level leading to a decrease in photosynthesis and associated carbon and nitrogen metabolism. The growth inhibition could be attributed to shrinkage of cells and to the fact that the turgor pressure against cell walls relaxes. Because turgor reduction is the earliest significant biophysical effect of water stress, turgor-dependent activities such as leaf expansion and root elongation are the most sensitive to water deficits. Cell expansion is a turgor-driven process and is extremely sensitive to water deficit so that a decrease in turgor causes a decrease in the growth rate (Taiz and Zeiger, 2007).

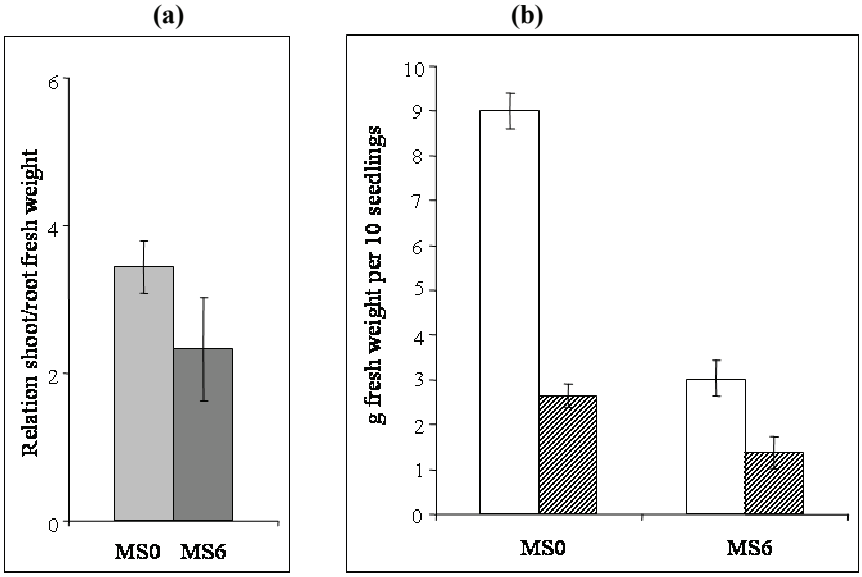

Fig. 3. Comparison of shoot and root development of 10-days-old seedlings of *Helianthus annuus* L c.v. Peredovik grown in MS media with (MS6) and without (MS0) drought stress. Means and standard deviations are given (n for MS0=9, n for MS6=10). **(a)** Fresh weight ratios shoot/root, **(b)** Fresh weight per 10 shoots (white columns) and fresh weight per 10 root systems (grey columns).

For root fresh weight in relation to shoot fresh weight, no significant difference could be detected (Fig. 3b). Karrenberg et al. (2006) investigated responses to salinity in the homoploid species *Helianthus paradoxus* and its progenitors *H. annuus* and *H. petiolaris*. They reported that growth reduction in the

progenitors *H. annuus* and *H. petiolaris* affected roots more than shoots as indicated by a decrease in root mass fraction. In the homoploid hybrid species *H. paradoxus* root biomass allocation did not change in response to salt stress so the relationship between root to shoot growth seems to differ from species to species. On the other hand, development of an optimal root/shoot ratio in relation to water availability is very important for the crop yield. Under natural conditions, plants are able to improve uptake of water by developing an extensive root system, which enables plants to grow into deep soil region with sufficient or improved water supply. Therefore, changes in relative root and shoot growth, leading to an increased root/shoot ratio were often observed with drought stressed plants (Verslues et al., 2006; Sharp et al., 2004). Additional tissue water storage capacity and thickness of the cuticula and water permeability are also of potential importance. Of these, changes in root growth to maximize water uptake are of the greatest importance for crop plants. In our PEG 6000-based hydroponics we observed, for shoot as well as root development, reduced growth in stressed plants (fresh weight, Fig. 3a) that leads to an unaffected root/shoot ratio. This may be due to the fact that PEG 6000-mediated drought stress conditions represent a severe stress.

Accumulation of osmolytes represents one of the central acclimation reactions in drought-stressed plants. Osmolyte analyses, which were done by HPLC and GC, obviously indicate an average accumulation of substances such as glucose (25-30fold), inositol (20-30fold), proline (10-20fold), fructose (3-6fold) and sucrose (4-5fold) in drought-stressed sunflower plants (Fig. 4.).

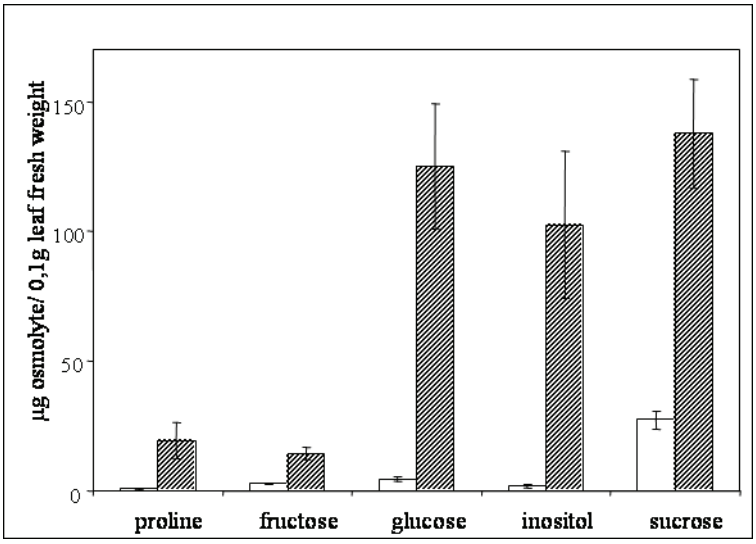

Fig. 4. Osmolyte accumulation in leaf extracts of *Helianthus annuus* L c. v. Peredovik determined by GC. Plants were grown on MS0 medium (white columns) or on MS6 medium (grey columns), respectively. Means of four analyses and their standard deviations are shown.

As expected, decreased water availability requires the accumulation of solutes by cells to decrease cell water potential, which enables plants to absorb water. This osmotic adjustment is a net increase in solute concentration per cell that is independent of the volume changes that result from loss of water. This can be accounted for by an increase in the concentration of a variety of common solutes, including sugars, amino acids, organic acids, polyols and inorganic ions (Taiz and Zeiger, 2007). In the case of our PEG-mediated drought stress system, the hexose glucose, the polyol inositol and sucrose seem to represent the main contributors to osmotic adjustment in primary leaves of *H. annuus* c.v. Peredovik followed by proline and fructose. Sharp et al. (2004), who investigated osmotic adjustment in roots of maize, found that in the maize primary root tip, hexoses are the dominant osmolytes in the basal region of the growth zone, while, in the apical zone proline concentration increases dramatically in water-stressed roots. One of the most frequently found solutes in water-stressed plants is the amino acid proline. Additionally, proline may act as a regulatory or signalling molecule to activate multiple responses that are part of the acclimation process (Maggio et al., 2002). Also, proline is a reliable indicator of the environmental stress imposed on plants (Claussen, 2005). Chechin et al. (2006) found in greenhouse-grown *H. annuus* c.v. Catissol-01 plants a 7-fold increase in proline content in young stressed leaves in comparison to non-stressed plants, but in the case of mature stressed leaves the proline content was increased four fold. In our study, proline was not the dominating compatible compound. These differences may be related to the

use of different sunflower varieties or culture conditions. Myo-inositol and its derivates are commonly studied with respect to cell signalling and membrane biogenesis, but they also participate in response to salinity in plants. Non-methylated inositols are found in all plants but are especially common in legumes. Pinitol and ononitol (methylated derivates) have been reported as a salt-induced response in *M. crystallinum* (Thomas and Bohnert, 1993). The already high concentration of cyclitols in unstressed soybean (*Glycine max*) is further increased in drought-stressed plants, underlying the important role of unmethylated and methylated inositols as osmoprotectants (Schneider et al., 2007; Gagneul et al., 2007).

Adaptation to drought stress requires alterations in the cell machinery that result directly from modifying gene expression. Functional gene expression profiles can be best achieved by proteome analysis. Furthermore, proteins undergo significant levels of post-translational modification of their primary sequences and are readily subjected to targeted proteolysis. Thus, a quantitative analysis of gene expression at the protein level is essential for dissecting responses to drought stress. We used the most common tool for revealing the expression of intact proteins, the two-dimensional gel electrophoresis (2D-PAGE). After staining proteins with colloidal Coomassie stain nearly 250 protein spots could be visualized in sunflower (Fig. 5). Protein pattern of control and drought-stressed sunflower plants were compared. So far, two regions marked on the gels were identified, where remarkable changes in protein expression became obvious. By using same sample replicates (4-5 times), several protein spots could be found, which showed an accumulation in drought-stressed plant material. However, a lot more spots seem to be present in smaller amounts in the drought-stressed plants compared to the control plants. In future experiments, the proteins of these stress-induced protein spots as well as decreased spots will be identified by excising gel plugs from the gel for MALDI-TOF analysis.

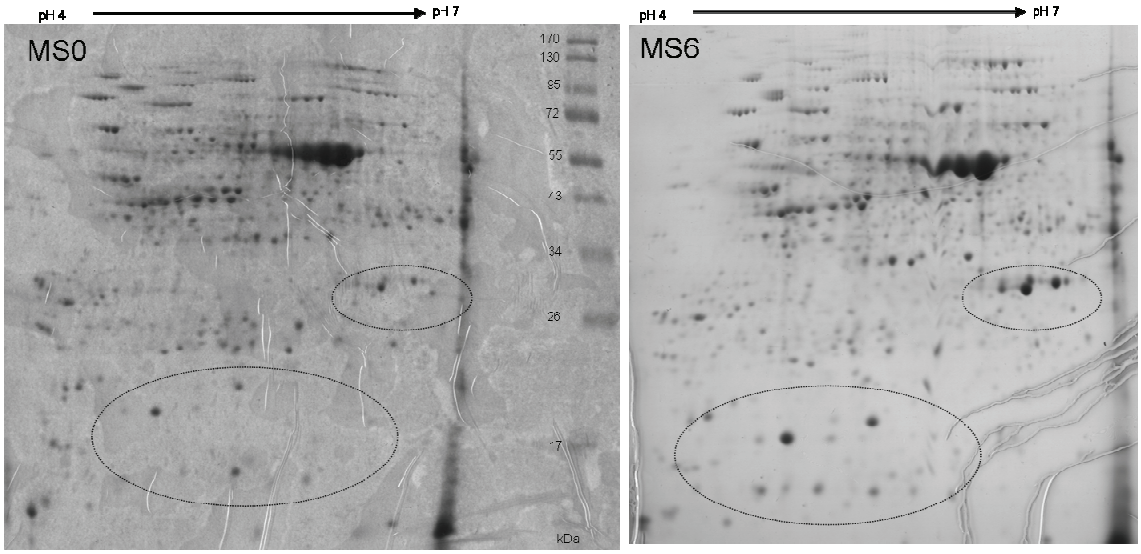

Fig. 5. 2D-PAGE gels of control and drought-stressed protein extracts from *Helianthus annuus* L. c.v. Peredovik. Soluble proteins were separated on 18 cm IPG pH 4-7 strip according to their isoelectric point in the first dimension and then on a SDS-PAG according to their molecular weight in the second dimension. The 2D-PAGE gels were stained with colloidal Coomassie blue. Protein samples were diluted to a load of 400 µg in rehydration buffer.

REFERENCES

Bailly, C., J. Leymarie, A. Lehner, S. Rousseau, D. Come, and F. Corbineau. 2004. Catalase activity and expression in developing sunflower seeds as related to drying. J. Exp. Bot. 55:396:475-483.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye-binding. Anal. Biochem. 72:248-254.

Cechin, I., S.C. Rossi, V.C. Oliveira, and T.F. Fumis. 2006. Photosynthetic responses and proline content of mature and young leaves of sunflower plants under water deficit. Photosynthetica 44:143-146.

Cellier, F., G. Conejero, J.C. Breitler, and F. Casse. 1998. Molecular and physiological responses to water deficit in drought-tolerant and drought-sensitive lines of sunflower. Plant Physiol. 116:319-328.

Cellier, F., G. Conejero, and F. Casse. 2000. Dehydrin transcript fluctuations during day/night cycle in drought-stressed sunflower. J. Exp. Bot. 51:299-304.

Chen, T.H.H., and N. Murata. 2002. Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. Curr. Opin. Plant Biol. 5:250-257.

Claussen, W. 2005. Proline as a measure of stress in tomato plants. Plant Sci., 168:241-248.

Fulda, S., F. Huang, F. Nilsson, M. Hagemann, and B. Norling. 2000. Proteomics of *Synechocystis* sp. strain PCC 6803: Identification of periplasmic proteins in cells grown at low and high salt concentrations. Eur. J. Biochem. 267:5900-5907.

Fulda, S., S. Mikkat, F. Huang, J. Huckauf, K. Marin, B. Norling, and M. Hagemann. 2006. Proteome analysis of salt stress response in the cyanobacterium *Synechocystis* sp. strain PCC 6803. Proteomics 6:2733-2745.

Gagneul, D., A. Ainouche, C. Duhaze, R. Lugan, F.R. Larher, and A. Bouchereau. 2007. A reassessment of the function of the so-called compatible solutes in the halophytic Plumbaginaceae *Limonium latifolium*. Plant Physiol. 144:1598-1611.

Kane, N.C., and L. H. Rieseberg. 2007. Selective sweeps reveal candidate genes for adaptation to drought and salt tolerance in common sunflower, *Helianthus annuus*. Genetics 175:1823-1834.

Karrenberg, S., C. Edelist, C. Lexer, and L. Rieseberg. 2006. Response to salinity in the homoploid hybrid species *Helianthus paradoxus* and its progenitors *H. annuus* and *H. petiolaris*. New Phytol. 170:615-629.

Kavas, M., G. Kalemtas, U.C. Akcay, A.T. Bayrac, E. Ozgur, C. Balolu, O. Ercan, M. Yucel, and H.A. Oktem. 2006. Effect of drought stress on the antioxidant systems of two sunflower (*Helianthus annuus*) cultivars. 2. Environmental stress and mechanisms of biochemical adaptation, Platform presentation. ESCPB 2006.

Korell, M. 1997. Zur Entwicklung eines Selektionssystems *in vitro* auf osmotische Anpassungsfähigkeit interspezifischer Sonnenblumenlinien als Grundlage für die Züchtung auf Trockenstresstoleranz. Promotion, Universität Gießen.

Liu, X., and W.V. Baird. 2003. Differential expression of genes regulated in response to drought or salinity stress in sunflower. Crop Sci. 43:678-687.

Liu, X., and W.V. Baird. 2004. Identification of a novel gene, HAABRC5, from *Helianthus annuus* (Asteraceae) that is upregulated in response to drought, salinity and abscisic acid. Am. J. Bot. 9:184-191.

Maggio, A., S. Miyazaki, P. Veronese, T. Fujita, J. I. Ibeas, B. Damsz, M.L. Narasimhan, P.M. Hasegawa, R.J. Joly, and R.A. Bressan. 2002. Does proline accumulation play an active role in stress-induced growth reduction? Plant. J. 31:699-712.

Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Poormohammad Kiani, S., P. Grieu, P. Maury, T. Hewezi, L. Grenzbittel, and A. Sarrafi. 2007. Genetic variability for physiological traits under drought conditions and differential expression of water stress-associated genes in sunflower (*Helianthus annuus* L.). Theor. Appl. Genet. 114:193-207.

Schneider, S., A. Schneidereit, P. Udvardi, U. Hammes, M. Gramann, P. Dietrich, and N. Sauer. 2007. *Arabidopsis* INOSITOL TRANSPORTER2 mediates H^+ symport of different inositol epimers and derivatives across the plasma membrane. Plant Physiol. 145:1395-1407.

Schoor, A., N. Erdmann, U. Effmert, and S. Mikkat. 1995. Determination of cyanobacterial osmolyte glycosylglycerol by high-performance liquid chromatography. J. Chromatogr., A 704:89-97.

Sharp, R.E., V. Poroyko, L.G. Hejlek, W.G. Spollen, G.K. Springer, H.J. Bohnert, and H.T. Nguyen. 2004. Root growth maintenance during water deficits: physiology to functional genomics. J. Exp. Bot. 55:2343-2351.

Taiz, L., and E. Zeiger. 2007. Plant Physiology. Fouth edn., Spektrum Akademischer Verlag, Heidelberg.

Thomas, J.C., and H.J. Bohnert. 1993. Salt perception and plant growth regulators in the halophyte *Mesembryanthenum crystallinum*. Plant Physiol. 103:1299-1304.

Verslues, P., M. Agarwal, S. Katiyar-Agarwal, J. Zhu, and J.K. Zhu. 2006. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. Plant J. 45:523-539.

Wang, W., B. Vinocur, and A. Altmann. 2003. Plant responses to drought, salinity and extreme temperatures: toward engineering for stress tolerance. Planta 218:1-14.

Yordanov, I., V. Velikova and T. Tsonev. 2003. Plant responses to drought and stress tolerance. Bulg. J. Plant Physiol., Special Issue 2003, 187-206.

Zhu, J.K. 2002. Salt and drought stress signal transduction in plants. Annu. Rev. Plant Biol., 53:247-73.

Development and validation of a model of lodging for sunflower

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ABSTRACT

Root and stem lodging cause significant yield losses in sunflower (*Helianthus annuus* L.) production in Argentina. Lodging is defined as the permanent displacement of the stem from its vertical position without any possibility of recovery. Few studies have investigated the mechanistic processes of root or stem lodging and not one has attempted to interrelate the complex interactions between climate and soil variables, husbandry practices and plant characteristics for sunflower lodging. A lodging model was developed for wheat and barley and can be used as a basis for a sunflower lodging model. The objectives of this work are to develop a root/stem lodging model for sunflower and to validate it. The root lodging model calculates the root failure wind speed using as its main variable the root plate diameter. The thickness of the epidermis+cortex measured in the lower third of the stem was the main variable used as input by the stem lodging model for the calculation of the stem failure wind speed. This model was tested against individual field experiments, in which natural root or stem lodging had occurred at different crop development stages under different husbandry practices, and it could recreate the differences observed in the field between hybrids and crop population densities in relation to lodging susceptibility. A parametric analysis showed the root plate diameter and epidermis+cortex as the main variables of the model and indicated that sunflower could be more susceptible to root than stem lodging.

Key words: epidermis+cortex – failure wind speed – model testing – root lodging – root plate diameter – stem lodging.

INTRODUCTION

Root and stem lodging cause significant yield losses in sunflower (*Helianthus annuus* L.) production in Argentina. About 10% of sunflower crop lodges annually, representing an estimated loss of US$40 million (Bragachini et al., 2001) due to the impossibility of harvesting the lodged plants. Lodging is defined as the permanent displacement of the stem from its vertical position without any possibility of recovery.

Root lodging is usually associated with rain (Baker et al., 1998) that weakens plant soil-root system (Pinthus, 1973) combined with a wind-induced force acting on the upper sections of the plant (root failure wind speed) that result in a bending moment at its base that exceeds the root failure moment (Berry et al., 2004). Few studies have investigated the mechanistic processes of root or stem lodging in sunflower. Ennos et al. (1993) observed that the most important anchorage component in sunflower was the resistance to the pulling of the roots on the windward side of the plant. Sposaro et al. (2008) found that anchorage strength was determined by the size of the root plate diameter. Stem lodging occurs when wind exerts a force which breaks the stem at its base (stem failure wind speed) that exceeds the stem failure moment. No studies have attempted to interrelate the complex interactions between climate and soil variables, husbandry practices and plant characteristics for sunflower lodging.

Models of lodging have been developed for wheat (Baker et al., 1998; Berry et al., 2003a) and more recently for barley (Berry et al., 2006). By considering the cereal plants as acting as a damped harmonic oscillator subject to a stepped input (Baker, 1995), these models calculate the wind-induced base-bending moment (leverage) of a shoot from plant characteristics. The calculated base bending moment is compared with the failure moments (strength at the point of failure) of the stem base and of the anchorage system to estimate the risk of stem and root lodging, respectively. Although some of the principles of the wheat and barley model could be the same for sunflower, other traits and differences between these crops must be considered. There are important issues that have to be taken into account for a sunflower model. It must firstly be investigated as to whether the sunflower shoot behaves as a damped harmonic oscillator. The area of the plant that is loaded by the wind is very different from cereals because the capitulum is

disc shaped and the leaves present a much greater area. The importance of the root plate diameter in lodging susceptibility has recently been studied (Sposaro et al., 2008). It is also uncertain whether the stem has the strength properties of a cylinder or whether the central pith provides significant strength. It is also possible that there are changes in stem internal anatomy during grain filling due to remobilization.

The objectives of this work are to develop a root/stem lodging model for sunflower and to validate it. This model was tested against individual field experiments in which natural root or stem lodging had occurred at different crop development stages under various crop population densities.

MATERIALS AND METHODS

In order to develop the lodging model a method to estimate the root and stem failure moments was developed during various years of experimentation.

Measurements of root failure moment (B_R)

Root failure moment values (B_R) were obtained by Sposaro et al. (2008). These measurements were carried out for two commercial hybrids (CF29 and Zenit), four crop population densities (3, 5.6, 10 and 16 plant m^{-2}) and three developmental stages (R2, R5.9 and R8 on Schneiter and Miller [1981] scale) in two different soil types (Typic Argiudoll and Typic Hapludoll) during three years.

Measurements of stem failure moment (B_S)

The values of stem failure moments (B_S) needed for the lodging model were obtained during two years of experiments: 2004/05 (E1) and 2005/06 (E2). In both experiments, two hybrids (Experimental Stay green (SG), Advanta Semillas, Argentina and Zenit, Sursem, Argentina), with different stem lodging susceptibility were planted using two plant population densities (5.6 plants m^{-2} [E1] and to 5.6, 10 and 16 plants m^{-2} [E2]) A randomized complete block design with three replications was used.

Measurements were performed at three stages of crop development: when the grain reached: a) 50% of its final dry grain weight (R7); b) 90% of its final dry grain weight (R8); and c) harvest maturity. These stages were selected because it is recognized that during grain filling and harvest maturity are the most stem lodging susceptible stages in sunflower (Abelardo de la Vega, personal communication).

An instrument especially constructed, the same used by Sposaro et al. (2008), was used to measure the force (F, N) required to induce stem lodging by pushing individual stems gradually from their vertical position until stem breakage occurred. The height of the bar that pushed the plant was adjustable, and set at 60% of the plant height (h). The stem failure moment (B_S, N m, i.e., the moment (Nm) needed to induce stem lodging) was obtained as the product of the force F (N) by $0.6h$ (plant height, m). Once the stem broke, the thickness of the epidermis plus the cortex (Ep+Co, m) was measured at the place where the stem failure occurred.

Plant area expected to be hit by wind gusts (A)

In order to understand how to estimate the area of the plant that is hit by wind gusts, we studied the shape of the leaves and capitulum through development. We measured the diameter and thickness of the capitulum and the length and width of the leaves in the upper third of the stem. The area formed by the leaves and the capitulum in the upper third of the plant was used for the estimation of the area that hits wind gusts.

Plant natural frequency (n)

Natural frequency (n) measurements were carried out for each hybrid at different stages of development. Each individual plant was pulled 10º from its vertical position and allowed to oscillate freely. The time taken for each plant to stop oscillating and the number of oscillations were recorded, and then transformed into number of oscillations per second (Hz) known as natural frequency (Berry et al., 2003b).

Model development

The sunflower model was developed based on exiting models for wheat (Baker et al., 1998; Berry et al., 2003a; Sterling et al., 2003) and more recently for barley (Berry et al., 2006). By considering the plants to act as a damped harmonic oscillator subject to a stepped input (Baker, 1995) these models calculate the wind-induced base-bending moment (leverage) of a shoot from B_R, B_S and plant characteristics. The calculated base bending moment is compared with the failure moments (strength at the point of failure) of the stem base and of the anchorage system to estimate the risk of stem and root lodging, respectively.

Point of application of the wind

In keeping with the work of Finnigan (2000), it was assumed that the top one third of the plant experienced significant wind loading. Hence the overall point of application was changed to 5h/6, where *h* is the height of the plant; this differs from the wheat model which assumes the point of application is at the centre of gravity.

Model validation

During season 2006/07 four meteorological stations (Vantage Pro2^{TM}, Davis Instruments Corp. 3465 Diablo Ave. Hayward, California 94545 USA) were installed at four different locations: Faculty of Agronomy, University of Buenos Aires (FAUBA), Advanta Semillas Research Centre, Venado Tuerto (VT), Daireaux, Buenos Aires; General Pico, La Pampa. At FAUBA, the meteorological station was installed during 2005/06, too. Three sunflower hybrids with different root or stem lodging susceptibility by four crop population densities were implanted at the four sites. The stations registered precipitation, wind speed and direction. At each location the percentage of root or stem lodging was recorded and was represented as a lodging index. The lodging index values are between 0 (no lodging) and 1 (the entire plot lodged).

RESULTS AND DISCUSSION

Methods for calculating root and stem failure wind speed

The root failure wind speed (minimum wind speed that could cause root lodging) V_{gR} (m s^{-1}) can be calculated using Eq. 1:

$$V_{gR} = \sqrt{\frac{2B_R}{\left(\rho A C_d h\left(1+\left(g/\left(2\pi n\right)^2 h\right)\right)\left(1+e^{-\pi\delta\left(\sin\left(\pi/4\right)/\left(\pi/4\right)\right)}\right)\right.}} \quad \text{Eq. 1}$$

Where $B_R = 0.2382x$, being x the root plate diameter cubed multiplied by the soil shear strength (sd^3, Nm, i.e. anchorage strength) (Fig. 1). This robust association between B_R and anchorage strength (sd^3) that held across hybrids, soil types, stages of development and crop population densities (Sposaro et al., 2008) allows a comparison to be made with the same relationship used in the previous lodging model for other crops, specifically wheat and barley. In wheat, the slope of the B_R / sd^3 relationship was 0.39 (Crook and Ennos, 1993) or 0.43 (Baker et al., 1998), while that for barley was 0.58 (Berry et al., 2006). These values contrast with the slope of 0.24 we found in sunflower (Fig. 4), suggesting that sunflower has an inherently lower B_R than the winter cereals for a given root plate diameter (Sposaro et al., 2008).

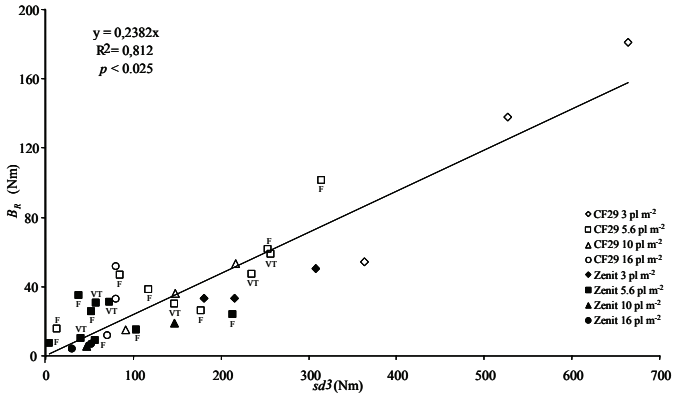

Fig. 1. Relation between B_R (Root failure moment) (Nm) and sd^3 (Plant anchorage) (Nm). Each point is the value for each d^3 (root plate diameter cubed) multiplied by s (soil shear strength) corresponding to four plant densities (3-5.6-10 and 16 plants m^{-2}), two genotypes (Zenit and CF29) and two soil types FAUBA (F), Typic Argiudoll and Venado Tuerto (VT) Typic Hapludoll against each respective value of B_R (from Sposaro et al., 2008).

In the same way the stem failure wind speed (minimum wind speed that could cause stem buckling) V_{gS} (m s^{-1}) can be calculated using Eq. 2:

$$V_{gS} = \sqrt{\frac{2 B_S}{\left(\rho A C_d h\right)\left(1+\left(g/\left(2\pi n\right)^2 h\right)\right)\left(1+e^{-\pi\delta\left(\sin\left(\pi/4\right)/\left(\pi/4\right)\right)}\right)}} \quad \text{Eq. 2}$$

Where $B_S = 5980.2x$, being x the Ep + Co (m) measured in the lower third of the stem (Fig. 2).

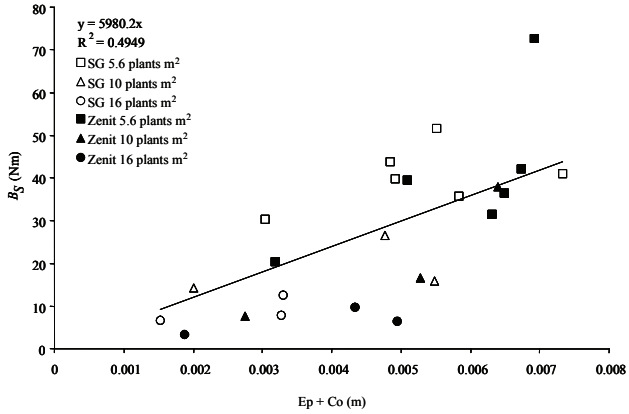

Fig. 2. Relation between B_S (Stem failure moment) (Nm) and Ep+Co (m) from lower third of the stem.

This relationship shows that hybrids with a better maintenance of the stem internal structures during grain filling (i.e. Stay green hybrids) could be more resistant to stem failure at these phenological stages. These results are important in determining the importance of the behavior of the stem as a cylinder providing significant resistance to buckling. Our results are consistent with those of Berry et al. (2003) and Berry et al. (2006) for wheat and barley, respectively.

In Eq. 1 and 2, n is the measured plant natural frequency, A the area expected to be hit by wind gusts, h the plant height, ρ air density, C_d sunflower drag coefficient (0.5), δ the damping ratio (0.08) and g the acceleration due to gravity (9.81 m s^{-2}).

Model validation

The model has been tested against various lodging events observed in sunflower experimental plots. Four storms that caused root lodging were recorded: 30/01/05, 17/12/06 (this lodging occurred at a neighbor's experimental plot [Hybrid 1] at FAUBA and was recorded too), 03/03/2007 at FAUBA and 01/12/06 at VT. Stem lodging was registered: 02/03/2007 at FAUBA. The plant parameters (mean of 10 plants per plot) were used with soil information to predict the failure wind speed for each experimental plot lodged. Table 1 shows the recorded and predicted root or stem failure wind speed and the lodging index for each hybrid, crop population density at the location where lodging occurred. If the recorded failure wind speeds were greater than those predicted, root or stem lodging could be expected for that plot. It is remarkable that the predictions of the model recreated the differences in lodging susceptibility between hybrids and crop population densities (Sposaro et al., 2008). For Zenit and CF29 the predicted root failure wind speed diminished when crop population density increased while for Stay green hybrid (SG) stayed stable, which was expected due to the maintenance of the integrity of the Ep+Co of the stem.

The results of the failure wind speed for root or stem lodging are consistent with the lodging index recorded in each combination of hybrid and crop population density. In the cases when the recorded failure wind speed was less or similar than that predicted by the model minimum or no lodging was recorded, and when the differences between recorded and predicted failure wind speed were greater, the recorded lodging index increased.

Parametric analysis

A parametric analysis was made in order to describe what the response of predicted failure wind speed was with changes in each separated variable of the model and to detect which one was the most important in determining lodging susceptibility. Each parameter varied throughout its entire range (between percentile 0 and 100) of values for all hybrids, stages of development and crop population densities.

Table 1. Lodging index, recorded and predicted (n = 10; ± 1 standard error) root or stem lodging wind speed and type of lodging (R, root; S, stem), for the lodged plots at different location, dates and crop population densities.

Hybrid	Crop population density (plant m^{-2})	Date	Location	Type of lodging	Lodging index	Recorded failure wind speed ($m\ s^{-1}$)	Predicted failure wind speed ($m\ s^{-1}$)
SG	5.6	30/01/2005	FAUBA	R	0.5	14.7	6.6 ± 0.7
Zenit	5.6	30/01/2005	FAUBA	R	0.8	14.7	5.7 ± 0.56
CF29	5.6	30/01/2005	FAUBA	R	0	14.7	14.4 ± 0.06
Zenit	3	01/12/2006	VT	R	0.3	13	7.4 ± 0.77
Zenit	5.6	01/12/2006	VT	R	0.37	13	6.1 ± 0.65
Zenit	10	01/12/2006	VT	R	0.6	13	5.6 ± 0.48
Zenit	16	01/12/2006	VT	R	0.85	13	4.3 ± 0.22
CF29	3	01/12/2006	VT	R	0	13	25.2 ± 1.22
CF29	5.6	01/12/2006	VT	R	0	13	16.7 ± 0.85
CF29	10	01/12/2006	VT	R	0.05	13	12.1 ± 0.27
CF29	16	01/12/2006	VT	R	0.05	13	11.5 ± 0.69
Hybrid 1	5.6	17/12/2006	FAUBA	R	1	14.8	6.1 ± 0.52
Zenit	3	03/03/2007	FAUBA	R	0	8.3	8.8 ± 0.78
Zenit	5.6	03/03/2007	FAUBA	R	0.3	8.3	7 ± 0.46
Zenit	10	03/03/2007	FAUBA	R	0.5	8.3	2.6 ± 0.25
Zenit	16	03/03/2007	FAUBA	R	0.95	8.3	2.5 ± 0.24
CF29	3	03/03/2007	FAUBA	R	0	8.3	12.7 ± 0.9
CF29	5.6	03/03/2007	FAUBA	R	0	8.3	10.9 ± 0.57
CF29	10	03/03/2007	FAUBA	R	0.05	8.3	8.6 ± 0.21
CF29	16	03/03/2007	FAUBA	R	0.1	8.3	7.5 ± 1.2
Zenit	5.6	02/03/2007	FAUBA	S	0	8.9	9.9 ± 0.39
Zenit	10	02/03/2007	FAUBA	S	0.95	8.9	5.3 ± 0.61
Zenit	16	02/03/2007	FAUBA	S	0.5	8.9	5.9 ± 0.65
SG	5.6	02/03/2007	FAUBA	S	0	8.9	8.7 ± 0.3
SG	10	02/03/2007	FAUBA	S	0	8.9	8.9 ± 0.48
SG	16	02/03/2007	FAUBA	S	0	8.9	8.9 ± 0.66

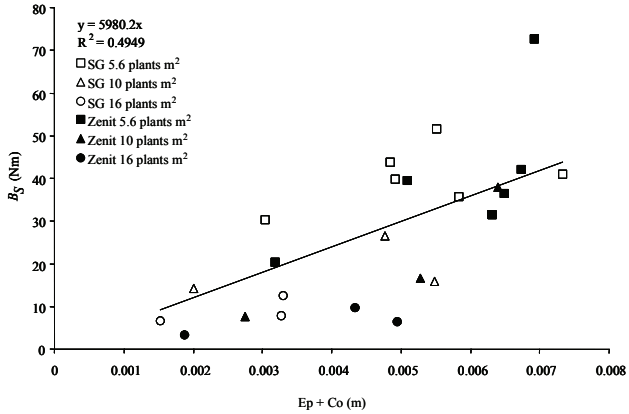

Fig. 3. Parametric analysis for root and stem lodging model. a) Predicted root failure wind speed ($m\ s^{-1}$) and b) stem failure wind speed ($m\ s^{-1}$) for variations between 0 (minimum value) and 1 (maximum value) in model variables: *h*, plant height (m); *A*, area expected to be hit by wind gusts (m^2); *n*, natural frequency (Hz); a) root plate diameter and b) *Ep+Co*, thickness of the epidermis plus the cortex of the stem.

Predicted root failure wind speed (Fig. 3a) was affected mostly by changes in root plate diameter (i.e. root failure wind speed increased in a greater proportion when root plate diameter was higher and decreased when it was lower). Stem failure wind speed (Fig. 3b) was affected mostly by variations in thickness of *Ep+Co* in the same way as the root plate diameter. The area expected to be hit by wind gusts (*A*) was an important variable too for the determination of the failure wind speed, but only when its values were the lowest. Although variations in height (*h*) and natural frequency (*n*) modified the results for failure wind speed, these were of a lesser importance than the other parameters. This is an important finding because most farmers think that plant height is the most important trait that determines lodging susceptibility. These results are similar to those of Berry et al. (2003) for wheat; the root plate diameter and the thickness of the stem wall (i.e. the same as Ep+Co in sunflower) being the most important parameters affecting the predicted failure wind speed. Fig. 3 a) and b) show that the value of the average stem failure wind speed is higher (8.5 m s^{-1}) than the root failure wind speed (7.8 m s^{-1}), indicating that sunflower could be more susceptible to root than stem lodging.

To summarize, the results of this study show, for the first time in sunflower, the developing of a model that can accurately predict the root/stem failure wind speed for crops growing under various husbandry practices (hybrids, crop population densities, soil types). The variables that are inputs of the model can be used in breeding programs to select hybrids that could be more resistant to stem or root lodging.

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REFERENCES

Baker C.J., P.M. Berry, J.H. Spink, R. Sylvester-Bradley, R.K. Scott, and R.W. Clare. 1998. A method for the assessment of the risk of wheat lodging. J. Theor. Biol. 194:587-603.

Berry, P.M., M. Sterling, C.J. Baker, J. Spink, and D.L. Sparks. 2003a. A calibrated model of wheat lodging compared with field measurements. Agr. Forest Meteorol. 119:167-180

Berry, P.M., J.H. Spink, M. Sterling, and A.A. Pickett. 2003b. Methods for rapidly measuring the lodging resistance of wheat cultivars. J. Agron. Crop Sci. 189:390-401.

Berry, P.M., M. Sterling, J.H. Spink, C.J. Baker, R. Sylvester-Bradley, Mooney, S.J., Tams, and A.R. Ennos. 2004. Understanding and reducing lodging in cereals, Adv. Agron. 84:217-271.

Berry, P.M., M. Sterling, and S.J. Mooney. 2006. Development of a model of lodging for barley. J. Agron. Crop Sci. 192:151-158.

Bragachini, M., A. von Martini, and A. Mendez. 2001. Pérdidas de cosecha. Evaluación y tolerancias en cosecha de Soja, Maíz, Girasol y Trigo. Proyecto agricultura de precisión. INTA E.E.A. Manfredi.

Crook, M.J., and A.R. Ennos. 1993. The mechanics of root lodging in winter wheat, *Triticum aestivum* L. J. Exp. Bot. 44:1219-1224.

Ennos, A.R., M.J. Crook, and C. Grimshaw. 1993. A comparative study of the anchorage systems of himalayan balsam Impatiens glandulifera and mature sunflower *Helianthus annuus*. J. Exp. Bot. 44:133-146.

Pinthus, M.J. 1973. Lodging in wheat, barley and oats: the phenomenon, its causes and preventive measures. Adv. Agron. 25:210-263.

Schneiter, A.A., and J.F. Miller. 1981. Description of sunflower growth stages. Crop Sci. 21:901-903.

Scott, D.I., A.R. Tams, P.M. Berry, and S.J. Mooney. 2005. The effects of wheel-induced soil compaction on anchorage strength and resistance to root lodging of winter barley. Soil Tillage Res. 82:147-160.

Sposaro, M.M., C.A. Chimenti, and A.J. Hall. 2008. Root lodging in sunflower. Variations in anchorage strength across genotypes, soil types, crop population densities and crop developmental stages. Field Crops Res. 106:179-186.

Exploring genotypic strategies for sunflower drought resistance by means of a dynamic crop simulation model

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ABSTRACT

Although sunflower is often reported as a drought-tolerant crop, it suffers from intense and frequent periods of water deficit in Europe because it is mostly grown on shallow soils, under low rainfall and in rainfed areas. Given the limitations of experimental trials to explore a large number of drought scenarios, a dynamic crop simulation model was developed to determine different phenological (duration of post-anthesis period), morphological (leaf area) and physiological (rate of stomatal closure) putative traits of drought resistance in sunflower. A virtual experimental network was built by combining 4 locations (N-S gradient), 3 soil depths, over 36 weather seasons. In each of the 432 trials, 12 synthetic varieties were evaluated, differing by earliness at maturity (2 levels), leaf area (3 levels), leaf expansion and stomatal regulation sensitivity to progressive soil drying (2 levels: early or late response). This simulation study suggests that the varieties with early stomatal closure could result in the best yields in drought-prone environments, this trait being more determining than leaf area or earliness. In the most productive locations, late varieties, with large leaf area and late stomatal closure should result in the best yield. It is concluded that an additional variety screening including the response to water deficit could improve the choice of optimal sunflower cultivars in France.

Key words: drought resistance – leaf area – phenology – simulation model – stomatal regulation – varietal choice.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is often reported as a drought-tolerant crop (Unger, 1990; Connor and Hall, 1997). However, in southern Europe it suffers from intense and frequent periods of water deficit because it is mostly cultivated in low rainfall areas, without irrigation, and on shallow soils.

Ludlow (1989) reviewed three main genotypic adaptations to water-limited environments: (a) drought escape, whereby the crop completes its life (or oversteps a critical growth stage) before the onset of severe drought, (b) drought avoidance, where the crop maximizes its water uptake and minimizes its water loss, and (c) drought tolerance, where the crop continues to grow and function at reduced water contents. To these plant strategies, crop management offers additional opportunities (Debaeke and Aboudrare, 2004): (d) drought alleviation or moderation, by the means of irrigation, (e) optimal crop water use pattern, by reducing soil evaporation and increasing the contribution of transpiration during grain filling period (through crop density and N fertilization).

In commercial fields, drought resistance should be achieved by combining optimal cultivar choice and crop management. But limited information is available to characterize the response of sunflower cultivars to water stress, as drought resistance is a complex trait which cannot be evaluated accurately at field level. Field trials where water deficit occurs are generally banned from the official evaluation network because of poor statistical value.

Given the limitation of experimental trials to explore a large number of drought scenarios, dynamic crop modelling may be an alternative to arduous experimentation and is recognized as an adequate tool to identify genotype x environment x cultural practice combinations to achieve the most stable yield over a wide range of soil water availabilities (Agüera et al., 1997; Sinclair and Muchow, 2001; Chapman et al., 2002; Soriano et al., 2004).

Although several models are available for sunflower crop (e.g. Chapman et al., 1993; Villalobos et al., 1996; Pereyra-Irujo and Aguirrezabal et al., 2007), a new simulation framework was developed to represent more explicitly the varietal differences and to support cultivar choice decision in relation with water availability (Casadebaig, 2008). The main original point comes from using genotypic parameters that are measured directly from field or greenhouse trials.

The objective of this communication is to examine, by means of this dynamic simulation model, whether different varietal types defined by earliness, architecture, and response to soil desiccation should

be recommended in France over the sunflower production area when natural water availability (precipitation, soil depth) is changing.

MATERIALS AND METHODS

Model and varietal parameters

The simulation model equations are described in detail in Casadebaig (2008): the daily step model simulates dynamically achene yield and oil concentration as a function of classic weather data (temperature, precipitation, ET_{ref}), soil data (available soil water content, N mineralization), crop management (sowing date, plant density, N fertilization, irrigation) and varietal characteristics (phenology, leaf area dynamics, leaf expansion and transpiration rate response to soil water deficit, biomass allocation).

Phenological parameters are: growing degree days (Tbase: 4.8°C) to reach different characteristic growth stages: emergence (A2), star bud (E1), early anthesis (F1), early grain filling (M0), physiological maturity (M3).

Leaf area (LA) index evolution is simulated on an individual leaf scale basis (Lizaso et al., 2003) and modulated from the measurement of 3 architectural parameters at anthesis: total leaf number, position and length x width of the largest leaf.

The extinction coefficient (k) is determined either directly or through a statistical adjustment using the previous LA parameters.

Genetic harvest index and oil concentration are determined in dense and unstressed sunflower stands.

Modules for development, biomass accumulation and allocation to the achenes were built using robust representations from the literature.

An original screening method was developed in greenhouse to parameterize leaf expansion and stomatal closure response to soil water content. Thresholds were calculated for a range of genotypes from different sources of selection (Casadebaig et al., 2008).

Phenotypic database

A database was built to gather the results of numerous experiments conducted by INRA and Cetiom from 2001 to 2007 on sunflower phenotyping (Debaeke et al., 2004). More than 20 cultivars representing the genetic progress from 1960 onwards (Vear et al., 2003) were fully described.

A virtual multi-environment trial network

From the phenotypic database, 12 virtual cultivars (Table 1) were created by combining 2 variants of earliness (E, early: 1750 °C.day from A2 to M3; L, late: 2160°C.day), 3 levels of plant potential leaf area (S, small: 4000 cm²; M, medium: 8000 cm² ; L, large: 12000 cm²) and 2 extreme levels of plant regulation in response to soil drying (E: early control of leaf expansion and stomatal closure, at a relatively high soil water content ; L , late control, at a rather low soil water content) (Fig. 1). In this study, the term regulation was used to reflect the effects of both response traits (leaf expansion and transpiration control). It was assumed that all the characters of drought resistance were independent.

Table 1. Combination of the 3 phenological, morphological and physiological traits to build 12 virtual varieties

Variety	Code	Earliness	Leaf Area	Regulation
1	ESE	Early	Small	Early
2	ESL	Early	Small	Late
3	EME	Early	Medium	Early
4	EML	Early	Medium	Late
5	ELE	Early	Large	Early
6	ELL	Early	Large	Late
7	LSE	Late	Small	Early
8	LSL	Late	Small	Late
9	LME	Late	Medium	Early
10	LML	Late	Medium	Late
11	LLE	Late	Large	Early
12	LLL	Late	Large	Late

Four regions were selected to sample the main French sunflower cropping area: Midi-Pyrénées (South-West), Provence (South-East), Poitou-Charentes (Center-West) and Parisian Basin (Center-North). Each region was described by one climate station and 3 soil types. The following climate stations from INRA were chosen: Auzeville (Department 31), Avignon (84), Lusignan (86) and Versailles (78). Each climate series was composed of 36 x 365 daily recordings (1971 – 2006). Solar radiation and climatic water deficit were the highest in Avignon and the lowest in Versailles as expected.

The 3 soil types differing by soil depth and available soil water content (ASWC) were extracted from a soil data base from INRA (Brisson et al., 2006): S1 (ASWC: < 60 mm), S2 (80-120 mm), S3 (130-150 mm).

Sunflower crop management was the same in the 12 environments: sowing date on 20 April, 60 kg N/ha applied 15 days after emergence, no supplemental irrigation.

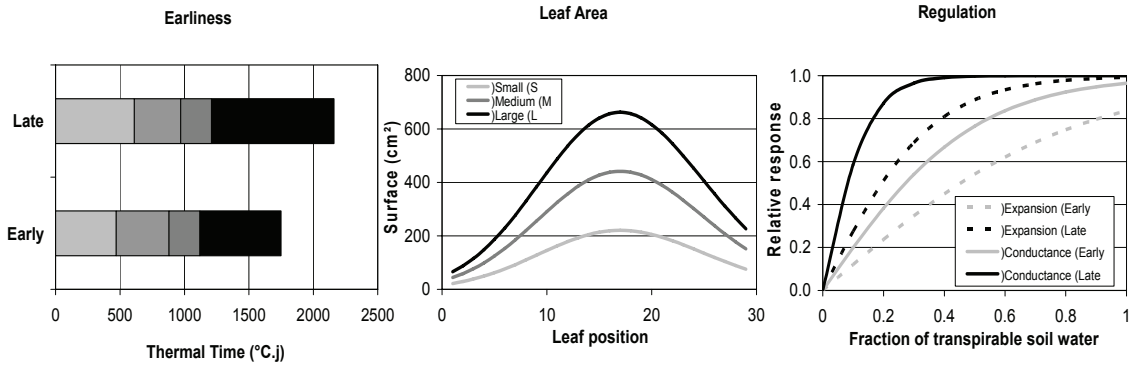

Fig. 1. Extreme values of phenological, morphological and physiological traits (maturity earliness, total leaf area, thresholds for stomatal closure and leaf expansion decline) among a range of 20 cultivars.

RESULTS

The application of the model resulted in different yield performances of the 12 varieties with season and pedoclimatic environment.

The northern situations (LUS, VER) resulted in higher yield levels, whatever the soil depth (Fig. 2). Grain yield was more stable in VER location (especially on S3) and more variable in Auzeville (AUZ). Average grain yield over 36 years ranged from 14 to 28 q/ha depending on soil type and climate. In France, average grain yield in national surveys ranges from 18 to 23 q/ha (at 0 % grain moisture).

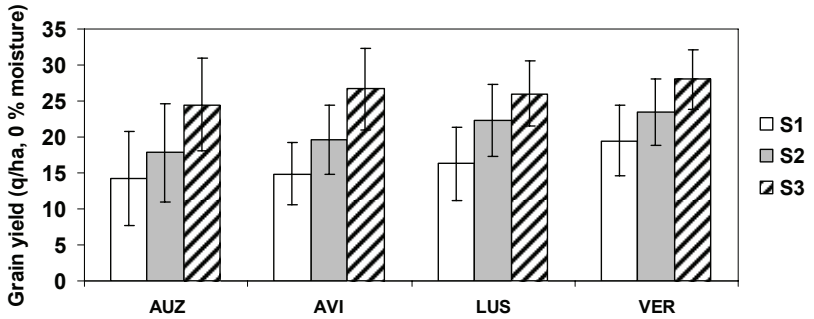

Fig. 2. Effects of location and soil type on grain yield (mean values and S.D over 36 years)
S1 = shallow ; S2 = medium ; S3 = deep soil

The mean effects of variety, earliness, leaf area and regulation on grain yield were displayed on Fig. 3. Late maturation, high leaf area index and early stomatal closure all increased grain yield: the latter trait was the most influential one (+ 3.8 q/ha vs 0.9-1 q/ha for the two other traits). The combination of the 3 traits resulted in GY variations from 18.8 (var. 2) to 24.4 q/ha (var. 11).

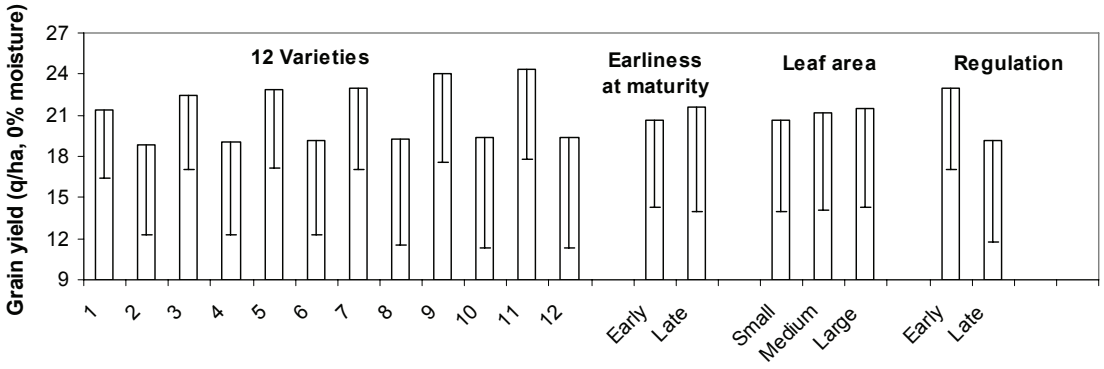

Fig. 3. Grain yield for the 12 virtual varieties and the 3 morpho-physiological traits (mean values and SD over 36 years)

The 3 traits (maturity earliness, leaf area, regulation) had a significant effect on yield ($P < 0.001$) but the 'trait x environment' interactions are not of the same level: highly significant for regulation ($P < 0.001$), significant at $P < 0.1$ for earliness, but not significant for leaf area.

To quantify the importance of a genotypic trait in a given location, an analysis of variance (ANOVA) was attempted as follows: Yield ~ Yr + E + LA + R + Yr*E + Yr*LA + Yr*R, in each environment (Table 2).

Table 2. Variances and statistical significance (*P<0.05; **P< 0.01; ***P<0.001) of the single effects (genotypic traits) and the 'year x trait' interactions in each of the 12 environments.

Location	Soil	Year (Yr)	Earliness (E)	Leaf Area (LA)	Regulation (R)	Yr x E	Yr x LA	Yr x R
AUZ	S1	374 ***	2 ns	12 ***	4043 ***	8.0 ***	0.4 ns	19.8 ***
AUZ	S2	376 ***	7 *	2 ns	5574 ***	5.3 ***	0.7 ns	20.4 ***
AUZ	S3	405 ***	163 ***	12 ***	1757 ***	8.9 ***	2.3 ***	37.7 ***
AVI	S1	183 ***	109 ***	63 ***	1089 ***	2.4 ***	0.3 ***	8.3 ***
AVI	S2	196 ***	63 ***	4 ***	2633 ***	2.4 ***	0.5 ***	4.9 ***
AVI	S3	319 ***	77 ***	2 *	1885 ***	9.7 ***	2.8 ***	8.3 ***
LUS	S1	201 ***	63 ***	12 ***	3283 ***	7.6 ***	0.7 ns	11.6 ***
LUS	S2	211 ***	87 ***	2 ns	1850 ***	7.3 ***	1.0 ns	22.3 ***
LUS	S3	185 ***	249 ***	22 ***	753 ***	5.0 ***	1.1 *	19.2 ***
VER	S1	217 ***	90 ***	82 ***	996 ***	6.2 ***	1.7 **	24.0 ***
VER	S2	204 ***	112 ***	60 ***	364 ***	7.1 ***	4.3 ***	19.1 ***
VER	S3	156 ***	567 ***	407 ***	33 ***	3.9 ***	1.5 ***	1.9 ***

If the advantage conferred by the R character was major whatever the environments (except for VER_S3 and VER_S2, where water stress was minimum), the impact of the other characters was dependent on the environment. Earliness (E) played a role in the medium and deep soils, where late varieties can take advantage of a longer growing duration. Leaf area, although its effect was significant in most of the environments, played only a significant role in the most extreme environments (AVI_S1, VER).

All the characters exhibited significant interactions with year, although Yr x R interactions were the most important. The existence of such interactions between the traits and the climate suggests that a varietal choice based only on the mean performance of a variety in a given location might be irrelevant in some years.

The R character was obviously the most determining one to explain yield variations in this simulation exploration: depending on the regions, regulation though leaf expansion and stomatal closure may be responsible for mean gaps of 7 q/ha in South-West (between varieties differing only by this character) but of 1.7 q/ha in the Parisian Basin region. These gaps were related to the intensity of soil water deficit, stomatal closure being a response to soil desiccation. Earliness had a lower influence: from 0.4 q/ha (South-West) to 1.8 q/ha (Parisian Basin). Concerning leaf area, the mean gap between two modalities ranged from 0.2 q/ha (South-West, Center-West) to 1.7 q/ha (Parisian Basin). Increasing LA had a negative impact on yield in the most drought-prone environments (AUZ, AVI) but only for varieties with late stomatal closure. The model suggested that « early regulation » has more impact than a variation in potential LA in these environments. In dry environments, reducing LA might not be a good strategy as potential yield would be reduced too much and soil evaporation could increase as well.

From the ANOVA, the best varietal choice (combination of 3 traits) among 12 candidates was determined for each of the 12 environments on the basis of 36 virtual trials (climate series) (Table 3).

At a regional level, the variety "11" or LLE (late maturing, large leaf area, early stomatal closure) would be systematically the best choice. The ideotype LLE was relevant 5 years out 10 in South-East and Parisian Basin and 7 years out 10 in the South-West and Center-West regions. Three years out of 10, the ideotype LLL, with late stomatal closure, would be a better choice in the Parisian Basin and the ideotype LSE, with a smaller LA, would be a better choice in South-East.

The soil type had no marked effect on the best choice within a region. But the frequency of the best yielding cultivar changed from one environment to another (from 46 % to 89 %). In general, early regulation should be recommended in shallow soils, because delaying soil water depletion in this way is a good strategy to sustain a large leaf area (and light interception). On the contrary, in the Parisian Basin region, in deep soils (VER_S3), where water deficit and global radiation are the lowest, the model selected a late maturing ideotype, with a late stomatal closure when exposed to water deficit (more photosynthesis in spite of more water transpired in the first part of the cycle) and a large LA value.

Table 3. Potential yield, mean GY value of the best ranked variety, and best ranked varieties in each simulated environment[1]

Location	Soil	Best year (q/ha)	Best variety (q/ha)	1st rank for variety n (%)	1st choice	2nd choice
AUZ	S1	27.0	18.0	71	**11** - 9 - 5	7 - 3 - 1
AUZ	S2	30.0	22.3	66	**11** - 9 - 7	5 - 3 - 1
AUZ	S3	34.1	28.1	63	**11** - 9	7 - 5 - 3
AVI	S1	25.4	17.7	89	**11**	9 - 5 - 3 - 7
AVI	S2	29.9	22.7	51	**11** - 9 - 7	5 - 3 - 1
AVI	S3	36.7	29.6	43	9 -11 - **7**	5 - 3 - 1
LUS	S1	26.4	20.4	83	**11** - 9	7 -5 -3 - 1
LUS	S2	30.9	25.7	66	**11** - 9 - 7	5 - 3 - 1
LUS	S3	33.1	29.0	71	**11** - 9	7 - 5 - 3
VER	S1	29.6	22.8	74	**11** - 9	5 - 3 - 7
VER	S2	32.6	26.0	46	**11** - 9	5 -7 - 3
VER	S3	35.0	30.5	63	11 - **12**	10 - 9

[1]The underlined variety number corresponds to the best ranked one in term of frequency over 36 years.

DISCUSSION

According to the model and to its multi-environment application, early regulation would be a relevant physiological trait to select in sunflower. According to Casadebaig et al. (2008), early stomatal closure is not frequent among commercial cultivars. This behaviour is closer to what is observed on isohydric species such as sorghum and maize. Conversely, Sinclair and Muchow (2001) did not simulate a significant increase in grain yield in sorghum, by manipulating this trait, as this crop was already well adapted to production under water stress.

In sunflower, changing cultivar earliness (from early to late type) did not result in huge differences in grain yield over the French cropping area, contrary to what is reported in Mediterranean environments (Debaeke and Aboudrare, 2004). In this paper, only differences in the anthesis-maturity were explored. The date of anthesis (about 10 days from early to late type) could have been modulated as well but probably with minor consequences on drought escape at anthesis. However, choosing an early maturing variety appeared as a good decision in the most stressful environments (AUZ_S1). Sowing date would probably have more effect on drought escape, especially sowing in autumn instead of spring as practised in the most southern regions of Europe (Soriano et al., 2004).

The optimal level of leaf area index results from a trade-off between transpiration, soil evaporation and light interception. In general, the lowest values of LA were not optimal in France even in the most stressed environments; plant density should rather be increased in this case.

Other traits have been reported as influencing grain yield in drought-prone environments: water extraction pattern and early vigour (Sadras and Hall, 1989; Agüera et al., 1997; Sinclair and Muchow, 2001). These traits could be explored by the model provided that experimental evidence of genotypic variation could be supplied.

The simulation of virtual genotypes, which is of interest for testing new combinations of traits, was based on characters expressing a sensitivity to water stress. From a practical angle, the farmer's decisions are based on cultivar potential productivity and disease tolerance (which were not considered in the simulations). Potential productivity corresponds to the LLL type in environments where water is not a limiting factor. With the exception of earliness, the characters involved in water stress resistance are not

evaluated in the official trials and for that reason they cannot be exploited by the advisers. As cultivar choice results from a complex evaluation of a range of characters (some are measured, others result from expertise), the varietal supremacy of LLE type would be probably less visible in trials. The interaction with the weather may change the optimal choice. For that reason, 2 or 3 years of field evaluation as currently practised are not sufficient to explore the advantages and limits of a new variety. The simulation of varietal strategies may help the adviser to promote a cultivar with a stable yield over a wide range of pedoclimatic conditions.

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REFERENCES

Agüera, F., F.J. Villalobos, and F. Orgaz. 1997. Evaluation of sunflower (*Helianthus annuus* L.) genotypes differing in early vigour using a simulation model. Eur. J. Agron. 7:109-118.

Brisson, N., F. Huard, A.I. Graux, C. Lebas, and P. Debaeke. 2007. Impacts de la variabilité des facteurs responsables de la sécheresse agricole à l'échelle du territoire par une approche de modélisation biophysique. Note Technique d'Agroclim n°1, 33 p.

Casadebaig, P. 2008. Analyse et modélisation dynamique de l'interaction génotype-environnement-conduite de culture: application au tournesol (*Helianthus annuus* L). PhD Thesis, INP Toulouse, 134 p.

Casadebaig, P., P. Debaeke, and J. Lecoeur. 2008. Thresholds for leaf expansion and transpiration response to soil water deficit in a range of sunflower genotypes. Eur. J. Agron. 28: 646-654.

Chapman, S., G. Hammer, and H. Meinke. 1993. A sunflower simulation model: I. Model development. Agron. J. 85: 725-735.

Chapman, S., M. Cooper, and G. Hammer. 2002. Using crop simulation to generate genotype by environment interaction effects for sorghum in water-limited environments. Aust. J. Agric. Res. 53: 379-389.

Connor, D.J., and A.J. Hall. 1997. Sunflower physiology. p. 113-182. In: A.A. Schneiter (ed.), Sunflower Production and Technology. Agronomy Monograph 35. ASA, CSSA and SSSA, Madison, WI, USA.

Debaeke, P., and A. Aboudrare. 2004. Adaptation of crop management to water-limited environments. Eur. J. Agron. 21: 433-446.

Debaeke, P., J. Lecoeur, A.M. Triboi-Blondel, and F. Vear. 2004. Crop physiological determinants of yield in old and modern sunflower hybrids. p 267-273 In: Proc. 16th Int.Sunfl.Conf., Fargo, ND, USA., Int.Sunfl.Assoc.Paris, France.

Lizaso, J.I., W.D. Batchelor, and ME. Westgate. 2003. A leaf area model to simulate cultivar-specific expansion and senescence of maize leaves. Field Crops Res. 80: 1-17.

Ludlow, M.M. 1989. Strategies of response to water stress. p. 269-281. In: K.H. Kreeb, H. Ritcher and TM Hinckley (eds). Structural and Functional Responses to Environmental Stresses, SPB Academic Publishing, Gravenhage, The Netherlands.

Pereyra-Irujo, G.A., and L.A.N Aguirrezabal. 2007. Sunflower yield and oil quality interactions and variability: Analysis through a simple simulation model. Agric. Forest Meteor. 143: 252-265.

Sadras, V., and A. Hall. 1989. Patterns of water availability for sunflower crops in semi-arid Central Argentina. A simulation-based evaluation of their interactions with cropping strategies and cultivar traits. Agric. Syst. 31: 221-238.

Sinclair, T.R., and R.C. Muchow. 2001. System analysis of plant traits to increase grain yield on limited water supplies. Agron. J. 93: 263-270.

Soriano, M.A., Orgaz, F., Villalobos, F.J., and E. Fereres. 2004. Efficiency of water use of early plantings of sunflower. Eur. J. Agron. 21: 465-476.

Unger, P.W. 1990. Sunflower. p. 775-794. In: B.A. Stewart and D.R. Nielsen (eds), Irrigation of Agricultural Crops, Agronomy Monograph N°30, ASA-CSSA-SSSA, Madison (WI), USA.

Vear, F., H. Bony, G. Joubert, D. Tourvieille de Labrouhe, I. Pauchet, and X. Pinochet. 2003. 30 years of sunflower breeding in France. OCL 10: 66-73.

Villalobos, F.J., A.J. Hall, J.T. Ritchie, and F. Orgaz. 1996. OILCROP-SUN: A development, growth, and yield model of the sunflower crop. Agron. J. 88: 403-416.

Dynamics of dry matter accumulation in sunflower

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ABSTRACT

This paper deals with the effect of sowing density on dynamics of dry matter accumulation in sunflower. The experiment was established on the chernozem soil, in a six-crop rotation system, in the two-factorial split-plot design. The main plots were three sunflower hybrids: NS-Dukat, NS-H-111 and NS-H-103. The subplots were six densities: 30000, 40000, 50000, 60000, 70000 and 80000 plants per hectare. The dynamics of dry matter accumulation and the distribution of dry matter among the various plant organs were dependent on competition between the plants. The largest amount of dry matter was accumulated by the flowering stage, while the peak of accumulation occurred 30 days after the flowering. The period of the most intensive dry matter accumulation was from budding till flowering.

Key words: dry matter – stand density – sunflower.

INTRODUCTION

Since sunflower hybrids differ in plant height, number, size and position of leaves, lodging and disease resistance, vegetative space, nutrient and water uptake as well as photosynthetic activity, it is necessary to establish optimal plant density for each hybrid. Different sunflower hybrids react differently to the environmental conditions. This leads to a different realization of yield potential.

Control of dry matter distribution between different plant organs is the basis for crop yield (Wardlaw, 1990). Special conditions for nutrient uptake, its distribution between different organs, and photosynthesis efficiency are created under the influence of different vegetative space and plant number. This is one of the causes of differences in sunflower yield in the same agro ecological conditions (Hall et al., 1990). Villalobos et al. (1992) found that, in order to create a model of crop growth, it is necessary to have a knowledge of assimilate distribution between plant parts and how the environment affects dry matter distribution and yield.

It was found that semi-dwarf sunflower hybrids had a higher dry matter content in head and a lower one in the stem, when compared with the hybrids with the standard height (Maid and Schneiter, 1988). No differences were observed in total dry matter accumulation in the hybrids of different heights, although taller hybrids formed a larger amount of dry matter.

There is increased competition between plants at high stand densities. As the competition between plants should be decreased in order to promote efficient use of water, nutrients and sunlight, it is necessary to determine optimal plant density in order to maximize the expression of the yield potential.

The aim of this experiment was to determine optimal plant densities for sunflower hybrids with a high yield potential which differ significantly in their growing habits.

MATERIALS AND METHODS

Field experiments were conducted at the Rimski Sancevi experiment field of the Institute of Field and Vegetable Crops, Novi Sad, Serbia, for three years. The experiment was established on a chernozem soil, in a six-crop rotation, following a two-factorial split-plot design. The main plot contained three cultivated sunflower hybrids: NS-Dukat, NS-H-111 and NS-H-103.

NS-Dukat is an early hybrid that matures in 90 to 95 days. The average stem height is 145 to 155 cm, the genetic potential for seed yield 4 t ha^{-1}, and the seed oil content from 47 to 49%. The hybrid is genetically resistant to downy mildew, broomrape and the sunflower moth. It is recommended for late sowing (15 May to 15 June) in fields that could not be sown before for some reason.

NS-H-111 is a medium early hybrid that matures in 105 to 115 days. The stem is firm, 165 to 185 cm tall on average. The genetic potential for seed yield is 5 t ha^{-1}, the seed oil content from 48 to 50%. The hybrid is genetically resistant to downy mildew, rust, broomrape and the sunflower moth, and tolerant to *Phomopsis.* The hybrid is adaptable to a wide range of agroecological conditions.

NS-H-103 is an experimental hybrid that matures in 120 to 130 days. The stem is firm, 90 to 100 cm tall on average. The genetic potential for seed yield is 4 t ha^{-1} and the seed oil content ranges up to 50%. The hybrid is genetically resistant to downy mildew, rust, broomrape and the sunflower moth.

The experiment subplots were six stand densities: 30,000, 40,000, 50,000, 60,000, 70,000 and 80,000 plants per hectare. Manual planting was done in early April, by placing 3-4 seeds per hill. At the stage of 1-2 pairs of leaves, the stand was thinned to one plant per hill, to obtain the desired number of emerged plants. Timely cultural practices were performed, applying the conventional technology. The experiment was conducted in four replications. The elementary plots consisted of six 10-meter rows.

Dynamics of dry matter accumulation during vegetative period of sunflower plants was observed at the main stages of the plant development:

1. 6 pairs of leaves
2. budding
3. flowering
4. seed forming
5. 30 days after flowering

Samples were taken from 12 plants (3 plants from each repetition) from the following variants: 30000, 80000 plants/ha in all three hybrids; 60000 plants/ha in NS-Dukat, 50000 plants/ha in NS-H-111 and 70000 plants/ha in NS-H-103. Average sample for each variant was used for the determination of dynamics of dry matter accumulation. Each sample was dried at 105° C, and dry matter percentage in different plant parts was determined.

RESULTS AND DISCUSSION

Dry matter accumulation dynamics varied depending on the development stage, plant density and the hybrid.

Dry matter accumulation at the stage of 6 pairs of leaves was low, and the plant density did not have any effect on plant dry matter production (Fig. 1). A higher proportion of total dry matter content in leaves compared to the stem was observed at this stage, especially in the hybrid NS-H-103 (Fig. 2). This is in accordance with the results of Horie (1977), who found predominance of assimilative distribution in the leaves at this stage.

Sunflower plants developed slower and accumulated less dry matter till the budding stage. Merrien (1986) explained this phenomenon by the fact that until the budding, most of the assimilates produced in the leaves are transported towards the root, which develops intensively during that stage. Bud appearance causes the inversion of the main direction of assimilate transportation. At that moment, the capitulum becomes the main assimilates consumer. In hybrids NS-Dukat and NS-H-111, the capitulum had a higher dry matter content than the leaves. According to Villalobos et al. (1994) and Trapani et al. (1994), this is the direct consequence of the increased competition between plants caused by increase in stand density. In contrast to NS-Dukat and NS-H-111, hybrid NS-H-103 dry matter content was higher in the leaves than in the capitulum.

The relationship between dry matter accumulation per plant and per acreage in different stand densities changed significantly at the budding stage. The highest dry matter content per plant was obtained at the lowest stand density, while the lowest dry matter content was recorded at the highest stand density. This was not the case with the dry matter accumulation per acreage, where the opposite trend was observed. Relations between dry matter production per plant and per hectare at the different stand densities are affected by competition between plants. Competition between plants starts at the stage of the intensive growth. The competition starts at the 13-14 pair of leaves stage, first in the stands with a higher number of plants, and, at the later stages of the development, in the stands with the lower plant densities. Percentage of dry matter formed at the budding stage increased with the increase in the plant density in all three tested hybrids.

The highest quantity of dry matter was accumulated from emergence till the flowering stage. Similar results were obtained by Merrien (1986). Dry matter accumulation was most intensive between budding and flowering, which is in accordance with the results of De Giorgio et al. (1990) and Sfredo et al. (1985).

Competition between plants for light, water and nutrients was more evident at the flowering stage. Till flowering, relations between plants were such as to enable the development of each plant even at higher stand densities, i.e. the plants developed faster and formed a larger quantity of dry matter at the stages of development in which the competition between plants was lower.

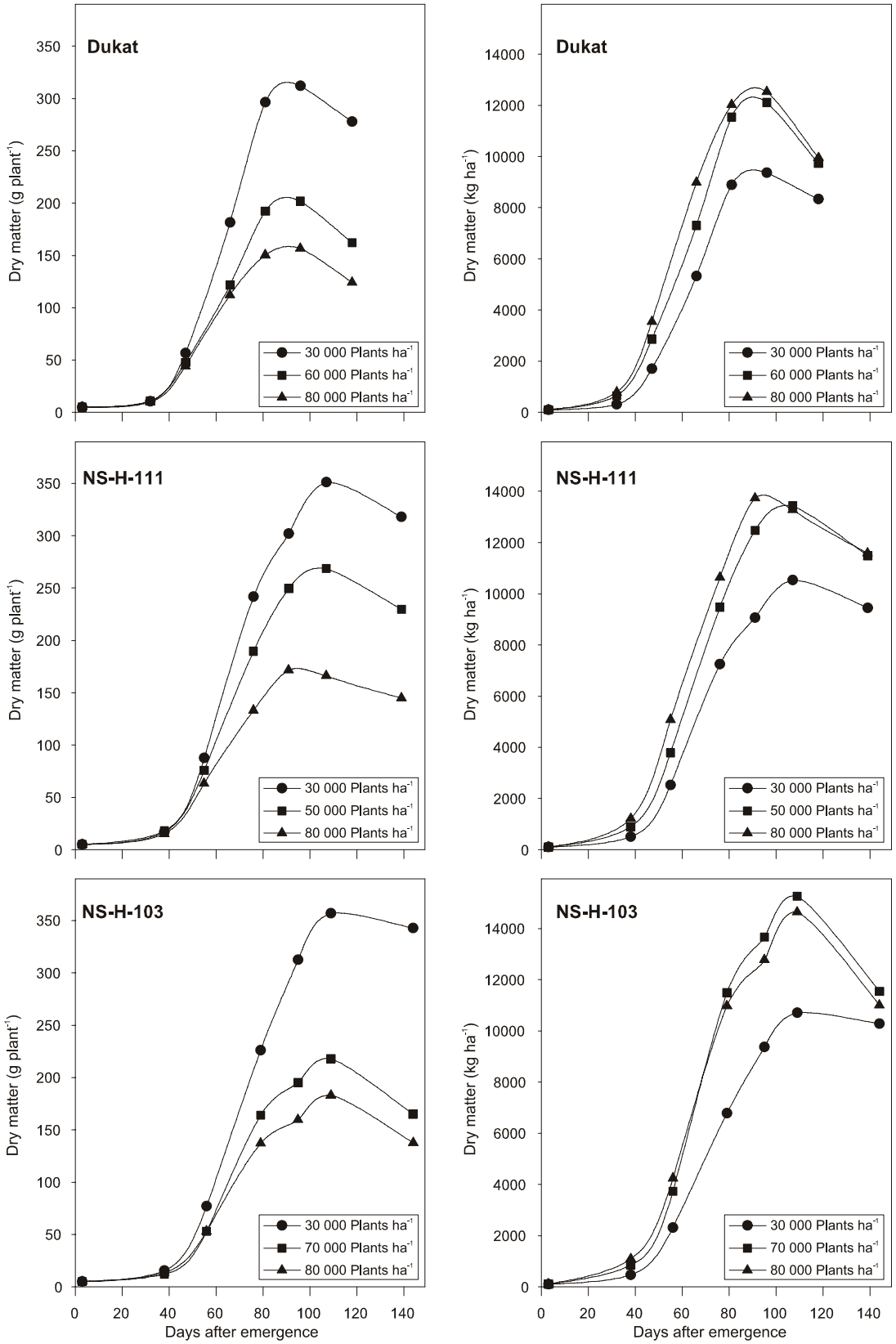

Fig. 1. Dynamics of dry matter accumulation during vegetative period of sunflower plants at different plant densities.

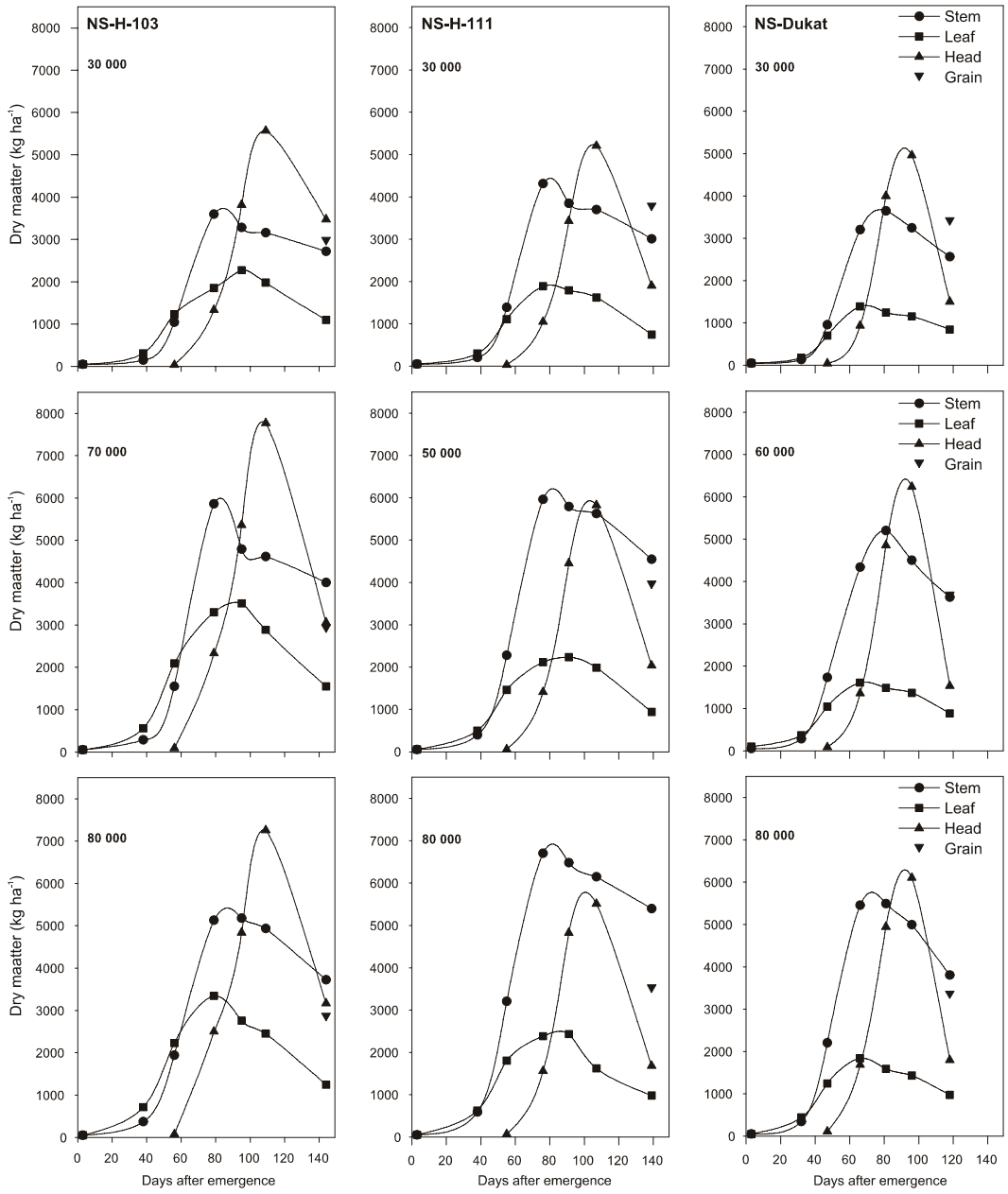

Fig. 2. Dry matter distribution between plant organs of sunflower plants at different plant densities.

Similar results were obtained by Blanchet et al. (1988). At this stage, in all three tested hybrids, the highest dry matter content was found in the stems, followed by the dry matter content in the leaves. In hybrids NS-Dukat and NS-H-111 dry matter content increased in the stem and decreased in the leaves with the increase in plant density. This is in agreement with the results of Horie (1977), Villalobos et al. (1994) and Trápani et al. (1994). In NS-H-103, the opposite trend of dry matter accumulation was observed. According to De Giorgio et al. (1990) and Sfredo et al. (1995), when there is no competition between plants caused by the size of vegetative space, the highest dry matter content can be found in the leaves, followed by the dry matter content in the stems.

Intensive dry matter accumulation continued from flowering till 30 days after flowering. This was especially the case of the hybrid NS-Dukat, in which, due to its growing habit, dry matter accumulation was not that much affected by competition. Proportion of stem in total dry matter content was still significant, but lower compared to the flowering stage. Significant decrease in dry matter, compared to

the flowering stage, was observed in leaves as well. Proportion of head in total dry matter content increased significantly at the expenses of assimilates from stem and leaves, as well as current assimilation. Similar results were obtained by De Giorgio et al. (1990). Proportion of stem in total dry matter content increased with the increase in stand density, while the proportion of leaves and capitulum decreased.

Maximal dry matter content was observed at the stage of 30 days after flowering. This is in accordance with the results of Vrebalov et al. (1983). Dry matter distribution between plant organs had the same tendency as in the previous developmental stage. Similar to the results of Sfredo et al. (1985), the proportion of dry matter of stem and leaves in total dry matter content decreased, and the proportion of dry matter in the capitulum increased.

The highest dry matter content in the stem was observed in NS-H-111 at all stages of the development. The other two tested hybrids had similar dry matter content in the stem, although it was non-significantly higher in NS-Dukat. Hybrid NS-H-103 had the highest dry matter content in leaves, NS-H-111 being the second and NS-H-Dukat the third.

In our work, we have found that the dynamics of dry matter accumulation and dry matter distribution between different sunflower plant organs depended on the competition between plants, which started from the budding stage. Dry matter content per plant decreased and dry matter per acreage increased with the increase in stand density. At the initial stages of the dry matter accumulation, leaves had priority in dry matter distribution. At the later stages of the development, that priority was derived to the stem till flowering, and later on to the capitulum. In all tested hybrids, the maximum dry matter accumulation was at the stage of 30 days after flowering. The most intensive period of dry matter accumulation was from budding till flowering stage.

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REFERENCES

Blanchet, R., M. Piquemal, G. Cavalié, M. Hernandez, and H. Quinones. 1988. Influence des contraintes hydriques sur la répartition des assimilats entre les organes du tournesol. p. 124-129 (vol. 1). In: Proc. 12th Int. Sunflower Conf.

De Giorgio, D., D. Ferri, and G. Lopez. 1990. Relazione tra dinamica dellazoto nel sistema pianta-suolo e caratteristiche quanti-qualitative del girasole (*Helianthus annuus* L.) Riv. Agron. 24:257-265.

Hall, A.J., D.M. Whitfield, and D.J. Connor. 1990. Contribution of pre-anthesis assimilates to grain filling in irrigated and water-stressed sunflower crops. Field Crops Res. 24:273-294.

Horie, T. 1977. Simulation of sunflower growth. I. Formulation and parametrization of dry matter production, leaf photosynthesis, respiration and partitioning of photosynthates. Bull. Natl. Inst. Agric. Sci. Ser. A 24:45-70.

Majid, H.S., and A.A. Schneiter. 1988. Semi-dwarf and conventional height sunflower performance at five plant populations. Agron. J. 80:821-824.

Merrien, A. 1986. Cahier technique cetiom tournesol-phisiologie. Ed. Cetiom, p. 1-47.

Sfredo, G.J., J.R. Sarruge, and R.J. Compo. 1985. Dry matter production and macro nutrients concentration in two cultivars of sunflower (*Helianthus annuus* L.) under field conditions. p. 183-188. In: Proc. 11th Int. Sunflower Conf.

Trápani, N., A.J. Hall, and F.J. Villalobos. 1994. Pre-anthesis partitioning of dry matter in sunflower (*Helianthus annuus* L.) crops. Field Crops Res. 37:235-246.

Villalobos, F. J., A. Soriano, and E. Fereres. 1992. Effects of shading on dry matter partitioning and yield of field-grown sunflower. Eur. J. Agron. 1:109-115.

Villalobos, F.J., V.O. Sadras, A. Soriano, and E. Fereres. 1994. Planting density effect on dry matter partitioning and productivity of sunflower hybrids. Field Crops Res. 36:1-11.

Vrebalov, T., Ž. Rajkoviæ, and Bogdanoviæ Darinka 1983. Akumulacija suve materije i usvajanje NPK od strane suncokreta NS-H-26 RM. Zbornik radova Instituta za ratarstvo i povrtarstvo Poljoprivrednog fakulteta Novi Sad 13:91-102.

Wardlaw, I.F. 1990. The control of carbon partitioning in plants. New Phytol. 116:341-381.

IAA/GA_3 quantitative ratio of some sunflower genotypes representing CMS-Rf system

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ABSTRACT

Quantitative ratio of IAA/GA_3 (indole-3-acetic acid/gibberellic acid) was studied during the growth and development of different sunflower genotypes that represent a CMS-Rf system. It has been shown that IAA/GA_3 ratio is variable and depends on ontogenesis stages, organs and genotype. Thus, IAA/GA_3 ratio had maximal values for male sterile line and minimal ratio for restorer line RW637Rf. The highest IAA/GA_3 ratio was registered in cotyledon leaves and subsequently decreased during ontogenesis whereby the hormonal ratio in reproductive stages was higher in inflorescences than in leaves. Gibberellic acid exogenously applied increased the hormonal ratio in the male-fertile line. The specificity of IAA/GA_3 balance in male sterility-fertility phenotype expression and in GA-induced pollen sterility is discussed.

Key words: CMS-Rf system – gibberellic acid – IAA/GA_3 ratio – indole-3-acetic acid – male fertility – male sterility.

INTRODUCTION

Hormonal regulation of plant growth and development including interaction between different classes of hormones remains an important research topic in biology. Plant growth regulators, endogenous or exogenously applied, are involved in male reproductive development, regulating sex differentiation (Ciailahean, 1988) and male (genetic and cytoplasmic) sterility promoting (Luis and Durand, 1978; Kaul, 1988; Rastogi and Sawhney, 1990; Nakajima et al., 1991) at various species. Our previous work has shown that CMS sunflower lines contain lower amounts of gibberellins than fertile genotypes, including homozygote line with Rf genes (Duca et al., 2003). This evidence suggests an auxin and gibberellin interaction in microsporogenesis processes by their quantitative ratio.

It is known that auxin and cytokinin interaction plays a decisive role in cell division and elongation (Inoue et al., 1991), in induction of root and stem development (Jacobsen et al., 1995). Also, the gibberellin and abscisic acid interaction was shown to regulate the beginning of seed germination through gene expression regulation (Collett et al., 2000; Zentella et al., 2002). It is also known that GA induces synthesis and secretion of a number of hydrolytic enzymes in germinating seed endosperm (Muthukrishanan et al., 1984; Jacobsen et al., 1995), and GA activity can be suppressed by abscissic acid (White et al., 2000).

To reveal the functional role of IAA/GA_3 balance in male sterility-fertility phenotype expression, the hormonal quantitative ratio was studied during the growth and development of different sunflower genotypes, representing a CMS-Rf system.

MATERIALS AND METHODS

Plant material

Sunflower plants were cultivated in the experimental field of Moldova State University according to conventional technologies during four years. Two isonuclear lines MB514 and MB514CMS with mitochondrial *orfH522*, RW637Rf with nuclear homozygote restoration nuclear gene *Rf* and hybrid F_1 obtained by cross between these lines (MB 514 CMS x RW637Rf) with restored male fertility (*Rf_*) were chosen for analyses. For comparative studies, SW501CMS was additionally used. Phenocopies method was applied (Duca, 1998).

The treatment with exogenous gibberellic acid (GA_3) solution by plant spray was carried out at the development period of the inflorescence buds. At this stage, prior to the opening of the inflorescence, male meiosis occurs in disc flower anthers (Anaschenco, 1971). Non-GA_3 treated plants (control) were sprayed with distilled water. For assaying non-GA_3 treated plants (control) and GA_3 treated plants, 24 h post-treatment were used.

Chromatographic analysis

The plant material was collected at various vegetative stages that were correlated with development and microsporogenesis (Duca, 1998). Fresh plant material (about 10g) was homogenized and fixed in cold (-20°C) 80% acetone (1:30 ratio) and extracted over-night at 3-5ºC during 24h. After a series of organic extractions and purifications, the extracts were dried in vacuum at 40°C. The residue was dissolved in 0.1 ml N,O-bis(trimethylsilyl)-acetamid with the addition of 0.05 ml of trimethylclorosilan (1%) and then subjected to chromatography.

Quantitative analysis of phytohormones was performed using gas-liquid chromatographic method and indole-3-acetic acid and gibberellic acid (Sigma) as internal standards, as described previously by Cavell et al., 1967 with modifications (Duca et al., 1997).

The chromatograph FRACTOVAP 4200 equipped with a detector of flame ionization, line programs for temperature MOD 410, integrator MEGA SERIES SP 4270, rustproof column (2m x 4mm) with 5% SE-30 DMCS Cromoton W, 60/80 mesh (0.15-0.2 mm) was used for analysis with gas carrier N_2 - 25 ml/min. Air flow was maintained at 300 ml/min, while hydrogen flow was 25 ml/min. The injector temperature was + 210^{0}C, and the detector temperature was +210^{0}C. The phytohormones were determined in the following temperature regime: after injection, the temperature was maintained at 60^{0}C for 4 min, then the temperature rate increase was 12^{0}C/min until the temperature of 220^{0}C was achieved. This temperature was maintained until the end of the analysis. The phytohormones content was expressed in ng per gram of fresh weight (ng/g fwt).

Data are presented as means ± SE (standard errors) of three separate experiments (n = 6 for each experiment) and Student's *t* test ($P< 0.05$and $P<0.09$) was used to determine the statistical significance of differences between genotypes.

RESULTS AND DISCUSSION

In our previous work, it was shown that the IAA and GA_3 content in vegetative and reproductive tissues was variable and depends on ontogenesis stages, organs and genotype (Duca and Port, 2002; Duca et al., 2003). But the ascertained changes of IAA and GA_3 concentrations are insufficient for revealing their functional role in male fertility-sterility phenotype expression. For this purpose, IAA/GA_3 ratio was analyzed in ontogenesis of sunflower plants using CMS-Rf system.

The highest IAA/GA_3 ratio was found in the cotyledon leaves with maximal values higher than 9 (Table 1). But, the RW 637 Rf line, in contrast to the all studied lines, had the minimal hormonal ratio – 7.9 resulting from a higher gibberellins content only, because no obvious genotypic differences in auxin content were observed.

Table 1. IAA/GA_3 ratio in leaves of different sunflower genotypes

Genotype	Stages of plant growth and development				
	Cotyledons	First leaves	Bud developing	Active growth	Blossoming
F_1	9.5	4.5	4.4	4.3	2.5
MB 514	9.7	3.6	3.9	3.5	2.5
MB 514 CMS	9.7	5.9	6.1	4.6	2.9
RW 637 Rf	7.9	3.3	2.9	2.8	3.8
SW 501 CMS	-	6.6	7.4	5.5	3.6

The IAA/GA_3 ratio was twofold decreased during the first true leaves stage and its values subsequently decreased in ontogenesis down to the lowest values (2.5-3.6) ascertained at blossoming stage. Heterozygote hybrid F_1 with restored male fertility and self-pollinate homozygote male fertile lines showed almost similar values of IAA/GA_3 ratio, while male sterile plants showed the highest hormonal ratio (Table 1).

The IAA/GA_3 ratio in inflorescences tissues, as in leaves, showed higher values at male sterile plants, while male fertile genotypes had nearly the same values of IAA/GA_3 ratio (Table 2).

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Table 2. IAA/GA_3 ratio in inflorescences of different sunflower genotypes

Genotype	Reproduction stages		
	Bud developing	Active growth of inflorescence	Blossoming
F_1	4.4	4.9	4.9
MB 514	4.1	4.4	5.0
MB 514 CMS	10.2	11.0	7.7
RW 637 Rf	3.3	3.9	5.1
SW 501 CMS	10.3	10.1	6.9

Significant results were observed for the RW 637 Rf line, which showed the lowest values of this ratio both in the leaves and in the inflorescences, assayed at the stages of bud development and active growth of inflorescence, when flower development and microsporogenesis occurred.

Similar features of the variable ratio were also determined for disc flowers (Table 3). Thus, during the flower development, the values of the studied index decreased in male sterile lines. But in comparison with male fertile genotypes, IAA/GA_3 ratio in sterile flowers was higher in archesporogenesis and sporogenesis phases. Meanwhile, in the following reproduction stage (carpogenesis) almost the same values of IAA/GA_3 ratio were found for all studied genotypes.

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Table 3. IAA/GA_3 ratio in disc flowers of different sunflower genotypes

Genotype	Microsporogenesis stages		
	Arhesporogenesis	Sporogenesis	Carpogenesis
F_1	4.7	5.0	5.1
MB514	4.1	4.8	5.0
MB514 CMS	8.9	6.6	4.6
MB514 + GA_3	6.9	6.0	5.0
RW637 Rf	5.4	5.1	4.5
SW501 CMS	11.7	7.4	4.4

A special interest related to physiological and genetic aspects of this study represents the variation of the IAA/GA_3 ratio at isogenic lines under exogenous gibberellins treatment (Table 4).

Table 4. IAA/GA_3 ratio in plants treated with gibberellins

Lines		Post-treatment period, hours						
		0	24	48	72		96	
					leaves	inflorescence	leaves	inflorescence
MB 514	control	4.5	4.8	4.5	5.1	4.6	4.9	4.6
	+ GA_3		5.4	5.1	5.9	4.9	5.7	4.7
MB514 CMS	control	6.1	5.6	5.3	6.0	4.6	6.6	5.6
	+ GA_3		5.6	5.6	5.7	4.6	5.1	5.2

The GA3 exogenously applied at inflorescence bud developing stage increased the values of IAA/GA_3 ratio in leaves and inflorescences tissues of male fertile lines almost to the level of the values found for MB 514 CMS line. This increasing effect was significant after 72 and 96 hours post-treatment. The revealed variations in hormonal balance were not noticed for CMS plants (Table 4). In spite of these genotypes being considered hormone susceptible, the effect of GA-treatment on endogen IAA/GA_3 ratio was different. Similar effect of ``genotype correction`` to the normal hormonal status under exogenous phytohormone influence was also reported for several hormone metabolism mutants (Fadeeva et al., 1980).

The following analysis of IAA/GA_3 ratio at entire plant level (Fig. 1) provided the information on genetic and physiologic interactions in self-regulation of CMS-Rf system at sunflower (Fig. 1).

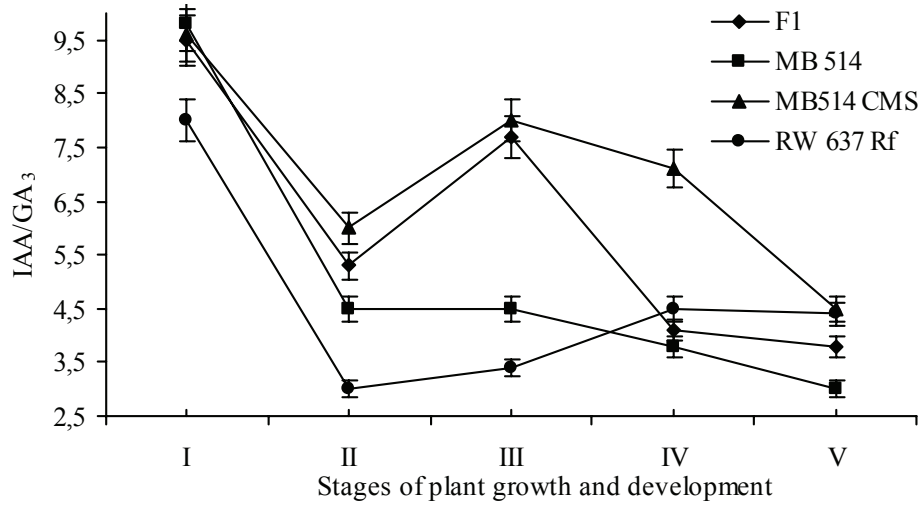

Fig. 1. Hormonal ratio of different sunflower genotypes at following stages of growth and development: I-cotyledons; II-first leaves; III- bud developing; IV- active growth; V – blossoming.

Thus, the most significant differences were revealed at the developing inflorescence bud stage. From the physiological point of view, this stage represents the stage of floral bud evocation and induction, because it was shown that microsporogenesis and microgametogenesis in sunflower occurs prior to the opening of inflorescence, when the diameter of the inflorescence bud reaches 2.5 – 4.5 cm (Smart et al., 1994). A high auxines/gibberellines ratio was ascertained at the stages of bud development (7.9) and active growth of the inflorescence (6.9) for the MB514CMS line, characterized by Srfrf genotype. A significantly lower ratio was observed for RW637 Rf (FRfRf) and MB514 (Frfrf), suggesting that the values of hormonal ratio of the CMS line are much higher than the optimal balance, which, according to our results, is approximately 4 for male fertile genotypes. The hybrid F_1 (SRf) contained sterile cytoplasm with nuclear Rf genes, which restore male fertility in homo- and heterozygote combination, resulting in the normalization of physiologic and biochemical processes in plants (Dmitreva et al., 1971). The fertility restorer gene presence in a genotype of these plants probably resulted in the IAA/GA_3 ratio decreasing at active growth and blossoming stages of reproduction development. Low values of this hormonal ratio are characteristic of fertile genotypes and high values are typical for sterile ones. The hormone ratio alterations observed at the critical stages of reproduction development, especially at microsporogenesis phases, reveal the phytohormonal mechanism of CMS-Rf genetic system control, because in F_1 the ratio of analyzed phytohormones is already restored at the next stage of the growth and development.

The hormonal balance and interactions between various plant hormones, as well as the cell capacity to receive the hormonal signal, play an important role in physiological spatial and temporal regulation of ontogenesis (Egorov et al., 1990; Braedford and Trewavas, 1994; Ross and Neill, 2001).

Our results have revealed the structural changes as a result of different auxins and gibberellins content and their ratio. Therefore, male sterile genotypes are characterized by a high IAA/GA_3 ratio. Also, the GA_3 treatment of fertile plants, resulting in phenotype male sterility, induced the increase in the IAA/GA_3 ratio, caused by the augmentation of endogen auxins and gibberellin amounts with a different intensity, which finally led to a ratio approximately similar to that in male sterile genotypes (Table 4).

It would seem that the hereditary cytoplasmic and GA-induced male sterility can be explained by the change in the phytohormone ratio and not in their concentration. It can be assumed that the phenotypic expression of the morphogenetic program, especially microsporogenesis realization, depends on the IAA/GA_3 ratio. The hormonal balance plays an essential role during the key stage of microsporogenesis (bud development and active growth of inflorescence).

These conclusions are sustained by the reported data. Thus, it was established that IAA/GA_3 regulates the primary differentiation of conductive fascicles, and, if this ratio is high, short phloem fascicles are developed (Roni et al., 1990). Also, it is well known that cytoplasmic and induced male sterility appear at the level of sporophyte tissues, because mononuclear microspores of the tetrads develop

normally up to the stage of binuclear pollen (Simonenko, 1982). This process is characterized by the break of interaction between the anther nests and parenchyma tissues of receptacle and an insufficient supply of nutritive substances (Frenchel, 1982), that finally cause tapetum tissue degeneration and the disruption of pollen formation (Roni et al., 1990).

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REFERENCES

Anaschenco, A.V. 1971. Osobennosti virascivanii podsolnicinica pri himicescoi castrati. Selectia I semenovodsta 2:36-38.

Braedford, K.T., and A.I. Trewavas. 1994. Sensitivity thresholds in plant hormone action. Plant Physiol. 105:1029-1036.

Cavell, B.D., J.M. Millan, R.J. Pryce, and A.S. Sheppard. 1967. Plant Hormones Thin layer and gas-liquid chromatography of the gibberellins: direct identification of the gibberellins in a crude plant extract by gas/liquid chromatography. Phytochem. 6:867-874.

Ciailahean, M.H. 1988. Reguliatia tvetenia vissih rastenii.Moskva, Nauka: 554 p.

Collett, C.E., N.G. Harberd, and O. Leyser. 2000. Hormonal interactions in the control of Arabidopsis hypocotyls elongation. Plant Physiol. 124: 553-561.

Dmitreva,A.H., and M. Ploleacov. 1971. Necotorie biohimiceskoe osobennosti protessov vostonovlenia fertilnisti pilti u pastenii cucuruzi s CMS, In Ghenetica i Selectia na Ucraine, p.1. Kiev: 157p.

Duca, M., 1998. Physiological and genetical aspects of ASC-Rf system at *Helianthus annuus* L. Abstract of Dr.hab. thesis in biology. Chisinau, p.1- 40.

Duca, M.V., and A.I. Port. 2002. Physiological indices variation during different sunflower genotypes ontogenesis (*Helianthus annuus L.*). Romanian AN. I.C.C.P.T. 69:232-243.

Duca, M., G. Duca, and O. Budeanu. 1997. Fitohormones identification method. BOPI. Brevet (MD), Nr. 788.

Duca, M., A. Port, and T. Rotaru. 2003. Influence of diverse factors on the variability in auxin and gibberellin contents in *Helianthus annuus* L. Helia 26: 121-126.

Egorov, I.V., V.I. Sutulova, and I.N Livova. 1990. Izmenenie pola rastenii razlicinih sistematiceschih grupp pod deistviem reguleatorov rosta. p. 73-87. In: Reguleatori rosta rastenii, Moscow, Russia.

Fadeeva, T.S., S.P. Sosnihina, and H.M. Ircaeva. 1980. Sravnitelinaea ghenetica rastenii. Leningradskogo universiteta, Leningrad (St. Petersburg), Russia, 234 p.

Frenchel, P., and A. Galun. 1982. Mehanisma opilenia, rasmnojenia i selectia rastenii. Kolos, Moscow, Russia, 383 p.

Inoue, T., M. Higuchi, Y. Hashimoto, M. Seki, and M. Kobayashi. 2001. Identification of CRE 1 as cytokinin receptor from Arabidopsis. Nature 409:1060-1063.

Jacobsen, J.V., F. Gubler, and P.M. Chandler. 1995. Gibberellin action in germinated cereals. p. 246-271. In: P.J. Davies (ed.), Plant Hormones, Boston, MA, USA.

Kaul, M.L. 1988. Male sterility in higher plants. p. 364-370. In: Monographs on Theoretical and Applied Genetics, vol. 10. Springer-Verlag, New York.

Luis, J.P., and B. Durand. 1978. Studies with dioecious angiosperm *Mercurialis annua* L. (2n=16). Correlation between genetic and cytoplasmic sterility, sex segregation and feminizing hormones (cytokinins). Mol. Gen. Genet. 165:309-322.

Muthukrishanan, S., G.R. Chandra, and E.S. Maxwell. 1984. Hormonal control of a-amylase gene expression in barley. J. Biol. Chem. 258:13637-13639.

Nakajima, M., I. Yamaguchi, S., Kizawa, N. Murofushi, and N. Takahashi. 1991. Semi-quantification of GA_1 and GA_4 in male sterile anthers of rice by radiommunoassay. Plant Cell Physiol. 32: 511-513.

Rastogi, R., and V.K. Sawhney. 1990. Poliamines and flower development in the male sterile stamenless-2 mutant of tomato (*Lycopersion esculentum* Mill.). I. Levels of polyamines and their biosynthesis in normal and mutant flowers. Plant Physiol. 93:493-445.

Roni, A., M. Tollier, and B. Monties. 1990. The role of auxin and gibberellin in controlling lignin formation in primary phloem fibers and in xylem of *Coleus blumei* stems. Plant Physiol. 2:1743-1754.

Ross J. O., and D. Neill. 2001. New interactions between classical plant hormones. Trends Plant Sci. 6:2-4.

Simonenko, V.K., and E.V. Carpovici. 1982. Citologhiceschoie proevlenie raslicinih tipov mujckoi sterilnosyi u podsolnicinica. N.T. Biul. VSTI 34:34-41.

Smart, C.J., M. Francoise, and J. Christopher. 1994. Cell/Specific regulation of gene expression in mitochondria during anther development in sunflower. Plant Cell 6:11-825.

White, C.N., W.M. Proesbsting, P. Hedden, and C.J. Rivin. 2000. Gibberellins and seed development in maize. I. Evidence that gibberellin/abscisic balance governs germination versus maturation pathways. Plant Physiol. 122:1081-1088.

Zentella, R., D. Yamauchi, and T.H.D. Ho. 2002. Molecular dissection of the gibberellin/abscisic acid signaling pathways by transiently expressed RNA interference in barely aleurone cells. Plant Cell 14:2289-2301.

Genetic-phytohormonal interactions in male fertility and male sterility phenotype expression in sunflower (*Helianthus annuus* L.)

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ABSTRACT

Amounts of indole-3-acetic acid (IAA) and gibberellic acid (GA_3) have been investigated in vegetative and reproductive tissues of diverse sunflower genotypes using gas-liquid chromatography. Quantification of endogenous GA_3 content from homozygote MB514 line, characterized by cytoplasmic male sterility (CMS), revealed a lower level in comparison to the fertility restorer RW637Rf line, which contained a higher hormone quantity. The largest amount of IAA was found in the heterozygote F_1 hybrid obtained by crossing these lines, regardless of the tissues and ontogenesis phases analyzed. Similar features were found in leaves, apex, inflorescence, and disc flowers in most of the variants investigated.

Key words: auxins – CMS-Rf system – gibberellins – *Helianthus annuus* L. – male fertility – male sterility.

INTRODUCTION

Genetic CMS-Rf system (*cytoplasmic male sterility – fertility restoration of pollen*) is a well known and versatile phenomenon that has been the subject of many studies due to its importance in commercial hybrid breeding and heterosis (Vrânceanu and Stoenescu, 1971; Voscoboinik, 1977). Besides, this genetic system represents a useful model for revealing nucleus-cytoplasm interaction mechanisms in male sterility-fertility expression. It has been shown that CMS in sunflower is associated with mitochondrial gene *orfH522* (Laver et al., 1991; Horn et al., 1994) that can be suppressed in F_1 hybrids based on CMS by the action of nuclear-encoded fertility restorer Rf genes both in homo – and heterozygous condition (Vrânceanu and Stoenescu, 1971; Anascenco and Duca, 1985). Also, male sterility can be induced by gibberelic acid (GA_3) treatment of plants and the same class of phytohormones restores male fertility to sterile plants (Anascenco, 1971). These phenomena sustain the hypothesis that plant hormones regulate the nucleus and other cell structure activity by the induction or suppression cytoplasmic systems of genes expression (Collett at al., 2000). It is assumed that the phenotype expression of hereditary male fertility/sterility trait is regulated by the phytohormones.

In the present research, the quantification of IAA and GA_3 endogen levels has been studied in several sunflower genotypes during their ontogenesis to reveal the interaction between genetic (nuclear and mitochondrial) factors and phytohormones in CMS–Rf phenotype expression and how these relationships influence the physiological and biochemical basis of microsporogenesis. Also, we studied two functional states of male gametophyte (male sterility/fertility) in the same nuclear context, using the phenocopies method, to obtain information on nuclear influence on the mitochondrial genome expression related to the CMS- Rf genetic system.

MATERIALS AND METHODS

Plant materials

Two sunflower isonuclear lines distinguished only by cytoplasm genes (MB514 and MB514CMS with mitochondrial *orfH522*), the line RW637Rf with nuclear homozygote restoration nuclear gene *Rf*, and the F_1 hybrid obtained by cross between these lines (MB 514 CMS x RW637Rf) with restored male fertility (*Rf_*) were used in this study. The plants were cultivated in the experimental field of Moldova State University according to conventional technologies during four years. Sunflower seeds were kindly provided by SRC ``Magroselect`` (Soroca, Republic of Moldova).

The apex decapitation at two leaves stage is a good way to obtain phenocopies, known as being convenient models for functional activity studies of a gene. As a result of apical domination excluding, two lateral sprigs have grown, one of them was treated with exogenous gibberellic acid. Thus, two inflorescences, fertile and with induced male sterility, were obtained from the same sunflower plant.

Treatment with exogenous gibberellic acid

GA_3 (Sigma) solution was prepared by dissolving GA_3 in the minimal amount of ethanol 96%, and bringing it up to remaining volume in distilled water to make a final concentration of 0.005%. The treatment with GA_3 solution by plant spray was carried out at the developing inflorescence buds period. At this stage, prior to the opening of the inflorescence, male meiosis occurs in disc flower anthers (Anascenco, 1971). Non-GA_3 treated plants (control) were sprayed with distilled water. For assaying non-GA_3 treated plants (control) and GA_3 treated plants, 24 h post-treatment were used.

Phytohormones extraction. The plant materials were collected at various vegetative stages that were correlated with development and microsporogenesis. Phytohormones assays were performed on cotyledons, apex with true 2-3 leaves, inflorescences without bracts, and inflorescence flowers without parenchyma tissues of peduncles. Slicing was performed from radial to head to analyze the anthers at different developmental stages on the single inflorescence. Fresh plant material (about 10g) was harvested in the morning. The samples were homogenized and fixed in cold (-20^{o}C) 80% acetone (1:30 ratio) and extracted over-night at 3-5^{o}C during 24h. After a series of organic extractions and purifications the extracts were dried in vacuum at 40^{o}C. The residue was dissolved in 0.1 ml N,O-bis(trimethylsilyl)-acetamid with addition of 0.05 ml of trimethylclorosilan (1%) and then subjected to chromatography.

Chromatographic analysis. Quantitative analysis of phytohormones was performed using gas-liquid chromatographic method and indole-3-acetic acid and gibberellic acid (Sigma) as internal standards, as described previously by Cavell et al. (1967) with modifications (Duca et al., 1997).

The chromatograph FRACTOVAP 4200 equipped with a detector of flame ionization, line programs for temperature MOD 410, integrator MEGA SERIES SP 4270, rustproof column (2m x 4mm) with 5% SE-30 DMCS Cromoton W, 60/80 mesh (0.15-0.2 mm) was used for analysis with gas carrier N_2 at a flow of 25 ml/min. Air flow was maintained at 300 ml/min, while hydrogen flow was 25 ml/min. The injector temperature was 210^{0}C, the detector temperature was 210^{0}C.

The phytohormones were determined in the following temperature regime: after the injection, the temperature was maintained at 60^{0}C for 4 min, from then on the temperature rate increase was 12^{0}C/min until the temperature of 220^{0}C was achieved. This temperature was maintained until the end of the analysis. The phytohormone content was expressed in ng per gram of fresh weight (ng/g fwt).

Data are presented as means ± SE (standard errors) of three separate experiments (n = 6 for each experiment) and Student's *t* test ($P< 0.05$and $P<0.09$) was used to determine the statistical significance of differences between genotypes.

RESULTS AND DISCUSSION

Plant hormone metabolism and maintaining the levels of hormonal balance in appropriate temporal and spatial patterns influence the intensity, localization, structure and quality of all morphogenetic processes. The pathway of GA biosynthesis and catabolism and their physiological role has been investigated over many years by a variety of approaches, including the application of active GAs, chemical inhibitors of GA biosynthesis, and the analysis of mutants of plants such as maize, pea, and Arabidopsis (Kende and Zeevaart, 1997).

IAA and GA_3 are essential hormones that act synergetically on diverse developmental processes in plants (Ross and O'Neil, 2001); moreover, auxins stimulate the gibberellin biosynthesis (Symoons and Reid, 2002). Based on this information, quantitative analyses of the hormonal balance variation have been performed in some sunflower genotypes including hybrid F_1 and parents lines, during different ontogenesis stages. Our results have shown the quantitative variation of IAA and GA_3 levels depending on the plant tissues, development stages (Duca and Port, 2002) and environmental factors (Duca et al., 2003).

The most interesting data obtained were related to hormone amounts in different sunflower genotypes making up the CMS-Rf genetic system. Thus, hybrid F_1 was shown to contain the highest IAA amount versus RW637Rf, male fertility restorer line, which had the lowest hormone level. These features were found for apex, leaves and inflorescences (Table 1). Hormone levels in roots showed no significant quantitative variations between studied genotypes.

It is known that IAA induces DNA replication. The highest IAA level of F_1 associated with increased mitotic activity (Capatana, 2004) and with other morphological and physiological indices (Duca and Port, 2002) suggests a correlation between IAA amount and heterosis. It is also possible that the low IAA amount at homozygote RW637Rf line is the cause of the reduced height of these plants.

Table 1. IAA amount of different sunflower genotypes, ng/g fwt

Genotype	Plants number: *fertile* / *sterile*	Phenotype Genotype	Ontogenetic phases			
			The first pair of true leaves		Inflorescence bud developing	
			Roots	Apex	Leaves	Inflorescence
F_1	*76* / *0*	fertile S Rf	32.79 ± 0.12	70.98 ± 0.31	62.99 ± 0.64	81.64±0.29
MB 514 CMS	*2* / *58*	sterile S rfrf	30.47 ± 0.24	57.03 ± 0.05	60.15 ± 1.17	77.40±0.83
RW 637 Rf	*78* / *0*	fertile F RfRf	31.71 ± 013	50.41 ± 0.25	54.99 ± 4.70	61.05±1.86
LSD	*0.95*		*0.093*	*0.432*	*0.265*	*0.367*
	0.99		*0.140*	*0.654*	*0.401*	*0.556*

S – male sterile cytoplasm. containing mitochondrial *orfH522*; F – male fertile cytoplasm.

It is important to accentuate that the gibberellin level in all studied tissues and genotypes of sunflower was four-six fold less than IAA, as has been shown for maize (Polevoi, 1992). The highest GA_3 concentration was found in the male fertile genotypes, F_1 hybrid and the RW637Rf line that was distinguished by the increased biosynthesis during ontogenesis (Table 2). The intensity of phytohormones accumulation, expressed by harmonic mean, was also significantly higher for RW637Rf line than for F_1 and MB 514 CMS line (Duca, 1998).

Table 2. Gibberellin content at different sunflower genotypes, ng/g fwt.

Genotype	Plants number: *fertile* / *sterile*	Phenotype Genotype	Ontogenetic phases			
			The first pair of true leaves		Inflorescence bud developing	
			Roots	Apex	Leaves	Inflorescence
F_1	*76* / *0*	fertile S Rf	2.04 ± 0.05	16.94 ± 0.03	18.07 ± 0.21	14,3 ± 0,24
MB 514 CMS	*2* / *58*	sterile S rfrf	0.36 ± 0.03	11.82 ± 0.87	7.21 ± 0.63	9,50 ± 0,42
RW 637 Rf	*78* / *0*	fertile F RfRf	0.97 ± 0.04	17.40 ± 0.20	17.30 ± 1.03	17,40 ± 0,52
LSD	*0.95*		*1.868*	*0.493*	*1.050*	*0.709*
	0.99		*2.828*	*0.747*	*1.590*	*1.074*

S – male sterile cytoplasm. containing mitochondrial *orfH522*; F – male fertile cytoplasm.

The gibberellins level showed maximum values in roots and leaves of heterozygote plants and in apex and inflorescences of homozygote plants, but these differences were not statistically significant, because they are not reliable either for 0.95 nor for 0.99 probability levels.

Isogenic lines and phenocopies of sunflower are a good experimental genetic system for investigation of phytohormones interactions and their role in gene expression. Thus, the IAA level during the ontogenesis of three sunflower lines: MB514, MB514 CMS and MB514 treated with exogenous GA_3 showed lower values in the homozygote line with male sterility than its male fertile analogue, characterized by normal bisexual flowers with fertile pollen (Table 3). As a result of an exogenous hormonal treatment, the microsporogenesis was blocked, this phenomenon being associated with significant increases in IAA amount during the inflorescence buds' developing and active growth stages.

Also, it was found that the nucleic acid level (especially of RNA) and protein biosynthesis was increased (Duca, 1998; Duca and Savca, 1998). But by blossoming phase the auxin content and the above mentioned parameters decreased as their levels became lower than those found at CMS lines (Table 3). At this reproduction stage, CMS plants and those treated with gibberellins displayed abnormally developed anthers and lack of pollen.

Table 3. Auxin content of three isogenic sunflower lines, ng/g fwt.

Genotype	Plants number	Phenotype Genotype	Ontogenetic phases		
			Bud development	Active growth	Blossoming
			Apex Inflorescence	Apex Inflorescence	Apex Inflorescence
MB 514	*60*	fertile	60.57 ± 1.14	61.50 ± 0.92	49,53 ± 2,49
		F rfrf	75.80 ± 1.23	76.90 ± 2.26	85,40 ± 0,28
MB 514 CMS	*58*	sterile	58.53 ± 2.08	59.13 ± 1.16	48,00 ± 2,08
		S rfrf	73.20 ± 2.22	74.10 ± 1.02	85,00 ± 1,41
MB 514 +CA_3	*10*	sterile	60.77 ± 0.94	85.57 ± 1.28	45,50 ± 0,59
		F rfrf	75.57 ± 0.77	88.47 ± 3.21	83,80 ± 0,71
LSD	*0,95*		*0,120*	*0.526*	*0.138*
			0,064	*0.241*	*0.037*
	0,99		*0,182*	*0.797*	*0.210*
			0,097	*0.365*	*0.057*

S – male sterile cytoplasm, containing mitochondrial *orfH522*; F – male fertile cytoplasm

Our results have shown that the maximum GA_3 content was in apex and inflorescence tissues of MB 514 line, and also in plants exogenously treated with GA_3 in contrast to the lowest hormonal content ascertained at the cytoplasmic male sterile analogous MB514 CMS (Table 4).

Table 4. Gibberellins content in three sunflower isogenic lines, ng/g fwt

Genotype	Plants number	Phenotype Genotype	Ontogenetic phases		
			Bud development	Active growth	Blossoming
			Apex Inflorescence	Apex Inflorescence	Apex Inflorescence
MB 514	*60*	fertile	17.90 ± 0.14	18.13 ± 0.47	24.50 ± 0.14
		F rfrf	18.50 ± 0.33	16.26 ± 0.32	18.37 ± 1.18
MB 514 CMS	*58*	sterile	9.50 ± 0.42	12.7 ± 0.19	16.70 ± 0.45
		S rfrf	7.20 ± 0.57	6.70 ± 0.47	12.33 ± 0.43
MB 514 +CA_3	*10*	sterile	17.90 ± 0.09	20.43 ± 0.58	18.40 ± 0.19
		F rfrf	18.50 ± 0.33	16.80 ± 0.19	13.40 ± 0.52
LSD	*0.95*		*0.786*	*0.570*	*0.506*
			1.082	*1.051*	*0.573*
	0.99		*1.190*	*0.863*	*0.766*
			1.693	*1.591*	*0.868*

S – male sterile cytoplasm, containing mitochondrial *orfH522*; F – male fertile cytoplasm

During blossoming stage, the gibberellins quantity of GA_3 treated MB514 line decreased by approximately 30% in comparison to CMS analogue and by 20% compared to untreated MB 514. MB 514 CMS plants had low concentrations of this hormone compared to male fertile plants during all the ontogenetic phases studied. Gibberellins content boost at MB514 fertile line happened during ontogenesis stages, reaching higher levels at blossoming stage (24.5 ng/g fwt). Exogenous gibberellins application changed its internal concentration. Maximum values of endogen IAA and GA_3 content were determined at inflorescence development stage, 24 hours post treatment, and also during active growth phase.

Data on isogenic lines study provided us with more complete information related to auxin-gibberellin regulation of generative differentiation processes in sunflower. A comparative analysis of endogen auxins and gibberellins levels at different microsporogenesis stages (Fig. 1 and 2) revealed that phytohormone concentration decreased in disc flowers from the centre of the inflorescence to the periphery during the microsporogenesis in all studied genotypes.

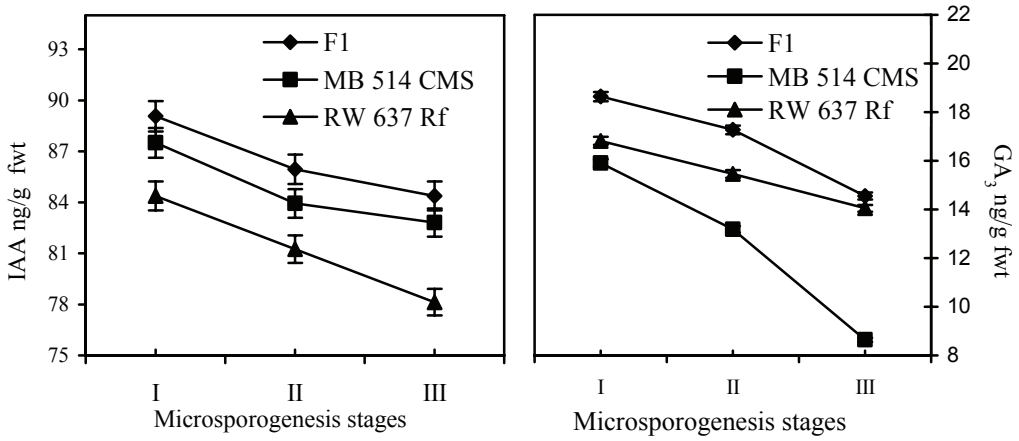

Fig. 1. Phytohormone levels in flowers at various microsporogenesis stages:
I – arhesporogenesis; II – sporogenesis; III - carpogenesis.

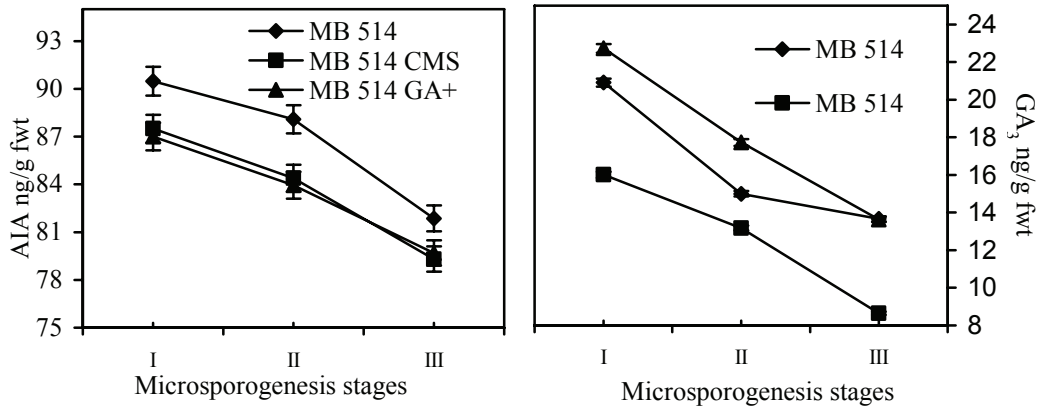

Fig. 2. Differences in phytohormone levels in flowers of three isogenic sunflower lines at following stages of microsporogenesis: I– arhesporogenesis; II – sporogenesis; III - carpogenesis

Our data support the findings related to the higher contents of both hormones in hybrid plants and the lowest IAA level in the male fertility restorer lines, but the lowest GA_3 level in the CMS plants.

Exogenous GA_3 treatment induced a decrease of the IAA concentration in disk flowers. The results obtained have shown no significant differences between plants with the male sterility induced and CMS plants. But the endogen gibberellin content in the treated genotype was higher than those detected in another two isogenic lines.

Besides the complex functional role of the studied parameters, the investigation of the IAA and GA_3 content in different sunflower genotypes during the ontogenesis has revealed several features regarding growth regulators levels and genetic CMS-Rf system. Thus, the outcomes showed a high level of GA_3 at RW 637 Rf line in comparison to other genotypes. MB 514 CMS line contained the lowest level of gibberellins, which increased during all the analyzed phases, even in disc flowers, where for RW 637 Rf line and F_1 a diminution in this hormone quantity was found. In fertile line MB 514 (as in other male fertile genotypes) there was a high auxin and gibberellin content during all studied phases in comparison to its male sterile analogue.

Genotypic peculiarities related to the auxin content were less considerable and less specific than those revealed by gibberellins content, which apparently verified their insignificant functional role in the phenotypic expression of CMS-Rf system. However, it could be supposed that a high gibberellin content is associated with restored male fertility, and a low auxin content with pollen sterility.

Evidence of the requirements of GAs in male reproductive development of flowering plants has resulted from genetic and physiologic studies of GA biosynthesis mutants. Typically, in addition to the dwarf stature, the GA-deficient mutants exhibit various defects of reproduction development (Kende and Zeevaart, 1997). Out of the majority of the plant growth regulators used as gametocydes (Frank et al., 1978), only gibberellins induce male sterility (Anascenco, 1971). These data together suggest that microsporogenesis development occurs normally at a sufficient level of GA. A low level of this hormone in the MB514 CMS line and a high level at RW 637 Rf line (and at all male fertile line) could support the hypothesis proposed. Also, these conclusions are sustained by the reported data that have shown that tomato sl_2 gene mutants (nuclear male sterility) contain a higher IAA and abscissic acid quantity but a lower gibberellin content (Santokh and Sowhneu, 1993).

Thus, it can be concluded that quantitative differences in auxins and gibberellins in various sunflower genotypes have revealed that self-regulation of the CMS-Rf system in sunflower is mediated by endogenous phytohormone concentration, depending on the genotype, ontogenesis phase and organ studied.

REFERENCES

Anascenco, A.V. 1971. Osobennosti virascivanii podsolnicinica pri himicescoi castrati. Selectia I semenovodsta 2:36-38.

Anascenco, A.V., and M.V. Duca. 1985. Izucenie gheneticeskoi sistemi CMS – Rf u podsolinicinica (*Helianthus annuus* L.). Soobscenie 2. Vostonovlenie fertilnosti v CMSr. Ghenetica, 12:1999-2004.

Capatana, A., 2004. The cytogenetic study of different sunflower genotypes. Roumanian Biotechnological Letters (Bucharest) 9:1763-1770.

Cavell, B.D., J.M. Millan, R.J. Pryce, and A.S. Sheppard. 1967. Plant Hormones Thin layer and gas-liquid chromatography of the gibberellins: direct identification of the gibberellins in a crude plant extract by gas/liquid chromatography. Phytochem. 6:867-874.

Collett, C.E., N.G. Harberd, and O. Leyser. 2000. Hormonal interactions in the control of Arabidopsis hypocotyls elongation. Plant Physiol. 124:553-561.

Duca, M. 1998. Physiological and genetical aspects of ASC-Rf system at *Helianthus annuus* L. Abstract of Dr.hab thesis in biology. Chisinau, p. 1- 40.

Duca, M.V., and A.I. Port. 2002. Physiological indices variation during different sunflower genotypes ontogenesis (*Helianthus annuus L.*). Romanian AN. I.C.C.P.T. 69:232-243.

Duca, M., and E. Savca. 1998. Exogenous gibberellins influence on nucleic acids contents at various sunflower genotypes. Moldova State University Anale, Chisinau, 132-135.

Duca, M., Gh. Duca, and O. Budeanu. 1997. Fitohormones identification method. BOPI. Brevet (MD), Nr. 788.

Duca, M., A. Port, and T. Rotaru. 2003. Influence of diverse factors on the variability in auxin and gibberellin contents in *Helianthus annuus* L. Helia 26:121-126.

Frank, J.S., F.S. Koves, and M. Szabo. 1978. Gibberellinel indukalt himsterilitas a napraforgonal.- Novenytermeles 27:487-492.

Horn, R., R. Kohler, and A. Lossl. 1994. Development and molecular analysis of all plasmatic male sterility in sunflower. Adv. Plant Breed. 18:89-110.

Kende, H., and J.A.D. Zeevaart. 1997. The five "classical" plant hormones. Plant Cell, 9:1197-1210.

Laver, H.K., and S.J. Reynolds. 1991. Mitochondrial genome organization and expression associated with cytoplasmic male sterility in sunflower. Plant J. 1:185-193.

Polevoi, V., and A. Polevoi. 1992. Andoghennie fitogormoni etiolirovannih prorostcov cucuruzi. Fiziologhia rastenii 39:1165-1174.

Ross, J., and P.D. O'Neil. 2001. New interactions between classical plant hormones. Trends Plant Sci. 6:2-4.

Santokh, S., and K.V. Sowhneu. 1993. Hormonal regulation of stamen development and male sterility in tomato. Plant Physiol. 1:65.

Symoons, G.M., and J.B. Reid. 2002. Auxin-Gibberellin interactions and their role in plant growth. J. Plant Growth Regul. 20:346-353.

Voscoboinik, L.K. 1977. Selkectia na heterozise v Bolgarii. Biol. NTI po maslicinim culituram. Krasnodar 1:14-20.

Vrânceanu, A.V., and F.M. Stoenescu. 1971. Pollen fertility restore gene from cultivated sunflower (*H. annuus* L.). Euphytica 20:44-55.

Direct and indirect effects of morphophysiological traits on seed yield of sunflower (*Helianthus annuus* L.)

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ABSTRACT

In this paper, we have studied the interdependence of seed yield per plant and the following morphophysiological traits: total leaf number per plant, total leaf area per plant, plant height, total seed number per head, head diameter, weight of 1,000 seeds and oil content. Path coefficient analysis was used to separate direct and indirect effects of studied traits on seed yield, and to identify traits that could be used as selection criteria in sunflower breeding. The research was conducted during two vegetation seasons on 21 experimental sunflower hybrids, produced within the breeding program at the Institute of Field and Vegetable Crops. Among the large number of examined traits, significant and highly significant correlations were found. A strong positive correlation between the weight of 1,000 seeds and seed yield (0.791^{**}) was determined. On the contrary, a strong negative correlation between oil content and seed yield was found (-0.649^{**}). The biggest highly significant positive effect on seed yield was determined for the following traits: the weight of 1,000 seeds (0.789^{**}), total seed number per head (0.473^{**}) and total leaf number per plant (0.199^{**}). Total leaf area per plant has demonstrated a significant direct positive effect on seed yield (0.139^{*}). The weight of 1,000 seeds and total seed number per head were the most important traits for seed yield. Based on the coefficient of determination in F_1 generation (R^2=0.92), it can be concluded that the influence of all traits involved in the study on total variability of seed yield per plant, was 92%.

Key words: correlations – hybrids – path analysis – quantitative traits.

INTRODUCTION

The basic direction in sunflower breeding at the Institute of Field and Vegetable Crops, Novi Sad is the creation of hybrids with high genetic potential for seed yield (above 5t/ha) and the seed oil content (>50%) providing oil yield per hectare over 2.5 t (Miklič et al., 2008).

Breeding for high seed yield, components of seed yield and creating new sunflower ideotypes demands an increase of genetic variability of sunflower by interspecies hybridization (Škorić et al., 2007).

High seed yield and oil content are the two important criteria for introducing new hybrids in the production. These two traits, however, pose problems for breeders because they are both characterized by low heritability and affected by genotype x environment interaction.

In sunflower breeding for productivity, it is important to find morphophysiological traits, which are easy to score and, at the same time demonstrate a causal connection with the seed yield, and, therefore which could be used as selection criteria (Škorić et al., 2002). The mutual connection of seed yield with morphophysiological traits is often studied by the simple correlation coefficient analysis (Škorić, 1974; Marinković, 1992; Hladni, 2006). Since the simple correlation analysis cannot fully explain the relationships between characters, the path coefficient analysis is introduced for more successful breeding work. This type of analysis enables the partition of correlation coefficients to their components, which, in turn, allows the separation of a direct effect of one variable from indirect effects of other variables, thus giving a clear picture of the individual contribution of each variable to the seed yield.

Positive direct effects of total leaf area and plant height (Hladni et al., 2004), total seed per head (Gonzales et al., 2000) and weight of 1,000 seeds (Marinković, 1992; Gonzales et al., 2000) on seed yield were found. However, different results were obtained for the effects of the oil content on the seed yield. Positive direct effects (Chaudhary and Anand, 1993; Razi and Assad, 1999) as well as negative direct effects (Doddamani et al., 1997) of oil content on seed yield per plant were established.

In this paper, we studied mutual relationships between several morphophysiological traits on one side and seed yield on the other, as well as the direct and indirect effects of these components on seed yield of sunflower hybrids of CMS inbred lines originating from interspecies crosses.

MATERIALS AND METHODS

In this research, 21 experimental hybrids, developed by using new divergent (A) CMS inbred lines were used. Female inbred lines (NS-GS-1, NS-GS-2, NS-GS-3, NS-GS-4, NS-GS-5, NS-GS-6, NS-GS-7) developed from interspecies hybridization and restorer inbred lines with good combining characteristics (RHA-R-PL-2/1, RHA-N-49, RUS-RF-OL-168) were created at the Institute of Field and Vegetable Crops, Novi Sad.

The experiment was set up at an experimental field of the Institute of Field and Vegetable Crops at Rimski Šančevi, in a randomized complete block system with three replications, during the period of two vegetation seasons. The soil was characterized by 2.8% humus content, moderate content of phosphorus and potassium and pH 6.92 (Vasin et al., 2002).

The basic sample for analysis of the examined trait consisted of thirty plants (ten plants per replication) sampled from middle rows of each block.

Plants in the flowering stage were transferred to the laboratory and the total leaf number per plant (TLN), as well as the total leaf area per plant (TLA; cm^2/plant) were measured on the leaf area meter (LI-300-LiCOR, USA). At the stage of physiological maturity the plant height (PH) and head diameter (HD) were measured (cm) in the field. After the harvest, the seed yield (SY) produced in free fertilization for every single plant was measured by technical balance in the laboratory. The number of full seeds per head (total seed number-TSN) was determined by counting. On a random sample of completely pure and air dried seed the weight of 1,000 seeds (M1000S) was determined (g). The analysis of oil content (OC) in seed was carried out nondestructively on a nuclear magnetic resonance (NMR) analyzer. The determination of main values and the correlation coefficients (r) was carried out according to Hadživuković (1991). The strength and the direction of the correlation was determined according to the Roemer-Orphalov scale.

Mutual relationships of the examined characteristics and direct and indirect effects on seed yield were analyzed by the path coefficient analysis (Wright, 1921; Dewey and Lu, 1952). Statistical analysis was performed using Mstat C (1991) and SAS System Software (2003) programs.

RESULTS AND DISCUSSION

Knowing the mutual relationships between different yield components as well as the dependence of seed yield on different yield components is an important precondition for a successful application of suitable selection criteria in sunflower breeding. Presence or absence of correlations can contribute to the right choice of examined traits so as to enhance the efficiency of some selection criteria.

Positive highly significant interdependence between SY and M1000S (0.791^{**}), TLA (0.623^{**}), HD (0.446^{**}), TSN (0.369^{**}), is shown in (Table 1). Similar results of highly significant correlations between SY and: M1000S, TSN (Dagustu, 2002; Dušanić et al., 2004), TLA (Merrien et al., 1982; Joksimović et al., 1999; Hladni et al., 2001), and HD (Hladni et al., 2003; Mijić et al., 2006) were obtained by others.

Table 1. Phenotypic coefficient of correlation among analyzed traits

Trait		TLA	PH	HD	TSN	M1000S	OC	SY
		X2	X3	X4	X5	X6	X7	y
TLN	X1	-0.202^{ns}	0.566^{**}	-0.452^{**}	-0.075^{ns}	0.010^{ns}	0.168^{ns}	0.087^{ns}
TLA	X2		-0.161^{ns}	0.602^{**}	0.253^{*}	0.461^{**}	-0.461^{**}	0.623^{**}
PH	X3			-0.544^{**}	0.040^{ns}	0.220^{*}	-0.011^{ns}	0.199^{ns}
HD	X4				0.297^{*}	0.291^{**}	-0.589^{**}	0.446^{**}
TSN	X5					-0.164^{ns}	0.090^{ns}	0.369^{**}
M1000S	X6						-0.786^{**}	0.791^{**}
OC	X7							$-0,649^{**}$

** F test significance at level P<0.01 * F test significance at level P<0.05 ns- not significantly different

X1 total leaf number (TLN)
X2 total leaf area per plant (TLA)
X3 plant height (PH)
X4 head diameter (HD)
X5 Total seed number per head (TSN)
X6 Mass of 1000 seed (M1000S))
X7 Oil content (OC)
Y seed yield per plant (SY)

Highly significant negative interdependence was established between SY and OC (-0.649^{**}), which is in agreement with the research of Doddamani et al. (1997), and in disagreement with the research of Chaudhary and Anand (1993) and Razi et al. (1999).

There was no correlation between TLN and SY, which is in disagreement with the research of Chaudhary and Anand (1993), El-Hosary et al. (1999) and Dagustu (2002), who determined a positive and significant correlation of TLN and SY. Significant positive interdependence was not established

between PH and SY which was detected by others (Marinković, 1992; Hladni i sar., 2003; Mijić et al., 2006).

A positive highly significant interdependence was established between TLN and PH (0.566**); TLA and HD (0.602**); TLA and M1000S (0.461**); HD and M1000S (0.291**). The positive significant connection between TLA and HD was determined by Hladni et al. (2004).

Negative highly significant interdependence was established between M1000S and OC (-0.786**), HD and OC (-0.585**), PH and HD (-0.544**), TLA and OC (-0.461**), TLN and HD (-0.452**). These results are in agreement with the investigations of Punnia and Gill (1994) who determined the negative significant interdependence of M1000S and OC.

The correlation relations were further analyzed by using path coefficient analyses which include the involvement of correlation coefficients in direct and indirect effect on a specific trait (Table 2).

M1000S (0.789**) and TSN (0.473**) have the biggest positive effect on SY, which justifies the existence of a highly significant simple correlation and confirms that these two traits are important components of seed yield. These results are in agreement with the work of Marinković (1992) and Dušanić et al. (2004).

A positive direct influence of TSN on SY was also demonstrated by others (Škorić, 1974; Marinković and Škorić, 1988; Punia and Gill, 1994). A high direct effect of the M1000S on SY was noted both under good water supply conditions as well as under limited water conditions (Razi et al., 1999).

TLN has shown an important direct effect on SY. These results are in agreement with the research of Razi et al. (1999) and Nirmala et al. (2000), but are in disagreement with the results published by Marinković and Škorić (1988).

TLA has shown a positive significant direct effect on seed yield. These results are in agreement with the work by Alba et al. (1979).

Other traits in the investigation have shown a significantly lower direct influence, which means they had an indirect influence through other traits. The direct influence of HD and SY was not significant, which means that the HD had a high and indirect positive influence through M1000S and TSN. Different results were obtained by Green (1980) and Nirmala et al. (2000), who state that HD has a significant direct influence on SY, while according to Škorić (1974), Fick et al. (1974) and Hladni et al. (2004) that influence was negative.

Negative direct influences of OC on SY was not significant which means that OC had a high indirect influence and a negative one through M1000S.

Table 2. Path coefficient analysis of grain yield

Traits	Direct effects	Indirect effects via							r* SY
		TLN	TLA	PH	HD	TSN	M1000S	OC	
TLN	0.1990*		-0.0281	-0.0373	-0.0151	-0.0354	0.0079	-0.0038	0.082
T L A	0.1390*	-0.0402		0.0106	0.0201	0.1196	0.3637	0.0104	0.6232
P H	-0.0659	0.1126	-0.0224		-0.0182	0.0189	0.1736	0.0002	0.1988
H D	0.0334	-0.0899	0.0837	0.0358		0.1404	0.2296	0.0133	0.4463
TS N	0.4726**	-0.0149	0.0352	-0.0026	0.0099		-0.1294	-0.0020	0.3688
M1000S	0.7889**	0.0020	0.0641	-0.0145	0.0097	-0.0775		0.0178	0.7905
OC	-0.0226	0.0334	-0.0641	0.0007	-0.0197	0.0425	-0.6201		-0.6499

r*- Correlation coefficient

Determination coefficient: R^2= 0.918

The differences in acquired results can be explained by different plant material which the authors used in their research.

In sunflower breeding, attention should be paid to the ways in which the increase in morphophysiological components influences the SY.

In this research, with the increase in TLA, HD, M1000S ,SY also increased, but OC decreased. Similary the increase in HD led to the increase in TNL, M1000S and SY, and to the decrease in OC. The increase in TSN and M1000S would cause an increase in SY. In short, the increase in TLN, HD, TSN and M1000S influences the increase in SY.

Path coefficient analysis helped to separate direct and indirect effects of individual traits on SY and identify traits such as M1000S and TSN, which should be used as selection criteria in sunflower breeding.

CONCLUSIONS

A positive highly significant interdependence has been established between seed yield per plant and total leaf area per plant (0.623**), head diameter (0.446**), total seed number per head, (0.369**) and mass of 1000 seed (0.791**). Highly significant negative effect was established between seed yield per plant and oil content (-0.649**).

The path coefficient analysis applied gave a somewhat different picture from what the correlation analysis did. The path coefficient analysis partitioned the direct and indirect effects of the morphophysiological yield components on seed yield of sunflower. It allowed us to detect those components which exhibit the highest effect on yield expression. The data obtained in this investigation, as well as various literature data, indicate that the morphophysiological character: mass of 1000 seed, total seed number per head, total leaf number and total leaf area per plant are the main yield components which should be used as selection criteria in sunflower breeding .

The coefficient of determination (R^2) was 0.92 which indicates that the influence of all traits involved in the study affected 92% of total variability in seed yield per plant.

REFERENCES

Alba, E., A. Benvenuti, R. Tuberosa, and G.O. Vannozzi. 1979. A path coefficient analysis of some yield components in sunflower. Helia 2:25-29.

Chaudhary, S.K., and I.J. Anand. 1993. Correlation and path-coefficient analysis in F_1 and F_2 generations in sunflower (*Helianthus annuus* L.). Int. J. Trop. Agric. 11:204-208.

Dagustu, N. 2002. Correlations and path coefficient analysis of seed yield components in sunflower (*Helianthus annuus* L.). Turkish J. Field Crops 7:15-19.

Dewey, D.R., and K.H. Lu. 1952. A correlation and path–coefficient analysis of components crested wheat grass see production. Agron. J. 51:515-518.

Doddamani, I.K., S.A. Patil, and R.L. Ravikumar. 1997. Relationship of autogamy and self-fertility with seed yield and yield components in sunflower (*Helianthus annuus* L.). Helia 20(26):95-102.

Dušanić, N., V. Miklič, J. Joksimović, and J. Atlagić. 2004. Path coefficient analysis of some yield components of sunflower. p. 531-537 (vol. 2). In: Proc. 16th Int. Sunflower Conf., Fargo, ND, USA.

El-Hosary, A., B. El-Ahmar, and A.E. El-Kasaby. 1999: Assoaciation studies in sunflower. Helia 22:561-567.

Fick, G.N., D.E. Zimmer, and D.C. Zimmerman. 1974. Correlationof seed and oilcontent with other plant and seed characteristics. Crop Sci. 14:755-757.

Gonzales, J., N. Mancuso, and C. Oliva. 2000. Factores genéticos ambientales y correlaciones entre rendimiento y calidad de girasol. p. 105-110 (vol. 2). In: Proc. 15th Int. Sunflower Conf., Toulouse, France.

Green, V.E. 1980. Corelation and path coefficient analysis of the components of yield in sunflower cultivars (*Helianthus annuus* L.). p. 10-12 (vol. 3). In: Proc. 9th Int. Sunflower Conf., Torremolinos, Spain.

Hadživuković, S. 1991. Statistički metodi. Drugo prošireno izdanje. Poljoprivredni fakultet, Novi Sad.

Hladni, N., D. Škorić, and M. Kraljević-Balalić. 2001. Međuzavisnost prinosa i komponenti prinosa kod suncokreta. Zbornik radova sa 1. Međunarodnog Simpozijuma "Hrana u 21. veku", Subotica, Srbija i Crna Gora, 162-167.

Hladni, N., D. Škorić, and M. Kraljević-Balalić. 2003. Components of phenotypic variability for head diameter in sunflower (*Helianthus annuus* L.). Genetika 35:67-75.

Hladni, N., D. Škorić, M. Kraljević-Balalić, M. Ivanović, Z. Sakač, and D. Jovanović. 2004. Correlation of yield components and seed yield per plant in sunflower (*Helianthus annuus* L.). 491-496 (vol. 2). In: Proc. 16th Int. Sunfflower Conf., Fargo, ND, USA.

Hladni, N., D. Škorić, M. Kraljević-Balalić, Z. Sakač, and D. Jovanović. 2006. Combiling ability for oil content and its correlations with other yield componentes in sunflower. Helia 29(44):101-110.

Joksimović, J., J. Atlagić, and D. Škorić. 1999. Path coefficient analysis of some oil yield components in sunflower (*Helianthus annuus* L.). Helia 22(31):35-42.

Marinković, R., and D. Škorić. 1988. Path-coefficient analysis of components of sunflower seed yield (*H. annuus* L.). p. 406-407. In: Proc. 12th Int. Sunflower Conf., Novi Sad, Yugoslavia.

Marinković R. 1992. Path-coeficient analysis of some yield components of sunflower (*Helianthus annuus* L.). Euphytica 60:201-205.

Miklič, V, N. Hladni**,** S. Jocić, R. Marinković, J. Atlagić, D. Saftić-Panković, D. Miladinović, N. Dušanić, and S. Gvozdenović. 2008. Sunflower breeding at institute of field and vegetable crops.

Zbornik radova Instituta za ratarstvo i povrtarstvo, Novi Sad 45(1):31-63.
Mijić, A., M. Krizmanić, V. Guberac, and S. Marić. 2006. Path koeficijent analiza nekih komponenti prinosa ulja suncokreta (*Helianthus annuus* L.). Poljoprivreda, Osijek 12(1):11-15.
Merrien, A., R. Blanchet, N. Gelfi, J.P. Rellier, and M. Rollier. 1982. Voises d elaboration ru rendement chez le tournesol sous differents stress hidriques. p. 1-14 (vol. 2). In: Proc. 10th Int. Sunflower Conf.
MSTAT-C (Version 2.1.Michigan State University 1991)
Nirmala, V.S., A. Gopalan, and D. Sassikumar. 2000. Correlation and path-coefficient analysis in sunflower (*Helianthus annuus* L.). Madras Agric. J. 86:269-272 .
Škorić, D. 1974. Correlation among the most important characters of sunflower in F_1 generation. p. 283-289. In: Proc. 6th Int. Sunflower Conf., Bucharest, Romania.
Škorić, D., R. Marinković, S. Jocić, D. Jovanović, and N. Hladni, N. 2002. Dostignuća i dalji pravci u oplemenjivanju suncokreta i izbor hibrida za setvu u 2002. godini. Zbornik radova Naučnog instituta za ratarstvo i povrtarstvo 36:147-160.
Škorić, D., S. Jocić, N. Hladni, and G.P. Vannozzi. 2007. An Analysis of heterotic potential for agronomically important traits in sunflower (*Helianthus annuus* L.). Helia 30, 46:55-73.
Patil, B.R., M. Rudradhya, C.H.M. Vijayakumar, H. Basappa, and R.S. Kulkarni. 1996. Correlations and path coefficient analysis in sunflower. J. Oilseeds Res. 13:2:162-166.
Punnia, M.S., and H.S. Gill. 1994. Correlations and path coefficient analysis for seed yield traits in sunflower (*Helianthus annuus* L.). Helia 17(20):7-12.
Razi, H., and M.T. Assad. 1999. Comparison of selection criteria in normal and limited irrigtion in sunflower. Euphytica 105:83-90.
SAS Institute Inc. 2003. SAS for Windows (r) 9.1. Cary, NC. USA.
Vasin, J., P. Sekulić, and M. Belić. 2002. Characteristics of the chernozem soil at Rimski Šančevi experiments field of Institute of Field and Vegetable crops. Sesiunea anuala omagiala de comunicari si referate stiintifice " 80 de ani de la nasterea prof. dr. Iulian Dracea", 9:153-160.
Wright, S. 1921. Correlation and causation. J. Agric. Res. 20:557-585.

Determination of maximum achene size in sunflower

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ABSTRACT

It has been suggested that the maximum volume of grain could determine its potential dry weight, and that grain water content can be used as a surrogate of grain volume, but this issue has not been investigated in sunflower. The aim of this work was to examine the relationships between final achene weight and the dynamics of achene volume and achene, pericarp and embryo water contents. Seven experiments were conducted between 2002-6 in Argentina, testing a total of 10 sunflower genotypes. Achenes were collected every 2-3 days, and the fresh and dry weights of the achene, pericarp and embryo, achene dimensions (length, breadth, width) and volume were determined. The pericarp played a dominant role in achene water content throughout grain filling. Maximum achene and pericarp water contents were reached early in development, and these maxima were good predictors of final achene and embryo dry weights across genotypes. Achene water content and volume showed parallel increases between anthesis and peak achene water content, but achene volume and dimensions continued to increase for a few days after the time of peak achene water content. We have concluded that the relationship between final achene dry weight and peak values for achene water content is not causal, and that the strong correlation between these variables arises because of the correlation between achene water content and volume during the phase where both variables increase together and the fact that, at the time of peak achene water content, the achene has reached close to 90% of its final volume.

Key words: achene dimensions – achene size – pericarp – sunflower – water content.

INTRODUCTION

In sunflower, as in other grain species, dry matter accumulation in the achene is related to achene moisture dynamics, and physiological maturity for sunflower is achieved with a grain water concentration of 38% (Rondanini et al., 2007). It has been suggested that maximum grain water content may be used as surrogate for maximum grain volume and that maximum grain volume may limit maximum final grain dry weight (Borrás et al., 2004). The relationship between maximum achene water content and achene final dry weight has not been explored for sunflower. Because pericarp maximum dry weight in sunflower is achieved well before maximum embryo dry weight during grain filling (Mantese et al., 2006), and if the pericarp represents a physical restriction to the growth of whole achene, maximum pericarp volume could determine the final achene size across different sunflower genotypes. The ease (relative to cereal grains) with which pericarp of the growing sunflower achenes can be separated from its contents increases the possibility of studying this issue.

The aim of this work was to relate final sunflower achene weight to the dynamics of achene volume, achene dimensions, and achene, pericarp and embryo water contents.

MATERIALS AND METHODS

Seven experiments were conducted between 2002-6 at FAUBA (Buenos Aires) and the Advanta Semillas Biotechnology Centre (Balcarce). Ten genotypes were evaluated, having contrasting final achene size (30-105 mg achene^{-1}) and pericarp proportion (17-35 %). They included inbred lines (HA89, IM9), white striped hybrids (Paraiso30, M734, CF19, CF29) and black striped hybrids (Paraiso20, VDH488, Aguara, DK4050). Experimental units were individual plants, and each experiment had three replicates. Starting at anthesis, achenes were collected every 2-3 days, and their volume (in genotypes DK4050 and CF29 only), dimensions (length, breadth, width, in genotypes Paraiso30, HA89, DK4050, and CF29 only), fresh weight and dry weight (all genotypes) were determined. Water content in achene, pericarp and embryo was calculated as the difference between the respective fresh and dry weights.

RESULTS AND DISCUSSION

Pericarp and embryo contributions to whole achene water content dynamics

The dynamics of dry and fresh weight, as well as those of water content and concentration for the whole achene, pericarp and embryo exhibited consistent patterns across genotypes and environments, and are illustrated for the genotype CF19 in Fig. 1. Achene and pericarp fresh weight increased rapidly during early grain-filling, to fall after achieving peak values, while embryo fresh weight increased until physiological maturity (Fig. 1B). The pericarp was the dominant component of whole achene water content right up to physiological maturity (Fig. 1C), and exhibited a higher water concentration than the embryo throughout, even after achene water concentration had fallen to harvest maturity values (i.e., ca. 17%, Fig. 1D). Sunflower achene water dynamics (Fig. 1 C) contrast with those of wheat (which show an extended plateau) and soybean (which achieve maximum water content very close to physiological maturity) grains (Egli, 1998).

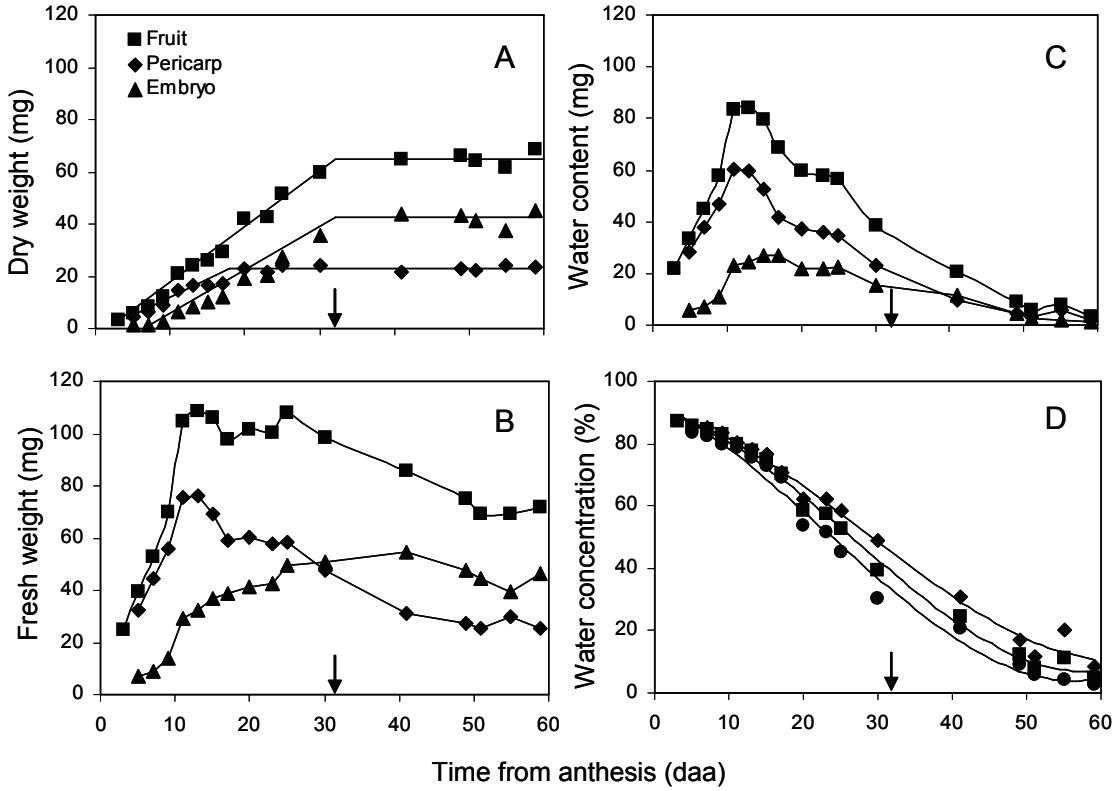

Fig. 1. Post-anthesis dynamics of whole achene, pericarp, and embryo dry weights (A), fresh weights (B), absolute water contents (C), and water concentration (fresh weight basis, D) for achenes from the peripheral position on the capitulum of hybrid CF19 (Exp. 1). Fitted functions are bi-linear (A) and cubic polynomials (D). Arrows on the "x" axis indicate PM. Each datum point is the mean of three replicates.

Averaged across genotypes, maximum pericarp water content was 1.9 times greater than maximum embryo water content, in spite of the fact that average embryo final dry weight was 3 times greater than average pericarp final dry weight (data not shown). Across genotypes, 62-78 %of the water in the achene was contained within the pericarp at the time of whole achene maximum water content (Table 1). This was unexpected, given that the pericarp constituted between 17-35 % of the final achene dry weight and, even at achene maximum water content, embryo dry weight was beginning to approach that of the pericarp.

Recently it has been shown that the achene dry weight/water concentration relationship (in both absolute and relative to maximum dry weight terms) for sunflower can be described using a trilinear relationship, in which the first section (for achene water concentrations in the 90-80% range) has a much steeper slope than the subsequent section (Rondanini et al., 2007). The slopes of the first two sections of the corresponding trilinear relationship for maize show the opposite behaviour (Borrás and Westgate, 2006). In this initial phase of the grain-filling process, most of the water in the achene is found in the pericarp. Thus, pericarp dominance of achene water content dynamics underpins the contrast between the

grain dry weight/water concentration relationships of sunflower and that of other grains like maize (Borrás and Westgate, 2006) and true seeds such as soybean (Egli, 1998).

Table 1. Achene water and dry weight contents at the time of achene maximum water content and proportions (%) of these water and dry matter contents present in the pericarp (P) and embryo (E). Values are means of three replicates.

Genotype	At the time of maximum achene water content					
	Achene water content			Achene dry weight		
	(mg)	Proportion in		(mg)	Proportion in	
		P	E		P	E
P30	140	65	35	30	58	42
M734	133	74	26	31	70	30
CF19	86	72	28	22	67	33
P20	83	75	25	18	71	29
VDH	80	74	26	19	67	33
Aguara	72	73	27	18	67	33
HA89	59	62	38	17	66	34
M734i	60	78	22	14	79	21

Associations between final achene and embryo dry weights and achene and pericarp maximum water contents

Achene final dry weight showed strong associations (r= 0.95) with achene and pericarp maximum water contents (Fig. 2). Given the dominant contribution of the pericarp to achene maximum water content (Table 1), the strong association between final achene dry weight and pericarp maximum water content (Fig. 2), and the fact that maximum pericarp dry and fresh weights are achieved before those of the embryo contained within the pericarp (Fig. 1), we also tested the association between embryo final dry weight and pericarp maximum water content. We found a strong relationship (r= 0.92) between these two variables (Fig. 2). Relationships between achene and pericarp final weights and their respective maximum fresh weights were also strong (data not shown).

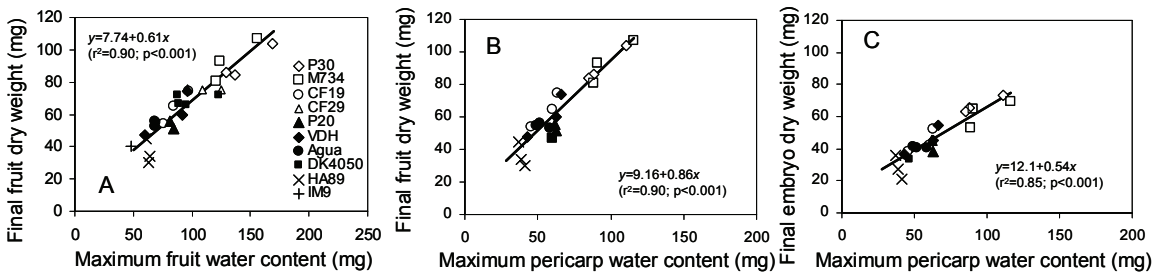

Fig. 2. Relationship between final achene dry weight and achene maximum water content (A), pericarp maximum water content (B), and relationship between final embryo dry weight and maximum pericarp water content (C) for 10 sunflower genotypes.

Dynamics of achene volume and achene dimensions

In the genotypes for which achene water content, volume and dimensions were followed simultaneously, achene water content reached its maximum value early during the development, some time before achievement of maximum achene volume (as illustrated for DK4050 in Fig. 3A). Achene volume peaked at 18 daa (Fig. 3B) and then decreased slightly. Maximum achene length was achieved before the maxima for other dimensions of the achene, whereas maximum achene breadth was determined later during the development, and matched to the timing of maximum achene volume (Fig. 3A and 3B).

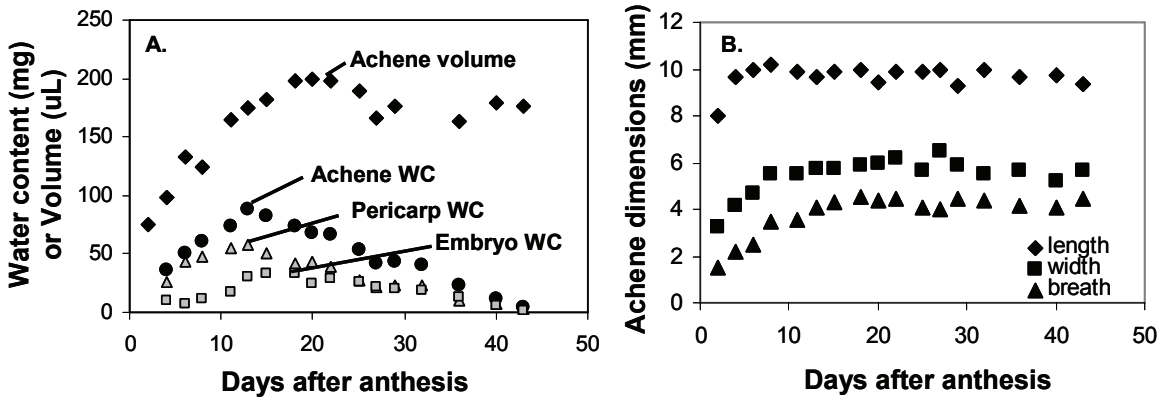

Fig. 3. (A) Dynamics of achene, pericarp and embryo water content (WC) and achene volume; and (B) dynamics of achene dimensions, in sunflower genotype DK 4050.

Plots of the variables shown in Fig. 3 in relative (to the respective maxima) terms against time from anthesis (Fig. 4) showed a strong parallelism for the trajectories of achene relative water content, relative volume and pericarp relative water content. At the time of peak achene and pericarp water contents, achene volume had reached 88-90% of its peak value (Fig. 4A). Achene dimensions showed different trajectories, with achene breadth being the last to achieve its peak value (Fig. 4B), coinciding with peak achene volume.

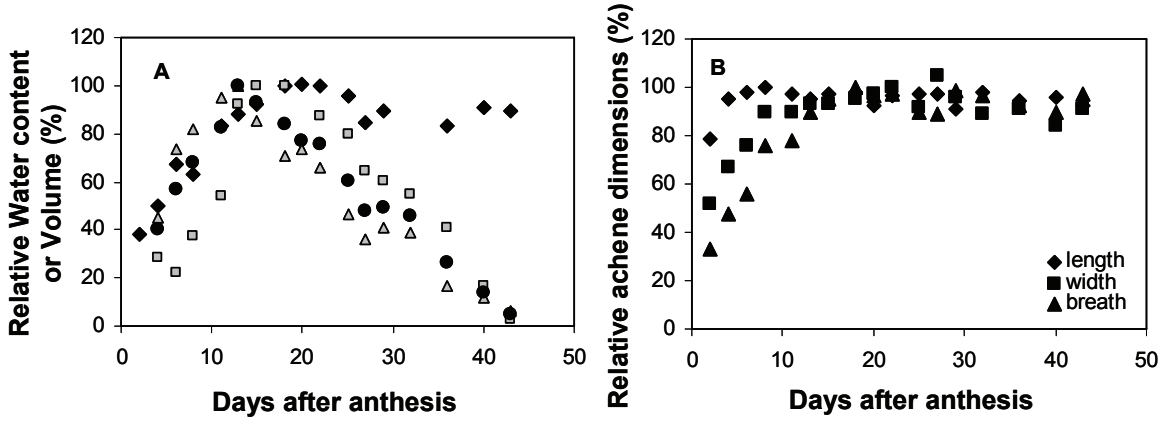

Fig. 4. (A) Relative (to the maximum value for each variable) dynamics of achene, pericarp and embryo water content (symbols as in Fig. 3 A); and (B) achene dimensions. Data for the hybrid DK 4050.

Achene volume and achene water content relationship

During the anthesis (maximum achene water content phase), volume and water content were positively associated (Fig. 5), but the relationship was not 1:1, indicating that achene volume is not all occupied by water.

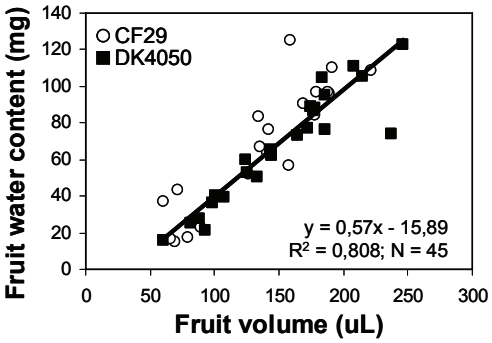

Fig. 5. Achene water content/volume relationship (for the anthesis/maximum WC phase) in two sunflower genotypes.

We conclude that, in sunflower, maximum achene water content is reached early in the development and that pericarp water content dynamics play a dominating role in achene water content dynamics (Fig. 1, Table 1). Differences in grain water content dynamics between sunflower and other grain crops (cereals, soybean) are probably due to a lesser importance of the pericarp in the structure of these latter grains. Because maximum achene water content and volume are determined fairly early during grain filling (e.g., over 60% of peak volume is achieved 10 days after anthesis, Fig. 4), achene capacity to compensate in size for early exposure to adverse environmental conditions later during grain filling may be limited, in contrast to that of other species such as soybean (Egli, 1998). Peak values for both achene and pericarp water content are good predictors of final achene and embryo sizes across genotypes (Fig. 2), However, the association between achene water content and volume (Fig. 5) only holds for the interval during which both variables increase in parallel (Fig. 4), and is clearly not causal, suggesting that not all the fruit volume is filled with water during the first phase of grain-filling. We speculate that the effectiveness of peak achene and pericarp water contents as predictors of final achene dry weight (Fig. 2) arises from the fact that peak values for the former two variables are achieved when achene volume has reached about 90% of its peak value (Fig. 4) and because achene volume is strongly associated with achene water content (Fig. 5). Complete resolution of the determinants of final achene size probably requires a study of the dynamics of pericarp epidermis cell division and expansion and of the contribution which cell destruction on the inner face of the pericarp during grain filling makes to the volume available for embryo growth.

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REFERENCES

Borrás L., and M.E. Westgate. 2006. Predicting maize kernel sink capacity early in development. Field Crops Res. 95:223-233.

Borrás L., G.A. Slafer, and M.E. Otegui. 2004. Seed dry weight response to source-sink manipulations in wheat, maize and soybean: a quantitative reappraisal. Field Crops Res. 86:131-146.

Egli, D.B. 1998. Seed Biology and the Yield of Grain Crops. CAB Int., Wallingford, UK, 178 p.

Mantese A.I., D. Medan, and A.J. Hall. 2006. Achene structure, development and lipid accumulation in sunflower cultivars differing in oil content at maturity. Ann Bot. 97:999-1010.

Rondanini D.P., R. Savin, and A.J. Hall. 2007 Estimation of physiological maturity in sunflower as a function of achene water concentration. Europ. J. Agron. 26:295-309.

Abscisic acid content of a nondormant sunflower (*Helianthus annuus* L.) mutant

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ABSTRACT

A sunflower (*Helianthus annuus* L.) mutant was observed in the progeny of a cross between the sunflower cultivar HA 89 and an amphiploid of a *H. divaricatus* L. × P21 cross that exhibited loss of dormancy induction in the developing embryo. Seeds of this mutant frequently germinate on the head about 40 d after pollination (DAP). In contrast to other nondormant sunflower mutants reported previously, the cotyledons of this mutant remain green, whereas other nondormant mutants exhibit loss of pigmentation. The objective of this investigation was to compare the level of abscisic acid, a plant hormone that induces dormancy in developing embryos, in the nondormant green mutant (*ndg*) and HA 89 from which *ndg* was derived. Immunoassays showed that abscisic acid was present in *ndg* and the levels decreased from a maximum at 5 to 20 DAP to basal levels at 25 DAP. The levels of abscisic acid were not significantly different from those in the control plant HA 89. We conclude that the nondormancy trait is due to a mutation that renders *ndg* insensitive to abscisic acid.

Key words: abscisic acid – *Helianthus annuus* – mutant – nondormancy – sunflower.

INTRODUCTION

Seed dormancy is a physiological strategy evolved by plants to ensure survival of the species. A dormant state prevents germination into a temporarily favorable, but unstable, environment that could become adverse shortly after germination and lead to plant death. Of the several types of dormancy, physiological dormancy is the most common mechanism that has evolved and is present in both gymnosperms and angiosperms. It can occur at a deep, intermediate, or non-deep level (Baskin and Baskin, 2004).

Cultivated sunflower seeds (*Helianthus annuus* L.) undergo a Type 2 non-deep physiological dormancy period (Baskin and Baskin, 2004). In Type 2 dormancy, seeds initially have greater germination potential at higher temperatures, but gradually improve their ability to germinate at lower temperatures during the progression from dormancy to nondormancy. Abscisic acid (ABA) is a known inducer of dormancy in sunflower as it is in many plants (Le Page-Degivry and Garello, 1992). Dormancy in sunflower seed can be broken by application of gibberellic acid or ethylene, by cold stratification, or by excision and culture of the embryo on appropriate medium (Fick, 1978; Corbineau et al., 1990; Jridi et al., 2004).

An albino sunflower mutant, *nd-1*, that exhibited loss of seed dormancy was previously reported by Fambrini et al. (1993). The mutant was found in the selfed progeny of an *in vitro*-regenerated plant and displayed visibly reduced pigmentation by carotenoids. Analysis showed that a defective ζ-carotene desaturase caused the loss of pigmentation (Conti et al., 2004). Because carotenoids are precursors of ABA, altered ABA biosynthesis was likely responsible for nondormancy in *nd-1*.

Within our sunflower germplasm enhancement program, we recently identified a nondormant sunflower mutant that occurred during an interspecific gene transfer from a wild *Helianthus* species into cultivated sunflower to find resistance to the newly evolved broomrape (*Orobanche cumana* Wallr.) race F in Spain. Resistance genes were found in an interspecific cross with the pedigree of *H. divaricatus* 830/P21 amphiploid//P21/2/HA 89. In 1999, a single plant among the sib-pollinated progeny with 2n=34 chromosomes of this pedigree was observed to have seed germinated on the head. The amphiploid *H. divaricatus* 830/P21 has 2n=68 chromosomes; therefore, it took several backcrosses and sib- or self-pollinations to reduce the 2n chromosome number to 34, the same as cultivated sunflower, while continuing to monitor the broomrape resistance. Continued self-pollination maintained the nondormancy trait until F_{14}, and one homozygous F_{14} nondormant line was selected in 2003 for this study.

In this mutant, dormancy was not induced in the developing embryo. Instead, developing seeds of the mutant sunflower began to germinate in the head about 40 d after pollination (DAP). This nondormant mutant differed from *nd-1* in that pigmentation appeared normal. Hence, we use the term *ndg* to describe this *nondormant green* sunflower mutant. Because ABA is known to induce dormancy in physiological dormancy, we investigated the levels of ABA in the developing seeds of *ndg* at various stages after

pollination. When the ABA levels in *ndg* were compared to those in HA 89, which is in the pedigree of *ndg*, we found that the ABA content of *ndg* was not significantly different from HA 89.

MATERIALS AND METHODS

Plant material

Both HA 89 and the mutant *ndg* sunflower were grown in the greenhouse (16-h light) and self-pollinated. After pollination, developing seeds of HA 89 and *ndg* were removed from the head of a single plant at 5, 10, 15, 20, 25, 30, 40, 50, and 60 DAP. At each harvest date, 20 to 40 achenes of *ndg* and 25 to 50 achenes of HA 89 were removed from the head and stored at -80° C prior to ABA analysis.

ABA determination

ABA content was determined in both mutant *ndg* and HA 89 achenes. The frozen achenes from each harvest date were thawed and the hulls separated from the kernels when possible. Both the hulls and kernels were weighed and placed in a desiccator (Moisture Gone desiccant, Hiatt Distributors, Ltd., Long Beach, CA, USA)[1] and allowed to dry overnight. For each sampling date the hulls and kernels were weighed separately and then the hulls or kernels from each were pulverized in liquid nitrogen using a mortar and pestle and extracted with 4 mL of 80% (v/v) aqueous acetone using a Polytron homogenizer. After evaporation of the acetone with a stream of nitrogen and extraction of oil with hexane, the homogenate was diluted with 5 mL of 1 M formic acid and partially purified by separation on an Oasis SepPak (Waters, Milford, MA). The ABA was eluted with 5 mL of methanol and the eluate was evaporated to dryness using a stream of nitrogen. The samples were reconstituted in 1.0 mL of Millipore-purified water. Dilutions of 10× or 100× in Millipore-purified water were used for immunoassay. Samples containing ABA were subjected to quantitative analysis for ABA content by an enzyme-linked immunosorbant assay (Suttle and Hultstrand, 1994; Walker-Simmons, 1987). The (±)ABA used for the standard curve was purchased from Sigma (St. Louis, MO, USA) and the (±)ABA concentrations were doubled for calculation of the physiologically active (+)ABA racemate. (+)ABA concentrations in the embryos and the hulls were expressed as nmol·g dry wt^{-1}. ABA analyses from at least three different plants were conducted for the time points between 5 and 25 DAP, and two ABA determinations were typically made for 30 to 60 DAP. Between 5 and 15 DAP, the ABA content of *ndg* was determined on the whole achene because the embryos were too small to be successfully separated from the hull. From 20 DAP and later, the ABA contents in the embryos and hulls were determined separately.

RESULTS

ABA levels in ndg mutant and HA 89 sunflower

The quantitation of ABA levels at the early stages of seed development showed high variability among samples, likely due to the small size of hulls and embryos and varying stages in initiation of development. Both HA 89 and the *ndg* mutant showed similar patterns of ABA levels in the achenes during the period following pollination (Fig. 1). ABA concentrations in each declined during achene development until basal levels were reached by 30 DAP. We detected a slightly higher ABA concentration in HA 89 compared to *ndg* at 15 DAP, but the differences were otherwise not statistically significant. For both HA 89 and mutant *ndg*, the concentrations of ABA in the hulls and embryos were the same (Fig. 2). By 40 DAP, the developed seeds of *ndg* typically began to germinate on the sunflower head.

DISCUSSION

Sunflower normally undergoes a dormancy period during and following seed development. The length of dormancy is dependent on cultivar and on the environment. Under normal conditions at room temperature, gradual dormancy release for cultivated sunflower typically begins about 45 to 50 DAP (Fick, 1978). For wild sunflower species, the dormancy period is often much longer and highly variable in length.

[1] Mention of trade names or commercial products in this paper is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Several studies on sunflower dormancy have been reported. By inhibiting ABA synthesis with fluridone, Le Page-Degivry et al. (1990) demonstrated that sunflower seed dormancy was dependent on ABA synthesis, but that dormancy induction was not concomitant with ABA accumulation. Their results for developing seeds showed an increase in embryo ABA content that reached a maximum at 13 DAP and then decreased to low levels by 25 DAP. They concluded that ABA induced embryo dormancy during seed maturation.

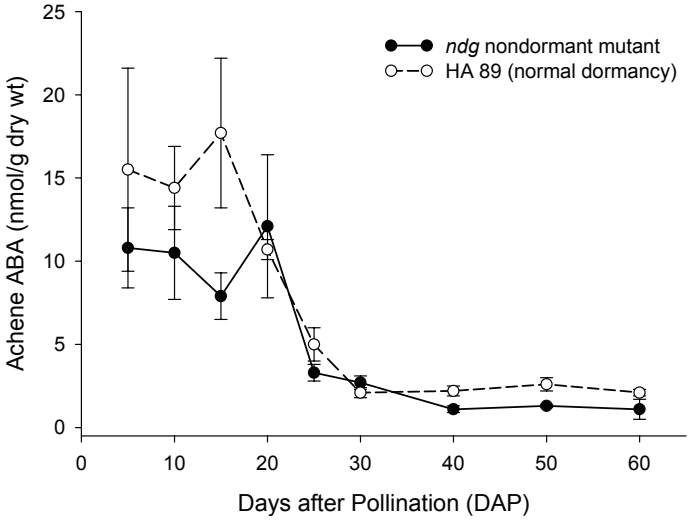

Fig. 1. ABA content (dry weight basis) of achenes of sunflower mutant *ndg* (●) and HA 89 (○) cultivated sunflower at various stages of achene development. Data are means of 3 or more individual plants for 5 to 25 DAP, and of 2 individual plants for 30 to 60 DAP ± SE.

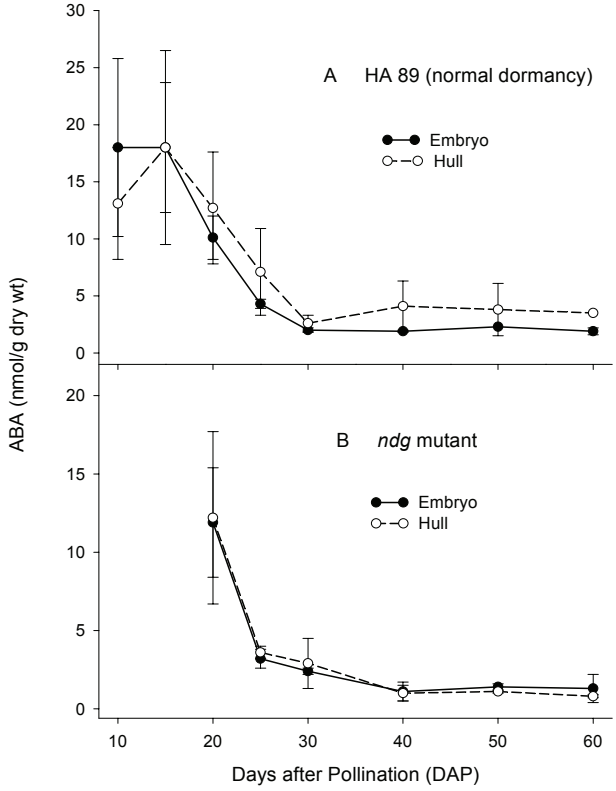

Fig. 2. ABA content (dry weight basis) of embryos (●) and hulls (○) in A) cultivated sunflower HA 89 and B) mutant *ndg* sunflower. Data are means of 3 or more individual plants for 5 to 25 DAP, and of 2 individual plants for 30 to 60 DAP ± SE.

Because expression of total ABA content in an embryo is dependent on the size of the embryo, we avoided using total ABA per embryo. Instead, we expressed ABA content on a dry weight basis of the whole achene so that equivalent comparisons could be made between ABA concentrations in the *ndg* mutant and HA 89. Our results clearly showed the presence of ABA during early achene development in both HA 89 and the *ndg* mutant (Fig. 1). The differences in ABA content between the *ndg* mutant and HA 89 were not statistically significant at most stages of achene development.

We believe that the nondormancy observed in the *ndg* mutant is due to loss of sensitivity to ABA. In our terminology, loss of sensitivity is used in the broad sense to include a defect in any component of the dormancy induction mechanism. A mutation in the ABA receptors that results in reduced affinity to ABA, or a mutation in any of the proteins involved in ABA signal transduction could lead to impaired transcriptional activation of ABA-inducible gene expression. Koornneef et al. (1984) have reported a similar ABA-insensitive mutant (*abi-3*) of *Arabidopsis thaliana* that is green and exhibits nondormancy that also is not reversed by exposure to ABA.

While the synthesis of ABA in the mutant *ndg* embryo appears to be normal, or at least near normal, we cannot rule out the effect of a slightly reduced capacity for ABA synthesis. White et al. (2000) proposed that an adequate ABA:GA ratio is critical for suppression of germination and induction of dormancy, rather than the absolute amounts of the two hormones. In the case of *ndg*, it may be that a slightly reduced content of ABA leads to a ratio shift in favor of GA, and the result is a failure to induce dormancy at a critical time during embryo development. Indeed, Fong et al. (1983), in a study on maize vivipary, proposed that there is a narrow window of embryo development in which ABA is able to induce dormancy. Accelerated catabolism of ABA during after-ripening or a reduced rate of seed desiccation might also result in loss of dormancy.

We did not investigate these alternative possibilities for the observed nondormancy of *ndg*. The aim of this study was to examine whether ABA synthesis was altered in seeds of the nondormant mutant. Our results showed that ABA levels in the mutant *ndg* were the same as in the control line HA 89. Thus, we believe that the *ndg* mutant is defective in the signaling pathway of ABA recognition and subsequent induction of gene expression leading to dormancy.

Introduction of the nondormancy trait into a breeding program could be a useful tool for sunflower breeders. We have determined that nondormancy in the *ndg* mutant is under the control of a dominant gene(s) (unpublished), and if introduced during development of a germplasm line could be a useful tool to advance generations quickly. Use of the nondormancy trait could circumvent the utilization of embryo rescue techniques to avoid the normal dormancy period between germplasm generation advances. Seeds could simply be transferred from the sunflower head directly to large pots without the need for chemical treatment to break dormancy. Finally, the nondormancy trait could be eliminated in the last phase of germplasm line development by selection for segregating lines having normal seed dormancy.

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REFERENCES

Baskin, J. M., and C.C. Baskin. 2004. A classification system for seed dormancy. Seed Sci. Res., 14, 1-16.

Conti, A., S. Pancaldi, M. Fambrini, V. Michelotti, A. Bonora, M. Salvini, and C. Pugliesi, 2004. A deficiency at the gene coding for ζ-carotene desaturase characterizes the sunflower *non dormant-1* mutant. Plant Cell Physiol., 45:445-455.

Corbineau, F., S. Bagniol, and D. Cóme. 1990. Sunflower (*Helianthus annuus* L.) seed dormancy and its regulation by ethylene. Isr. J. Bot., 39:313-325.

Fambrini, M., C. Pugliesi, P. Vernieri, G. Giuliano, and S. Baroncelli. 1993. Characterization of a sunflower (*Helianthus annuus* L.) mutant, deficient in carotenoid synthesis and abscisic-acid content induced by in-vitro tissue culture. Theor. Appl. Genet., 87:65-69.

Fick, G.N. 1978. Breeding and genetics. In: J. F. Carter, (ed.), Sunflower Science and Technology, 279-338. ASA, CSSA, SSSA, Inc., Madison, Wisconsin, USA.

Fong, F., J.D. Smith, and D.E. Koehler. 1983. Early events in maize seed development: 1-methyl-3-phenyl-5-(3-(trifluoromethyl)phenyl)-4-(1H)-pyridinone induction of vivipary. Plant Physiol., 73:899-901.

Jridi, T., H. Belguith, M. Hammami, and J.B. Hamida. 2004. Effect of the gibberellis acid on lipids reserves mobilization during the sunflower (*Helianthus annuus* L.) seeds germination. Rivista Italiana delle Sonstanze Grasse, 81:239-243.

Koornneef, M., G. Reuling, and C.M. Karssen. 1984. The isolation and characterization of abscisic acid-insensitive mutants of *Arabidopsis thaliana*. Physiol. Plant., 61:377-383.

Le Page-Degivry, M.-T., and G. Garello. 1992. *In situ* abscisic acid synthesis. Plant Physiol. 98:1386-1390.

Le Page-Degivry, M.T., P. Barthe, and G. Garello. 1990. Involvement of endogenous abscisic acid in onset and release of *Helianthus annuus* embryo dormancy. Plant Physiol., 92:1164-1168.

Suttle, J.C., and J. F. Hultstrand. 1994. Role of endogenous abscisic acid in potato microtuber dormancy. Plant Physiol., 105:891-896.

Walker-Simmons, M. 1987. ABA levels and sensitivity in developing wheat embryos of sprouting resistant and susceptible cultivars. Plant Physiol., 84:61-66.

White, C.N., W.M. Proebsting, P. Hedden, and C.J. Rivin. 2000. Gibberellins and seed development in maize. I. Evidence that gibberellin/abscisic acid balance governs germination versus maturation pathways. Plant Physiol., 122:1081-1088.

Producción de girasol (*Helianthus annuus* L.) en valles altos de México

Sunflower (*Helianthus annuus* L.) production at México highlands

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RESUMEN

El girasol (*Helianthus annuus* L.), por su uso integral, es un recurso genético potencial para valles altos, donde los cambios en la producción están ligados al manejo del cultivo y las condiciones ambientales. El objetivo de este estudio fue determinar la variación de la fenología, biomasa total, rendimiento de aceite y semilla, contenido de aceite de la semilla y su relación con factores e índices climáticos durante 1992 a 2001. El cv. Victoria de girasol se sembró en Montecillo México (clima BS1 y 2240 m de altitud) bajo condiciones de temporal, a la densidad de 5 plantas m^{-2} en surcos a 80 cm y con orientación este-oeste en las fechas siguientes: (I) 20/5/1992; (II) 01/5/1995; (III) 18/5/1996 ; (IV) 13/5/1997; (V) 01/6/1998; (VI) 26/5/1999; y (VII) 22/5/2001. La fenología mostró cambios debidos a fecha de siembra. El rendimiento de aceite mostró mayor variabilidad (cv=45%), seguido del de semilla (cv=40%), la biomasa total (cv=20%),el contenido de aceite de la semilla (6%) y la radiación fotosintéticamente activa (RFA, cv=8%), la evapotranspiración de cultivo (ETc, cv=6%) y las unidades calor (cv=4%), siendo en promedio de 100 g m^{-2}, 256 g m^{-2}, 1,224 g m^{-2}, 38%, 1,111 MJ m^{-2}, 360 mm, y 1402 UC, respectivamente. Los cambios en la biomasa total pueden estimarse mediante la ETc, y el rendimiento de semilla mediante la ETc, RFA y la humedad relativa mínima.

Palabras clave: biomasa – contenido de aceite - evapotranspiración - rendimiento - unidades calor.

ABSTRACT

The integral use of the sunflower (*Helianthus annuus* L.) is an important potential genetic resource for the highlands, where crop production changes are linked to management practices and environmental conditions. The aim of this study was to determine the phenology, total biomass, seed yield, oil yield and seed oil content of sunflower and their relation with climate factors and indices from 1992 to 2001. The sunflower cv. Victoria was sown in Montecillo Méx (climate BS1 and 2240 m of altitude) in the following dates: (I) 20/5/1992; (II) 01/5/1995; (III) 18/5/1996; (IV) 13/5/1997; (V) 01/6/1998; (VI) 26/5/1999; y (VII) 22/5/2001. The phenology was affected by sowing date. The oil yield variability was the highest (cv=45%), followed by seed yield (cv=40%), total biomass (cv=20%), oil seed (cv=6%), the radiation photosynthetically active (PAR, cv=8%), heat units (cv=4%) and the evapotranspiration (ETc, cv=6%) with mean values of 100 g m^{-2}, 256 g m^{-2}, 1224 g m^{-2}, 38% , 1111 MJ m^{-2}, 1402 UC and 360 mm, respectively. Total biomass changes can be estimated by the ETc, and seed yield by the ETc, PAR and minimum relative humidity.

Key words: biomass – evapotranspiration – heat units – oil seed - yield.

INTRODUCCIÓN

El girasol (*Helianthus annuus* L.) es un recurso genético cuyas semillas son ricas en aceite para consumo humano (como aceites para usos culinarios, elaboración de margarinas y confitería). Tiene también usos industriales (producción de ceras, fosfatina, lecitinas y tocoferoles), ornamentales y para alimento del ganado (Alba y Llanos, 1990) y aves. Además de uso potencial como biocombustible. Se estima que por hectárea se pueden producir 890 L de biodiesel, (Biodiesel Uruguay, 2007; Kavalov y Jensen, 2000). Rodríguez et al. (1998) mencionan el uso de los residuos de girasol aplicados al suelo para el control de la maleza que de manera natural emergen en los cultivos. No obstante el uso integral de la planta y de que las condiciones de clima y suelo no parecen ser limitantes para el girasol en México, su cultivo no es muy extensivo y solo se siembra en algunos estados del país (Baja California, Tamaulipas, Nayarit, Jalisco y Durango) con un rendimiento medio de semilla de 0.8 t ha^{-1} (SAGARPA, 2000), donde el manejo del

cultivo y las condiciones del clima son cruciales para determinar la producción de girasol. Así, el objetivo del presente estudio fue determinar el grado de variación de la fenología, biomasa total, rendimiento de semilla, contenido de aceite y rendimiento de aceite del girasol de temporal en valles altos y su relación con factores e índices del clima durante el período 1992-2001.

MATERIALES Y MÉTODOS

El estudio se realizó en Montecillo Méx., (clima BS1, el menos seco de los áridos y 2,240 m de altitud) donde una reproducción del cv. Victoria se sembró bajo condiciones de temporal, a la densidad de 5 plantas m^{-2} en surcos a 80 cm con orientación este-oeste y fertilización de 100-100-00 de NPK en las fechas siguientes: (I) 20/5/1992; (II) 01/5/1995; (III) 18/5/1996 ; (IV) 13/5/1997; (V) 01/6/1998; (VI) 26/5/1999; y (VII) 22/5/2001 en un suelo Fluvisol mólico (Flm) de textura arcillosa, 2% de materia orgánica, pH 7.5-8.0 y sin problemas de salinidad. En cada año se evaluó la fenología de acuerdo con el criterio de Schneiter y Miller (1981), biomasa total (materia seca, g m^{-2}), rendimiento de semilla (materia seca, g m^{-2}), el índice de cosecha (IC) como una medida de eficiencia de la acumulación de materia seca en la semilla en relación a la total , el contenido de aceite en la semilla (%) mediante resonancia magnética nuclear (Granlund y Zimmerman, 1975), utilizando un analizador modelo MKTIIA (Newport Instruments Bucks G. B.). El rendimiento de aceite se calculó mediante el rendimiento de semilla y el contenido de aceite de la semilla (%), la evapotranspiración del cultivo (ETc) como una medida que estima el requerimiento hídrico para el crecimiento, mediante la evaporación del tanque de tipo A (Ev), utilizando 0.6 como coeficiente de tanque (Kt) y 0.8 como coeficiente de cultivo (Kc) y el planteamiento: Etc= Ev*Kt*Kc (Doorenbos y Pruitt, 1986). Las unidades calor (UC) se calcularon mediante el método residual (Snyder, 1985) tomando como temperatura base 6 ºC. La radiación fotosintéticamente activa (RFA, MJ m^{-2}), la evaporación del tanque tipo A, la temperatura media máxima (Tmáx) y mínima (Tmín), y la humedad relativa máxima (HR máx) y mínima (HR mín) fueron proporcionadas por la estación agrometeorológica del Colegio de Postgraduados. A cada variable en estudio se le determinó la media y el coeficiente de variación (cv %) y un análisis de correlación entre las variables en estudio y regresión múltiple procedimiento paso a paso para determinar el mejor modelo que estime la producción de biomasa y el rendimiento.

RESULTADOS Y DISCUSIÓN

Factores e índices climáticos

En los años de estudio la oscilación de la media estacional de la Tmáx fue de 27.3 y 29.3 ºC; la T mín entre 6.6 y 9.4 ºC; la HR máx entre 93 y 98%, la HRmín entre 32.9 y 44.8%; la ETc fluctuó entre 329 y 393 mm, las UC entre 1353 y 1503 ºC y la RFA entre 1017 y 1259 MJ m^{-2} (Tabla 1).

Tabla 1. Factores e índices del clima durante el desarrollo del girasol (*Helianthus annuus* L.) cv. Victoria durante 1992-2001. Suma y promedio estacional

Experimento	ETc mm ∑	UC ºC ∑	RFA MJm^{-2} ∑	Tmáx ºC	Tmín ºC	HRmáx %	HRmín %
I) 1992	329	1353	1017	28.2	8.4	96.8	41.2
II) 1995	380	1450	1168	28.5	8.5	97.2	40.4
III) 1996	393	1371	1259	27.3	7.5	96.4	34.3
IV) 1997	367	1379	1143	28.5	7.9	95.3	32.9
V) 1998	331	1503	1036	29.3	9.4	93.0	37.5
VI) 1999	358	1374	1076	28.0	6.6	93.5	39.0
VII) 2001	362	1488	1079	28.8	8.0	98.0	44.8
Media	360	1417	1111	28.4	8.0	95.7	38.6
S	24	62	84	0.63	0.87	1.9	4.1
CV (%)	6	4	8	2.2	10.8	2.0	10.6

S=desviación estándar; cv (%)= coeficiente de variación

Fenología, biomasa, índice de cosecha, rendimiento de aceite y sus componentes

La fenología del girasol cv. Victoria mostró cambios entre años de estudio. Así, la emergencia (Ve) ocurrió entre los 7 a 12 días después de la siembra (dds), el inicio de floración (R5) entre los 77 y 84 dds y la madurez fisiológica (R9) entre los 113 y 120 dds (Ve, R5 y R9 son etapas fenológicas; Schneiter y Miller, 1981). En la Tabla 2 se observa que durante el período de estudio, el rendimiento de aceite mostró mayor variabilidad (cv=45%), seguida del rendimiento de semilla (cv=40%) , la biomasa (cv=20%) y el contenido de aceite de la semilla (cv=6%). El índice de cosecha (IC) mostró valores entre 11 y 23% (cv=23%), donde los valores más bajos de IC son indicativos de una posible limitada disponibilidad de agua durante el período reproductivo que generó una menor acumulación de materia seca en la semilla (Escalante, 1999). El promedio de biomasa total, rendimiento de semilla, de aceite y contenido de aceite durante el período de estudio fueron de 1,430 g m-^{2}, 256 g m-^{2}, 100 g m-^{2} y 38%, respectivamente. La más baja variabilidad en el contenido de aceite en la semilla, sugiere que para lograr incrementos en la producción de aceite a nivel de superficie, se requiere buscar incrementos en la acumulación de materia seca en la semilla.

Tabla 2. Biomasa total (g m^{-2}), índice de cosecha (IC), rendimiento de semilla (g m^{-2}),contenido de aceite (%) y rendimiento de aceite (g m-^{2}) en girasol, cv. Victoria, en Montecillo Méx. de 1992 a 2001.

Experimento	Biomasa g m^{-2}	Rendimiento de semilla g m^{-2}	IC (%)	Aceite semilla (%)	Rendimiento de aceite g m^{-2}
(I) 1992	1,050	120	11	36	43
(II) 1995	1,675	336	20	42	141
(III) 1996	1,840	370	20	41	152
(IV) 1997	1,511	352	23	39	137
(V) 1998	1,075	151	14	36	54
(VI) 1999	1,403	271	19	37	100
(VII) 2001	1,456	191	13	37	71
Media	1,430	256	17	38	100
S[1]	290	102	4	2	44
cv (%)	20	40	23	6	45

[1]S=desviación estándar; cv= coeficiente de variación.

Relaciones entre la biomasa, el rendimiento y la ETc, UC y RFA

Los resultados del análisis de correlación presentados en la Tabla 3, indican que la biomasa, el rendimiento de aceite y de semilla presentaron una correlación alta con la ETc y la RFA. Asimismo, el contenido de aceite en la semilla está altamente asociado con los cambios en la producción de biomasa y el rendimiento de semilla, lo que sugiere que la mayor producción de materia seca y de aceite en girasol cv. Victoria estuvieron afectados por la disponibilidad de agua y la capacidad del dosel en la intercepción de la radiación solar (Escalante, 1992).

Tabla 3. Coeficiente de correlación (r) entre la biomasa (Bio), índice de cosecha (IC), rendimiento de semilla (Ren) y aceite (RA), contenido de aceite en la semilla (aceite), evapotranspiración (ETc) y la radiación fotosintéticamente activa (RFA) estacional en girasol cv. Victoria. 1992-2001.

	Bio	Ren	IC	aceite	RA	ETc	RFA
Bio	-------	0.90***	0.73*	0.88**	0.92***	0.99***	0.94***
Ren	0.90**	-------	0.94**	0.86*	0.99***	0.91**	0.91***
IC	0.72*	0.94***	------	0.72*	0.92***	0.73*	0.73*
aceite	0.88***	0.87*	0.72*	-----	0.91**	0.89**	0.89**
RA	0.92***	0.99***	0.92***	0.91***	------	0.92***	0.92**
ETc	0.99***	0.91***	0.73*	0.89**	0.89**	-------	0.94**
RFA	0.95***	0.90**	0.73*	0.89**	0.89**	0.94***	------

*,**,*** Prob <0.05, 0.01, 0.001, respectivamente.

Por otra parte, la acumulación de calor estacional y la producción de biomasa y el rendimiento mostraron una relación cuadrática. Las ecuaciones que describen dichas relaciones son: Biomasa= -25254+356.8 UC-0.125 UC^2; R^2=0.63*; y el Rendimiento=-90898+128.3UC-0.045UC^2; R^2=0.73**.

Dicha relación también fue encontrada en frijol por Escalante et al. (2001) y sugiere la existencia de uno o más factores que limitan una mayor respuesta del girasol al calor acumulado, donde deben de considerarse en primera instancia ajustes en las prácticas de manejo del cultivo.

Modelo para estimar la biomasa , rendimiento de aceite y sus componentes en función de los índices y factores del clima

De los índices y factores del clima que mejor estiman la biomasa y el rendimiento de aceite y sus componentes tenemos a la ETc, RFA y HRmín. Las ecuaciones que mostraron un coeficiente de determinación (R^2) superior a 0.80 se presentan en la tabla 4.

Tabla 4. Modelo para estimar la biomasa, rendimiento de aceite y sus componentes en función de los índices y factores del clima. Selección con base al procedimiento "paso a paso".

Variable	Ecuación	R^2
Biomasa ($g\ m^{-2}$)	Biomasa=-2997.74+12.29 ETc	0.99***
Rendimiento de semilla ($g\ m^{-2}$)	Ren= - 428.2+6.6ETc-1.039RFA-14.38HRmín	0.97***
Indice de cosecha	IC=20.48+0.40ETc-0.95RFA-1.08HRmín	0.95***
Aceite en la semilla (%)	Aceite=9.68+0.025RFA	0.80**
Rendimiento de aceite ($g\ m^{-2}$)	RA=-441.01+0.4869RFA	0.85**

,* P<0.01, 0.001, respectivamente; Ren= rendimiento de semilla; ETc= evapotranspiración estacional; RFA=radiación fotosintéticamente activa; HRmín= humedad relativa mínima; IC=índice de cosecha; RA=rendimiento de aceite.

Finalmente, en concordancia con Dirks y Bolton (1981), este tipo de estudios pueden contribuir al conocimiento de los principales factores ambientales de riesgo para la producción en cada región agrícola, así como apoyar a la generación de modelos de predicción de rendimiento.

CONCLUSIONES

Durante el período de 1992 a 2001 en la región de influencia de Montecillo Méx., la ocurrencia de las etapas fenológicas es variable. Asímismo, la variabilidad del rendimiento de aceite y de semilla es mayor que la de la biomasa. El contenido de aceite de la semilla muestra mayor estabilidad. Los cambios en la biomasa presentan una relación alta con la evapotranspiración, de manera que éste índice puede considerarse como un estimador apropiado de la producción de materia seca total. El rendimiento del girasol cv Victoria puede estimarse en función de la evapotranspiración, radiación fotosintéticamente activa y la humedad relativa mínima.

REFERENCIAS

Alba, O.J., and M. Llanos. 1990. El cultivo del girasol. Agroguías Mundiprensa, Ediciones Mundiprensa. Madrid, Spain. 160 p.

Biodiesel Uruguay. 2007. Rendimiento de cultivos oleaginosos por hectárea aptos para biodiesel (en línea). Consultado 27 octubre 2007. www.ergomix.com.

Dirks, V.A., and E.F. Bolton. 1981. Climatic factors contributing to year to year variation in grain yield of corn on Brookston clay. Can. J. Plant Sci. 61:293-305.

Doorenbos, J., and W.O. Pruitt. 1986. Las necesidades de agua por los cultivos. FAO: Riego y Drenaje. Manual 24. Roma, Italy.

Escalante Estrada, J. 1992. Interacciones entre el nitrógeno y densidad de siembra en el cultivo del girasol (*Helianthus annuus* L.) bajo suministro limitado de agua. Tesis de Doctor Ingeniero agrónomo. Universidad de Cordoba. Córdoba, Spain.

Escalante Estrada, J.A. 1999. Área foliar, senescencia, y rendimiento del girasol de humedad residual en función del nitrógeno. TERRA 17(2):149-157.

Escalante-Estrada, J.A., L.E. Escalante-E. y M.T. Rodríguez. 2001. Producción de frijol en dos épocas de siembra: su relación con la evapotranspiración, unidades calor y radiación solar en clima cálido. TERRA 19(4):309-315.

Granlund, M., and D.C. Zimmerman. 1975. Oil content of sunflower seeds as determined by wideline nuclear magnetic resonance (NMR).Proc. ND Acad. Sci. 27:128-133.

Kavalov, B., and P. Jensen. 2000. Potencial de producción de biocombustibles en los países candidatos a la Unión Europea (en línea). Consultado el 27 octubre 2007. www.jrc.es.

Rodríguez, M.T., J.A. Escalante-E., and L. Aguilar. 1998. Control de maleza con productos de girasol (*Helianthus annuus* L.). p:24-26. In: Memorias del XIX Congreso Nacional de la Ciencia de la Maleza. Mexicali BC. México.

SAGARPA 2000. Anuario estadístico de la producción agrícola de los Estados Unidos Mexicanos. p. 358-359.

Schneiter, A.A., and J. Miller. 1981. Description of sunflower stages. Crop Sci. 21:901-903.

Snyder, R.I. 1985. Hand calculating degree days. Agric For. Meteorol. 35:353-358.

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