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Questions and answers to the challenges in agriculture development

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SUMMARY

Why can't we feed the people of the world? The answer is agricultural research and development Crisis in the World food supply is not one of production but of distribution and that the solution is political. Relevant investment in agricultural research is needed throughout the world in developed and developing countries to maintain food production and to achieve agricultural sustainability. Emerging fields of research such as genomics and nanotechnology, indicate that there will be ample opportunities to devise sustainable food production strategies capable of satisfying the needs of the world's growing populations without placing undo stress on the environment and natural resources. The goal should be to provide farmers in developing countries with the tools that they need to increase crop yields and to market their crops profitably. Agricultural development programs are briefly introduced which carried out in Egypt, Catalonia (Spain) and Uganda supported by University of California (US).

Keywords: agricultural research, food supply, USAID, innovation, Egypt, Catalonia, Uganda, UCI, water resources

CHALLENGES IN AGRICULTURE

Today the entire world is aware that our food supply cannot meet the demands of the world population. How can that be possible? In our high tech world, we can fix every technology, visit Mars, and cure diseases. Why can't we feed the people of the world? The answer is agricultural research and development. It is never complete and rarely current. About the time we think we have all the problems solved, there is a new pest or a new disease or just more people. World leaders and financial institutions tire of hearing about the problems facing farmers and food processors. Haven't we done all that? No we have not; it is a continual process. For the last 20 years support for agriculture has not been a priority. Now it is!

How do we feed the population of the world today? That is the challenge we face. More than a billion people suffer from chronic hunger. On top of that 2 billion people lack adequate or safe drinking water. It is a crisis with devastating and far reaching effects.

World consumption of food crops increases with population growth and with the increased demand for crop use to produce energy.

We are told repeatedly that the crisis in the World food supply is not one of production but of distribution and that the solution is political. Nevertheless, even if structural solutions improve food distribution, world population will soar from 6 billion to 10 billion, or thereabouts, by 2050. This increase in population will necessitate a vast increase in the amount of food produced. At the same time the area of useful agricultural land is shrinking and, in many cases, deteriorating in quality.

To maintain the historical gains in agricultural productivity, scientific knowledge through research must continue to advance. Relevant investment in agricultural research is needed throughout the world in developed and developing countries to maintain food production and to achieve agricultural sustainability.

History has many good reminders how difficult it is to adapt new scientific knowledge. One that impresses me is the very slow acceptance of hybrid corn. Hybrid corn was introduced in Iowa in 1928. It was superior in every way to the seed farmers were using at the time, but it was 1941 before 100% of the Iowa farmers were using hybrid corn.

When it comes to food security, there is both good and bad news. The good news is that, as recent history shows, progress is possible. Between 1990 and 2005, the percentage of people worldwide going to bed hungry each fell four percentage points from 20% to 16. The bad news is that hunger in poor countries remains stubbornly in place.

The progress that has been made in solving the hunger problem should be applauded. Yet, the number of people who continue to lead lives marked by chronic hunger and malnutrition remains shockingly high. Food experts estimate that the figure currently stands at more than 960 million people, including 300 million children. That is more than 16 percent of the global population. Even more ominously, over the past year, the percentage has been moving upwards due to rising food prices.

More often though, the fight against hunger is undermined by mundane yet misguided policies that make the vulnerability of the poor the norm. Take, for example, the recent flurry of global activity to convert millions of hectares of farmland from food to biofuels production. This global 'food-to-fuel' conversion, ignited by a spike in oil prices that made biofuels production both desirable and profitable in many developed countries, ultimately left an additional 30 million poor people without the means to acquire sufficient quantities of food to lead healthy and productive lives. High food prices and declining food supplies sparked protests and social unrest in 38 poor countries.

When we think of problems of hunger, we tend to focus on those individuals without enough to eat. And that is how it should be. Yet, historically, successful campaigns to end hunger have depended on the work of a sufficient number of well-trained agricultural researchers and the presence of adequate numbers of agricultural laboratories and training centres to ensure that first-class research and development can take place.

Agricultural research - the cornerstone of successful agricultural policies in the developing world between the 1960s and 1980s - lost some of its standing following the success of the green revolution. The agricultural research community did not do anything wrong. In fact, there was a prevailing belief that researchers had done their job and that the remaining challenges posed by hunger and malnutrition were largely due to inadequacies in distribution and marketing of products.

For Example, from a Personal Experience in Egypt

By the middle of the 20th century, it had became obvious that Egypt's food supply was in serious trouble. In 1960, for example, Egypt had been almost self-sufficient in wheat production. By 1980, the country was importing about three-fourths of its wheat needs. This alarming gap resulted in increased attention to agriculture. The 1982 U.S. Presidential Mission on Agricultural Development in Egypt focused major attention on the rapidly widening food gap in Egypt, and recommended a number of specific actions to deal with the problem. The recommendations included a 10 year research program lead by USAID called NARP.

During NARP, there was a sharp increase in production, with a distinct slowing in the rate of increase in food utilization. When projected to the year 2000, these changes showed a potential food gap of some 4.5 million tons. This gap was about 17 percent of the projected gap that would have occurred in 2000 based on extrapolations of the trends in 1980.

In 1994, During the assessment team's visit to Egypt, we were asked a very pertinent question by a USAID official: "If Egypt is making all these advances in agricultural production, why is there need to continue support for further research-related activities in agriculture. Isn't this task now done so that we can move on to address other needs?" The simple answer to that question is that research to improve or maintain efficiency and productivity in agriculture is never done-never finished. Today we are learning that lesson again. www.egyptianagriculture.com

Food for the Future

Emerging fields of research such as genomics and nanotechnology, indicate that there will be ample opportunities to devise sustainable food production strategies capable of satisfying the needs of the world's growing populations without placing undo stress on the environment and natural resources. Virtually all of the population growth between now and 2050 - indeed up to 99 percent, according to the Population Reference Bureau - will take place in the world's least developed countries (LDCs). That means a large portion of agricultural research must be directed towards the need of the LDC's.

The main challenge, then, lies in ensuring that the world's poorest countries have access to both conventional and cutting-edge agricultural technologies capable of increasing crop yields. It also requires that farmers in the poorest countries possess the prerequisite knowledge and skills to use these technologies effectively.

Growth in the supply of agricultural commodities is primarily driven by growth in productivity, especially as growth in the availability of land and water resources for agriculture has become more constrained.

Need Investment to at Least Double Food System Productivity

- Make presently unusable soils productive
- Reduce post-harvest loses
- Increase genetic potential (of individual crops and/or farming systems)
- Achieve as much of that potential as possible by:
    1. Improving nutrition of that crop
    2. Increasing water availability and control
    3. Reducing competition from weeds for water, nutrients and sunlight
    4. Reducing losses from disease and insects

The 3 Fronts of Agricultural Development

On the first front, agricultural research communities in the developing world must work more closely with their governments to convince public officials of the enormous value and impact that agricultural research and development have on society.

Successful agricultural policy depends on broad scientific and technological knowledge, and the ability to transfer such knowledge to farmers working in the field. It also depends on putting the right policies in place to ensure that the needs of all stakeholders, and especially those of resource-poor agricultural communities, are incorporated in agricultural research and development programs.

Historically, agriculture has been one of the prime areas of investment for research and development among governments in the developing world. That has certainly been the case in Egypt, which is home to some of the most respected agricultural research centres in the developing world, including Soil, Water, and Environment Institute and the Cotton Research Institute.

Yet over the past decade investments have failed to keep pace with the challenge. Even more ominously, agricultural research institutes in the South have been lagging behind their counterparts in the North. Governments in the developing world, unfortunately but understandably, have focused more attention on other critical concerns - for example, access to safe drinking water and adequate sanitation - which they have come to believe posed a greater threat to their nation's well being.

Boosting agricultural research in the developing world is one key to ensuring food security for the world's poorest (2).

Agricultural Research Potential (3)
Most productivity enhancement potential of Green Revolution technologies already exploited, but biotechnology opens new frontiers:

- Improve nutritional content of grains, etc.
- Increase tolerance to drought, wetness, temperature, salt, aluminum toxicity, (to increase yields and/or planted area under averse or variable conditions)
- Internalize resistance to diseases; viruses
- Reduce pesticide use, esp. insecticides
- Herbicide-resistant varieties
- Slow down product deterioration

On the second front, agricultural research institutions in developed countries must work closely with their counterparts in developing countries. Initiatives should be enacted to facilitate the transfer of cutting-edge agricultural technologies from the North to the South. The goal should be to provide farmers in developing countries with the tools that they need to increase crop yields and to market their crops profitably.

Who sets the agenda for the generation and transfer of new technologies and knowledge has been a common issue in debates dealing with the sharing and transfer of new knowledge, skills and technologies between the North and the South. This is a debate worth having.

Yet, it is important to note that growing North-South gaps in agricultural research are delaying the adaptation of new technologies in the South. And that, in turn, is undermining potential increases in crop yields and placing even more stress on efforts to feed the population.

With effective global policies in place, researchers in developing countries could serve as "transponders" - that is, as knowledge brokers with the skills and resources that are necessary to convey emerging agricultural technologies and state-of-the-art agricultural practices to farmers in the field. Farmers could then apply the knowledge they acquire to expand their crop yields.

Experience has shown that agricultural researchers in developing countries are more likely to have a deeper understanding and appreciation of the challenges farmers in their countries face than agricultural researchers in the North. Equally important, they carry more credibility among local farmers and therefore can have a greater impact on what happens on the ground.

The problem is that, increasingly, researchers in developing countries do not have sufficient knowledge of the emerging technologies to convey that information to the farmers. That is where Northern researchers and agricultural institutions can come into play.

This new agricultural research paradigm is based on North-South-South collaboration - a process of information exchange that moves from researchers in developed countries to researchers in developing countries to farmers in developing countries.

But there are three fronts at work in agriculture in the developing world, and we must do all that we can to close all three:

- First, there is the North-North front in agricultural technology and extension.
- Second there is the North-South front in capacity in agricultural research and development.
- Third there is the South-South front between research and application between scientists and farmers in developing countries.

We need to quickly narrow all three gaps if we hope to achieve a more equitable and peaceful world: A world in which all people have access to sufficient quantities of nutritious food, and a world in which hunger is a thing of the past.

California/Catalunya Cooperation Aims at the Third Front

Cooperative research between countries of the North enables scientists to take advantage of different environments and populations; to share facilities and to better understand the problems facing various regions of the world. It is a UC philosophy that the results from research must be extended to the people that can and will benefit from the new knowledge. Such development also improves the capability of both countries to meet the needs of countries of the south.

In 1995 the governments of Catalunya and California created a program to reinforce the ties between the 2 states in academic, scientific, technological and economic fields to consolidate the relationship between academic research and business organizations.

Student exchanges, joint faculty research projects, business ventures, conferences and individual consultations have expanded the mutual interests of the two regions.

Education Abroad

In a reciprocal manner the University of California sends about 500 undergraduate students to Spain each year through the Education Abroad Program (EAP). The EAP is the University's primary outreach to the international community. It offers UC students access to 6 locations in Spain. Other areas of the Mediterranean that have UC students include France, Italy, Turkey, Egypt and Israel.

Graduate Studies in Engineering

The School of Engineering at UC Irvine offers a scholarship program for the most talented young engineers and scientists from Catalonia in their pursuit of post-graduate degrees (M.S. and Ph.D.) and post-doctoral studies.

UCI is a major research campus of UC offering graduate student opportunities in a wide range of traditional academic disciplines and several programs unique to the campus. The campus provides access to the high-technology industry of Orange County and unparalleled cultural and recreational opportunities.

The School of Engineering offers graduate degree programs in Biochemical Engineering, Chemical Engineering, Civil Engineering, Computer Engineering, Electrical Engineering, Environmental Engineering, Mechanical and Aerospace Engineering and Materials Science and Engineering. In 1995, thanks to a generous endowment from Mr. Pete Balsells, Sr. and his family, the School of Engineering at the University of California, Irvine (UCI) established the Balsells Fellowship program. The Generalitat de Catalunya, through the Departament d'Universitats, Recerca i Societat de la Informació(DURSI) became an essential partner of the program.

The program's main goal is to prepare the most talented young engineers and scientists from Catalonia in their pursuit of post-graduate degrees (M.S. and Ph.D.) and post-doctoral studies in engineering. The program thus promotes the scientific and technological advancement of Catalonia and strengthens the collaborations between UCI and California on the one hand and Catalonia and its university system on the other.

For details please contact Professor R. H. Rangel, Director, Balsells Fellowship, University of California, Irvine.rhrangel@uci.edu; http://Balsells.eng.uci.edu

Engineering Innovation between Academia and Industry

The California-Catalonia Program for Engineering Innovation sponsors research partnerships between academia and industry involving both California and Catalonia. This program builds on the existing strength of the Balsells-Generalitat Fellowship program at the Irvine campus of the University of California. The California-Catalonia Engineering Innovation Program sponsors research partnerships between academia and industry involving both California and Catalonia. The research should explore new approaches to support and sustain innovations in the translation of research into results that benefit both California and Catalonia. The long term goal of the program is to stimulate the transformation of knowledge created at the academic level into innovations that create new wealth and build strong local, regional and inter-related national economies.

Development of Agriculture

The collaboration between the University of California and Catalunya in Agriculture is based in the Institut de Recerca i Tecnologia Agroalimentàries (IRTA) and dates back to 1986.

Over the years, this collaboration has resulted in many types of activities including scientific visits, training programs, sabbaticals, joint seminars and business contacts. Since 1988, over 40 visits have been made in either direction, over thirty doctoral and postdoctoral students have received training, eight sabbaticals and eight joint seminars have been held, and business trips and other initiatives have been organized.

Of the over one hundred activities that have taken place, sixty-one have been held in California and forty-three in Catalonia. Moreover, twenty-eight scientific articles have been published in collaboration between researchers from the University of California and IRTA, and various joint contributions have been presented in publications and at specialized conferences. The most significant areas of collaboration have involved biotechnology, although other contacts and activities have also taken place in fields including veterinary sciences, animal production and food technology. The agreement has gone beyond a purely scientific and technical collaboration based on student exchanges or postdoctoral training to encompass technological and business partnerships.

Over the years, a considerable number of business trips have been made in either direction, some of which have resulted in agreements between firms from California and Catalonia. The collaboration has led to the creation of the IRTA antenna in California. This antenna consists of the presence at University of California Davis of senior IRTA researcher Dr. Lluís Pérez Grau and the granting of different spaces at the Department of Plant Pathology thanks, in part, to the eminent plant virologist Dr. George Bruening. From his post, Dr. Pérez

Grau conducts research projects and helps to foster academic collaborations between Catalonia and California. http://www.IRTA.es

Water Resources, Conflict and Management

UNESCO reports that 1.2 billion people have no access to drinking water and 2.4 billion are deprived of water purification services. Fresh water must be recognized as a common good and the international community must prevent conflict over water allocation.

The Bank of America and the University of California have established a major international forum on water policy, which focuses on reducing water-related conflicts while encouraging environmental protection and economic growth. It is called the *Rosenberg International Forum on Water Policy* in honor of retired Bank of America Chairman and CEO Richard M. Rosenberg, who has played a significant role in mediating water issues in California.

The Rosenberg Forum was established with an initial grant from Bank of America and is permanently managed by the University's Division of Agriculture and Natural Resources. The Rosenberg Forum sponsors a major international conference of invited scholars and policy-makers every two years on water policy issues of global significance.

"Water resources are becoming increasingly scarce worldwide," said Henry J. Vaux, Jr., a University of California water expert and the forum's principal organizer. "We can no longer tolerate the fact that a billion people do not have access to safe drinking water and more than twice that many have no access to adequate sanitation services. Furthermore, we must plan now how to use water resources more wisely to feed the world's growing population while protecting the environment." http://rosenberg.ucanr.org/

New Challenge on the Horizon-A Changing World

Land Purchases

The issues of research and production of food may have been the easy issues. Now there is a new challenge on the horizon. The buying and developing of land in Africa for Agriculture by world corporations.

Food now rivals oil as a basis of power and economic security. Arable land has become the latest target for international investors, with more than 90 funds invested directly in farmland.

Over the next 40 years the world's population is projected to grow from 6 billion to 9 billion, doubling demand, while arable land and water become scarcer. As a result, the cost of farmland keeps rising.

With the current credit crunch, large companies are investing in farmland as a means of control over future food supplies when food security could become a major concern.

In June a Global Ag Investing 2009 Conference, held in New York, aimed at investors eager for opportunities to invest in agricultural lands, commodities and infrastructure. It brought together top players from the global agricultural and investing industries, including Soyatech, Altima Partners, Bayer CropScience, Brazil AgroLogic, DuPont, Rabobank and the World Bank. The participating firms own and/or manage over 11 million acres of productive farmland worldwide.

The International Food Policy Research Institute reports that 37 million to 49 million acres of land in poor countries, valued at $20 to $30 billion, were sold or under negotiation for sale to foreign buyers since 2006.

Another significant difference is the scale of these purchases. A "big land deal" used to be 240,000 acres. Now the largest ones are many times that size.

The investment firm Blackrock has set up a $200 million hedge fund to invest in land. Dow Chemical has invested its pension funds in farmland futures. Morgan Stanley bought nearly 100,000 acres of Brazilian farmland.

Multibillionaire George Soros is getting into the global land-buying business. Jim Rogers Jr., Soros' partner at the Quantum Fund, is involved with two farmland investment funds–Agrifirm and Agcaptia Farmland Investment Partnership. "I'm convinced that farmland is going to be one of the best investments of our time," Rogers told ContrarianProfits. (July 27)

Susan Payne, a British woman, is the CEO of the largest land fund in southern Africa, which currently includes 150,000 hectares (370,000 acres), mainly in South Africa, Zambia and Mozambique. Payne hopes to raise half a billion euros from investors. She talks about fighting hunger, but the headings on her PowerPoint slides, embellished with photos of soybean fields at sunset, tell a different story. One such heading refers to "Africa—the last frontier for finding alpha." The word alpha signifies an investment for which the return is greater than the risk. Africa is alpha country.

That's because land, which is extremely fertile in some regions, is inexpensive on the impoverished continent. Payne's land fund pays $350-500 per hectare ($140-200 per acre) in Zambia, about a tenth the price of land in Argentina or the United States. For a small farmer in Africa, the average yield per hectare has remained unchanged in 40 years. With a little fertilizer and additional irrigation, yields could quadruple - and so could profits.

UGANDA hydroelectric dam

Sithe and the Aga Khan Group, a private international development organization, have joined to build a huge hydroelectric dam in Jinja, Uganda, which is expected to cost $860 million. The project is one of the largest

infrastructure projects in Africa. It's also one of the largest private foreign investments on the continent. Uganda's government is counting on it to help it address an energy shortage that has stifled development in this country, where rolling blackouts are a recurring nuisance.

"Instead of talking to USAID, I'd rather be talking to a company like Nike," Mr. Barnes said. "Having a partner like that means jobs and economic growth, and you just don't get that from aid."

Foreign investments in agriculture are not new, but today they are more strategic than commercial, with many transactions intended to insulate the foreign investor's home country from future global food and energy crises. What happens to the local farmers and citizens? Who will be responsible for soil maintenance and overall environmental issues? We thought our challenge was to feed the world by improvements in water supply and food productivity!

One thing is for sure. We never run out of new challenges. You will always wake up to a new opportunity. Take advantage of your years as students and faculty to learn and question and challenge.

The work is never done; it is never easy but it is always fascinating.

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Botrytis cinerea group I isolates from different hosts in Hungary

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SUMMARY

Botrytis cinerea has been reported as a species complex containing two cryptic species, Groups I and II. Thirteen Group I B. cinerea isolates has been collected from rape and strawberry in Hungary during 2008. The identification of Group I strains were carried out with both the sequence analysis of β-tubulin gene fragment and the PCR-RFLP analysis of Bc-hch gene. Regarding to the temporal distribution, Group I isolates were present mainly at the beginning of the season. Based on the presence and/or absence of two transposons, Boty and Flipper, two transposon types were detected in Hungarian Group I strains: boty only and containing both (transposa).

Keywords: *Botrytis cinerea* Group I, β-tubulin, transposon, PCR-RFLP

INTRODUCTION

Botrytis cinerea (anamorph of *Botryotinia fuckeliana*) causes gray mold on a high number of crop plants including strawberry and rape. However, *B. cinerea* was proposed to be a species complex (Giraud *et al.,* 1997, 1999; Albertini *et al.,* 2002, Munoz *et al.,* 2002; Fournier *et al.,* 2003). Initially, two sympatric sibling species or transposon types were described: 1) *transposa* that contained two transposons *Boty* and *Flipper* and 2) *vacuma* which contained no transposons (Diolez *et al.*, 1995; Levis *et al*., 1997; Giraud *et al*., 1997). Recently, Fournier *et al*. (2005) showed that genetic differentiation determined from multiple gene sequences (genealogical concordance of the phylogenetic species recognition) was not concordant with either of the previously described transposon types (*transposa* or *vacuma*) and revised partitioning of *B. cinerea* into Group I and Group II phylogenetic cryptic species. Diagnostic molecular markers for these groups have been developed based on difference of digestion with *Hha I* restriction endonuclease of amplified *Bc-hch* gene (Albertini *et al.,* 2002; Fournier *et al.,* 2003). To date, *vacuma* transposon type has been detected within Group I and all transposon types (*vacuma*, *transposa*, *flipper*-only, and *boty*-only) have been detected in Group II (Fournier *et al.,* 2005, Ma and Michailides, 2005, Váczy *et al.*, 2008). *Botrytis cinerea* Group II has shown to be the predominant cryptic species however (Fournier *et al.,* 2005, Issenegger *et al*., 2008; Karchani-Balma *et al*., 2008; Váczy *et al*., 2008).

MATERIALS AND METHODS

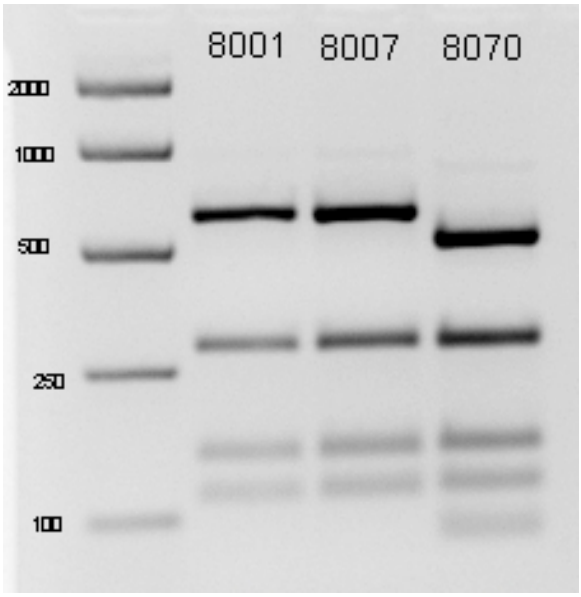
Strains of *B. cinerea* were isolated from infected plants in 2008. Single-spore isolates were prepared for DNA isolation. Dot-blot analysis was used for the detection of transposable elements. Purified PCR products were subjected to automatic sequencing at MWG-Biotech AG, Ebersberg, Germany.

RESULTS AND DISCUSSION

More than two hundred *B. cinerea* isolates were collected from infected rape, strawberry and raspberry in Hungary in 2008.

The electrophoresis profile of amplified *Bc-hch* fragment digested with *Hha I* restriction enzyme resulted two restriction patterns, differing in the size of the upper band, 601 bp for Group I and 517 bp for Group II, strains. The restriction enzyme *Hha* I had 5 restriction sites in the *Bc-hch 2* allele in Group II, whereas the *Bc-hch1* allele had only 4 in Group I strains. The restriction site in position 367 had mutated in the *Bc-hch* allele (Fournier *et al*., 2003), this was revealed by a 600 bp band detected in the DNA of strains from Group I (*Fig. 1*). The presence of 601 bp fragments of the *Bc-hch* locus PCR-RFLP profile showed 13 strains (5%) belonged to the Group I *B. cinerea* cryptic species. The remaining 239 isolates belonged to Group II *B. cinerea* (Fournier *et al.,* 2003) the most common cryptic species reported in *B. cinerea*. In contrast Group I *B. cinerea*, has so far only been found at low frequencies in France and England (Fournier *et al.,* 2003; Martinez *et al.*, 2005).

Figure 1: **Electrophoresis on agarose gel showing the result of the digestion of *Bc-hch* gene with *Hha I* restriction enzyme following PCR amplification of *Botrytis cinerea* Group I and Group II strains**



Left side: molecule marker from 2000 to 100 base pair length 8001; 8007: Group I; 8070: Group II strain

The β-tubulin sequences of *B. cinerea* isolates also resulted different patterns for Group I and II strains with fixed nucleotide differences (*Fig 2.*). The β-tubulin sequence analysis supported the results of *Bc-hch* PCR-RFLP results, identifying the same isolates as Group I cryptic species (*Table 1*).

Figure 2: **Differences in β-tubulin sequences of *Botrytis cinerea* Group I and Group II strains**

```
          TGCTTCGCGACAA  TCAGATTGCAACTAACCATATCACAGGCAAACTATCTCTGGCGAGCACGGTCTTGACGG TCCGGTGTGTAAGTA AATCACAAATTCTT   CTCGTA TTGAAAC TTACTGATA
AY770365 : ............CC..........................................................T............A............---.....C.......G.........
8020     : ............CC..........................................................T............A............---.....C.......G.........
8027     : ............CC..........................................................T............A............---.....C.......G.........
8034     : ............CC..........................................................T............A............---.....C.......G.........
8037     : ............CC..........................................................T............A............---.....C.......G.........
8061     : ............CC..........................................................T............A............---.....C.......G.........
AY770377 : ..............................................................................................................................
AY770375 : ..............................................................................................................................
AY770374 : ..............................................................................................................................
AY770357 : ..............................................................................................................................
8001     : ..............................................................................................................................
8002     : ..............................................................................................................................
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8029     : ..............................................................................................................................
8030     : ..............................................................................................................................
8031     : ..............................................................................................................................
8032     : ..............................................................................................................................
8047     : ..............................................................................................................................
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Botrytis cinerea Group I (GenBank accession numbers: AY770377, AY770375, AY770374, AY770357 from France, and strains 8001-8008, 8029-32 and 8047 from Hungary) and Group II (accession numbers: AY770365 from France, and strains 8020, 8027, 8034, 8061 from Hungary). The β-tubulin sequences – Upper row: consensus sequences; the match with the consensus is indicated by „.", gap is indicated by „–".

Dot blot analysis was used with the sequences of *Boty* and *Flipper* as probes, revealed DNA hybridization for the detection of the presence of the two different tranposons in Group I *B. cinerea* isolates. All of the thirteen Hungarian Group I isolates contained the transposable element *Boty* and one isolate also contained *Flipper*, showing that *Boty* only and *transposa* type (containing both transposable elements) strains occured in Hungarian Group I isolates. Our results proved new transposon types of within Group I. Earlier *vacuma* (not containing either *Boty* or *Flipper*) transposon type have only been detected with types in Group I (Fournier *et al.*, 2005; Martinez *et al.*, 2005).

Regarding the temporal distribution of *B. cinerea* isolates sampled in 2008 Group I were present mainly at the beginning of the season (*Table 1.*), in agreement with previous studies (Fournier *et al.*, 2005, Martinez *et al.* 2005).

CONCLUSIONS

In this work we found that both Group I and II types of *B. cinerea* occured in the Hungarian isolates. The identification of Group I strains based on PCR-RFLP of *Bc-hch* gene and sequence analysis of β-tubulin gene. The Group I (*Botrytis pseudocinerea*) detected in 5% of French isolates (Fournier *et al*., 2005, Martinez *et al*. 2005) was also found in 5% among the Hungarian isolates collected in 2008 from different hosts. That group earlier was characterized by the absence of the transposable elements *Boty* and *Flipper* (Fournier *et al*., 2005). However all the Hungarian Group I isolates contained the transposable element *Boty* and one isolate also contained *Flipper*, indicating a more diverse genetic structure of this criptic species, than earlier described. Group I strains were present mainly at the beginning of the season, in agreement with previous studies (Fournier *et al*., 2005, Martinez *et al.* 2005).

Table 1

Characteristics of *Botrytis cinerea* Group I strains

					Presence of transposon	
Strain	Isolation date	host	β-tubulin*	*Bc-hch* + HhaI*	Flipper	Boty
8001	24/04/2008	rape	**1**	**1**	-	+
8002	24/04/2008	rape	**1**	**1**	-	+
8003	24/04/2008	rape	**1**	**1**	-	+
8004	24/04/2008	rape	**1**	**1**	-	+
8005	24/04/2008	rape	**1**	**1**	-	+
8006	24/04/2008	rape	**1**	**1**	-	+
8007	24/04/2008	rape	**1**	**1**	-	+
8008	24/04/2008	rape	**1**	**1**	-	+
8029	18/05/2008	strawberry	**1**	**1**	-	+
8030	18/05/2008	strawberry	**1**	**1**	-	+
8031	18/05/2008	strawberry	**1**	**1**	-	+
8032	18/05/2008	strawberry	**1**	**1**	+	+
8047	07/06/2008	strawberry	**1**	**1**	-	+

* 1: Group I sequences

* * -: absence, +: presence of transposon

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Vegetative compatibility and fungicide resistance of *Botrytis cinerea* group I and II isolates in Hungary

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SUMMARY

The necrotrophic phytopathogenic ascomycete fungus Botrytis cinerea is responsible for the gray mold disease causing significant yield losses on many host plants including grape worldwide. However, B. cinerea is suggested to be a species complex containing two cryptic species, Groups I and II mostly determined by vegetative compatibility (VCG) and fungicide resistance. In our study we revealed the frequency of these groups in Hungarian B. cinerea isolates by identifying the VCG groups and studying the fenhexamid resistance.

Key words: *Botrytis cinerea* Group I, vegetative compatibility, fenhexamid resistance

INTRODUCTION

The necrotrophic phytopathogenic ascomycete fungus *Botrytis cinerea* Pers.:Fr. (teleomorph *Botryotinia fuckeliana* /de Bary/ Whetzel) is the causal agent of the gray mold disease worldwide. In viticulture, it is commonly known as "botrytis bunch rot"; in horticulture, it is usually called "grey mould" or "gray mold". Within the *Botrytis* genus, *B. cinerea* has the largest host range, including more than 230 plant species, mainly dicotyledonous (Prins, 2000). The great adaptability of the fungus is demonstrated by its polyphagous feature and the diversity of the target plant organs (leaf, berry, flower petal, and stem). The fungus infects flowers, setting fruits, mature fruits, and leaves as well. However it is also the agent of noble rot on grapevine, used for the elaboration of special sweet wine. Control of grey mould is based on an integration of several cultural methods with the use of fungicides belonging to several groups. However, *Botrytis* develops rapid resistance against chemicals due to their high genetic variability.

Several authors have shown that *B. cinerea* is a species complex with restricted gene flow between different cryptic genetic groups (Giraud *et al.*, 1997, 1999; Albertini *et al.*, 2002; Munoz *et al.*, 2002; Fournier *et al.*, 2003). Early they described two sympatric sibling species or transposons types: 1. *transposa* that contains *Boty* and *Flipper* transposons and 2. *vacuma* which contains no transposons (Diolez *et al.*, 1995; Levis *et al.*, 1997; Giraud *et al.*, 1997).

Recently molecular studies of different nuclear genes have suggested that *B. cinerea* populations grouped in two different clades in the different gene phylogenies, Group I and Group II, which are proposed to be phylogenetic species (Albertini *et al.*, 2002; Fournier *et al.*, 2003). As long as the Group I contains exclusively the *vacuma* transposon type, the Group II comprise both *vacuma* and *transposa* ones. DNA polymorphism and vegetative incompatibility studies revealed that the genetic diversity is lower in Group I. The two groups also show differences in morphology, phenology and host range characters, too. The asexual spores in Group I are significantly larger than in Group I as well as the Group I isolates are mainly found in Spring on grapevine, whereas Group II isolates are found both in spring and fall of leaves. As regard to the host preferences, in the Group I comprise less host plant species than in the Group II. Furthermore, the differences between the *vacuma* and *transposa* isolates may have been due to the differences between Group I and II, and the different proportion of *vacuma* and *transposa* isolates in the two cryptic species.

The Group I and Group II have also been shown to coincide with resistance to the fungicide fenhexamid (Fen), and synonymously known as FenR (resistant) for Group I, and FenS (sensitive) for Group II (Albertini *et al.,* 2002).

Vegetative compatibility gives an opportunity for population sub-structuring. Vegetative compatibility refers to the ability of individual fungal strains to undergo mutual hyphal anastomosis, which results in viable heterokaryons. When a heterokaryon is established, the participating isolates are placed in the same vegetative compatibility group /VCG/ (Leslie, 1993). Members of the same VCG can undergo hyphal fusion, with the potential for transferring nuclear and cytoplasmic elements. VCGs have been very useful for identifying clones of fungi that are largely asexual, and VCGs often correlate with pathogenicity and other traits (Korolev and Katan, 1999; Korolev *et al.*, 2000, 2001).

This paper reports the results of vegetative compatibility test of *Botrytis cinerea* isolates from Hungary. The aims of the investigation were to clarify the existence of different compatibility group as well as to determine the fungicide sensitivity for fenhexamid in Hungarian *B. cinerea* isolates.

MATERIALS AND METHODS

Strains

B. cinerea strains were collected from infected plant materials in 2008 in Hungary. The identification of Group I and II strains were carried out as described earlier (Fekete *et al.*, 2009).

Mycelial incompatibility (barrage)

Mycelial incompatibility was tested by observing the interaction zone between paired colonies of wild-type *B. cinerea* strains on malt-extract agar (MEA) amended with NaCl (Beever and Parkes, 1993, 2003). The pairings were examined 2 weeks after inoculation. Strains that formed dark pigmentation or exhibited sparse mycelium, with or without dark pigmentation, along the line of confrontation were considered incompatible. Incompatibility between two strains was registered as strong if it was evident and stable in replications; it was registered as weak if the line of confrontation was vague and/or it was inconsistent in the replications.

Fungicide resistant tests

Two fungicides were used in this study viz. fenhexamid and azoxystrobin.

To evaluate the resistance for fenhexamid, the mycelial growth of *B. cinerea* strains was measured on a solid minimal medium containing 0, 2, and 6 mg l^{-1} fenhexamid (Leroux *et al*., 1999). Each Petri dish was inoculated with an inverted mycelium plug (5 mm in diam.), cut from the margin of a 3-day-old colony.

RESULTS

Group I and Group II strains were collected from neighborhood territories during 2008 from different hosts (*Table 1*). The identification of Group I strains were carried out with both the sequence analysis of β-tubulin gene fragment and the PCR-RFLP analysis of *Bc-hch* gene (Fekete *et al.*, 2009).

Table 1

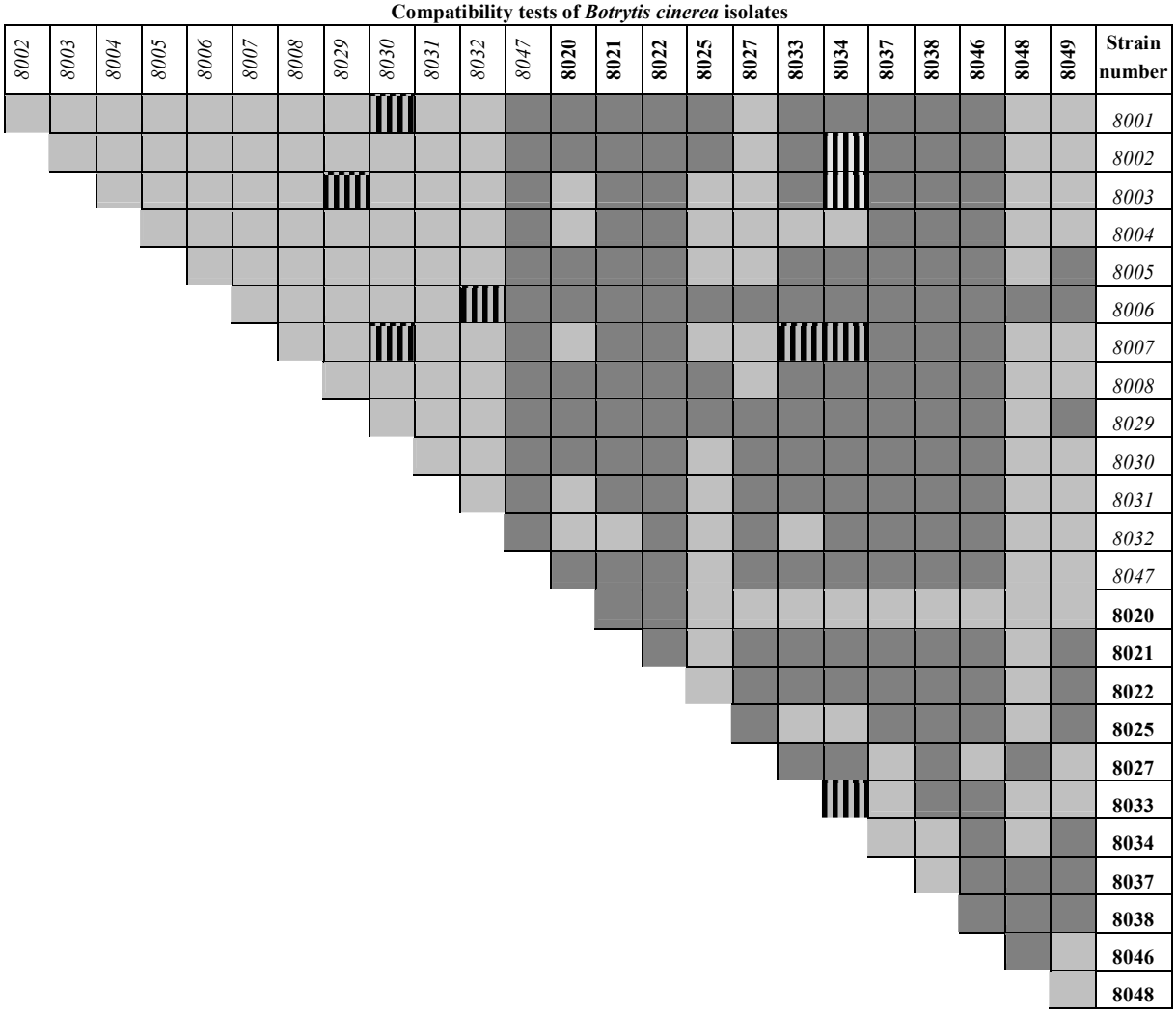
***Botrytis cinerea* Group I and Group II isolates**

Isolate	Host	Isolation date	Isolate	Host	Isolation date
Group I			Group II		
8001	rape	24/04/2008	8020	raspberry	18/05/2008
8002	rape	24/04/2008	8021	raspberry	18/05/2008
8003	rape	24/04/2008	8022	raspberry	18/05/2008
8004	rape	24/04/2008	8025	raspberry	18/05/2008
8005	rape	24/04/2008	8027	raspberry	18/05/2008
8006	rape	24/04/2008	8033	strawberry	18/05/2008
8007	rape	24/04/2008	8034	strawberry	18/05/2008
8008	rape	24/04/2008	8037	strawberry	18/05/2008
8029	strawberry	18/05/2008	8038	strawberry	18/05/2008
8030	strawberry	18/05/2008	8046	strawberry	07/06/2008
8031	strawberry	18/05/2008	8048	strawberry	07/06/2008
8032	strawberry	18/05/2008	8049	strawberry	07/06/2008
8047	strawberry	07/06/2008			

In vegetative compatibility tests the majority of Group I isolates formed a unique vegetative compatibility group (VCG). The 8047 Group I isolate however showed incompatibility with all Group I strains and compatibility with three (8025, 8048, 8049) Group II strains. Several VCGs were detected within group II, and there were VCGs overlapped between the two groups (*Table 2*).

Table 2

Compatibility tests of *Botrytis cinerea* isolates

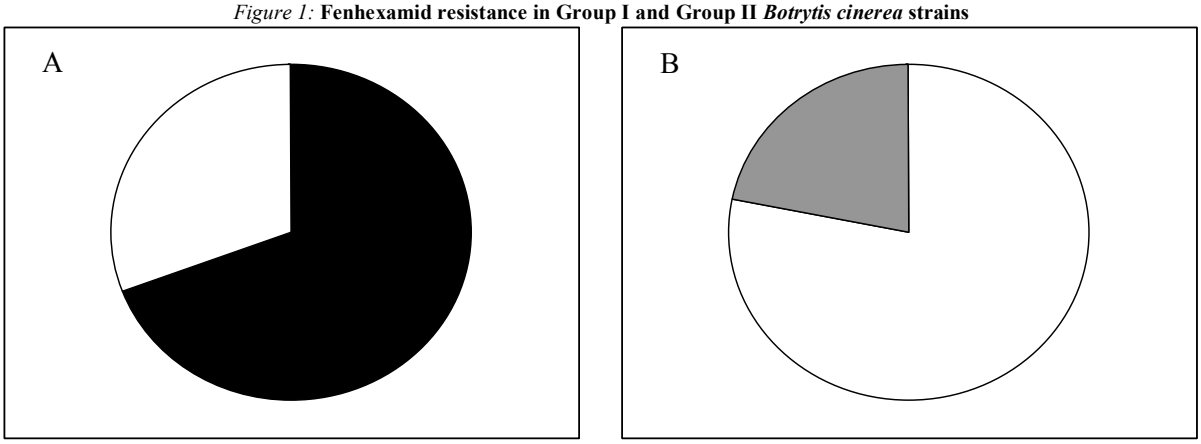


Italic numbers indicate *Botrytis cinerea* Group I strains (8001-8008, 8029-32, 8047), bold numbers indicate Group II strains (8020, 8027, 8034, 8061)

Light grey: compatible, dark grey: non compatible, barred: uncertain

To determine fungicide resistance profiles in Group I and Group II, sensitivity to fenhexamid of 25 *B. cinerea* isolates (Group I and II) were studied. According to in vitro responses of 25 field strains of *B. cinerea* towards fenhexamid, three phenotypes have been distinguished. Firstly, the sensitive one, which have the estimated effective concentration of resistance lower than 2 mg l^{-1}. The low resistant strains had the estimated effective concentration of resistance between 2 and 5 mg l^{-1}. The estimated effective concentration of resistance was higher than 5 mg l^{-1} in resistant isolates. Resistance could be detected only in Group I, while low resistance in Group II isolates. Sensitive isolates could be detected in both groups (*Figure 1*). The majority of Group II isolates were resistant to fenhexamid.

Figure 1: **Fenhexamid resistance in Group I and Group II *Botrytis cinerea* strains**



A: Group I, B: Group II
White: sensitive, grey: low resistant, black: resistant

CONCLUSIONS

Several previous genetic characterization of populations of the fungus *B. cinerea* revealed that this pathogen was genetically diverse and suggested that it was not a unique, large, panmictic population (Giraud *et al*., 1997, 1999; Albertini *et al.*, 2002; Munoz *et al.*, 2002; Fournier *et al.*, 2003). Recently *Botrytis cinerea* has been reported as a species complex containing two cryptic species, Groups I and II. The genetic diversity was lower within Group I, as revealed by DNA polymorphism and vegetative incompatibility tests (Fournier *et al.*, 2005). Group I have also been shown to coincide with resistance to the fungicide fenhexamid (Fournier *et al*., 2005; Martinez *et al*., 2005).

In this study, thirteen Group I and twelve Group II of *B. cinerea* strains were studied. Vegetative compatibility analyzes were used to characterize the biological diversity within each group. The results indicated that majority of Group I isolates formed a unique VCG, but VCGs overlapped between the two groups. This result indicated the possibility of information exchange between Group I and Group II strains.

The fenhexamid resistance studies supported previous results of Albertini *et al.*, (2002) and Fournier *et al.* (2005) as fenhexamid resistant strains could only be detected in Group I isolates. However 3 Group I strains were sensitive to this fungicide. Fenhexamid resistance can not be used for determination of Group I of *B. cinerea* isolates.

Before the introduction of the hydroxyanilide derivative, fenhexamid, numerous strains of *B. cinerea* highly resistant to this promising botryticide were detected (Leroux *et al*., 1999). However, this phenomenon was mainly expressed in mycelial growth tests whereas the germ-tubes produced by these strains appeared sensitive to fenhexamid. In the field, fenhexamid was found to act preventively against *B. cinerea* and this could explain why fenhexamid resistant strains do not apparently lead to practical resistance (Leroux *et al.*, 1999).

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Role of rDNA and protein-encoding genes in determining identity and phylogeny of fungi

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SUMMARY

The identification of fungal species had been based both on microscopic and macroscopic characteristics on host alone, and later on the trend has changed to study their features in pure culture. However on the basis of morphological studies on different culture media, several species described as independent ones were found actually to be identical. Therefore, lately introduced molecular studies have been applied to delimitate species and infer phylogenetic relationships within fungi. Molecular markers like RAPD and ITS-rDNA sequences were firstly used for identification and differentiation of fungal species and to understand the evolutionary relationship among these ones. The main goal of the present review is to introduce the applied molecular identification of fungi as a case study of Phoma.

Keywords: *phylogenetic analysis, ITS sequences, protein encoding genes, fungi, Phoma*

INTRODUCTION

The identification of taxa in fungi have been based on the microscopic and macroscopic characteristics on host or in culture, such as colony color, rate of growth, size and shape of asexual and sexual bodies (Rai, 1998), hyphal pigmentation (Besl and Bresinsky, 1997), secondary metabolite production (Frisvad and Filtenborg, 1990), growth at different temperatures or water activities (Pitt, 1979), biochemical characteristics (Boerema and Höweler, 1967; Dorenbosch, 1970; Monte *et al.*, 1990, 1991), production of crystals (Noordeloos *et al*., 1993), or physiological characteristics, like requirement of carbon and nitrogen resources by the fungi (Boerema *et al*., 2004). The strength of identification based on morphological characters is that species can be compared with other existing species without involving sophisticated equipment like PCR and comparatively cheaper to molecular methods. The major drawback of identification of species on the basis of morphological characters is that it is time-consuming and species identified by morphological characters often comprise more than one species when diagnosed by molecular markers, for example, in human pathogenic fungus *Histoplasma capsulatum,* six genetically isolated groups identified by congruence of gene genealogies were found in this one morphological species (Kasuga *et al*., 1999). Recent molecular studies (Irinyi *et al.*, 2009) revealed that *Phyllosticta sojicola* is grouped with *Phoma exigua* var. *exigua*, which clearly indicates that speciation based on shape and size of conidia or spore are variable on different media and under different culture conditions (Rajak and Rai, 1983; Rai, 1998).

Realizing the importance and need of molecular studies, Guarro *et al.* (1999) stated that although the current classification of fungi is commonly based upon morphology, molecular techniques include DNA sequencing are powerful tools and nowadays used in many fungal cases. After the development of the molecular markers, taxonomist now use these methods as supplementary tools for identification and delimitation of fungi (Bowman *et al.,* 1992; Bruns *et al.,* 1991, 1992; Gargas *et al.,* 1995; Kauff and Lutzoni, 2002; Tarkka, 2006; Takamatsu *et al*., 2008). Ribosomal DNA (rDNA) has been used as a potential marker by most taxonomists (Nazar *et al.,* 1991; Bruns *et al.,* 1992; reviewed in Avise, 2004). On the basis of DNA-based molecular methods identification and delimitation of the genera, species, races, and strains of fungi can be made (Glass and Donaldson, 1995). The methods which have often been used include Random Amplification of Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), and Amplified Fragment Length Polymorphism (AFLP) (Hibbet and Vilgalys, 1991; Guthrie *et al.,* 1992; Ellsworth *et al.*, 1993). But rDNA has several shortcomings, for example its repetitive nature and consequently its high intraspecific mutation rate among multiple copies due to weak selective constraints (Voigt *et al*., 1999). On the other hand, protein coding genes have less chance of mutation and are less variable, and therefore, the phylogenic analysis of multiple protein-encoding genes is proposed as a more robust way of determining and recognition species (Taylor *et al.,* 2000).

With the advent of Polymerase Chain Reaction (PCR) the molecular analyses have become easier and many fungi have been studied for their genome structure and molecular phylogeny (Innis *et al*., 1990; Jones *et al*., 1993; Glass and Donaldson, 1995; Guarro *et al.,* 1999; Lutzoni *et al*., 2004; Binder *et al.,* 2005; Tarkka *et al.,* 2006; Irinyi *et al.,* 2009).

The present review will focus on the use of rDNA markers and protein-encoding genes like tubulins, actin and translation elongation factors (*tef)* in molecular identification and phylogeny of fungi.

Ribosmal DNA

Ribosomal DNA (rDNA) has long been used as a potential marker for phylogenic studies (reviewed in Avise, 2004). Genes of eukaryotic rDNA are organized in a cluster that includes a small subunit (18S SSU) gene, a large subunit (28S LSU) gene, and the 5.8S gene that lies in between two internal transcribed spacers (ITS; White *et al*., 1990). The region that separates the cluster of three genes along the chromosome is called the non-transcribed spacer (NTS) and prior to where the 18S gene is transcribed, there is another small spacer region called the externally transcribed spacer region (ETS). Together the ETS and NTS regions comprise the intergenic spacer region (IGS). These components are repeated in a tandem array but they evolve as a single unit and vary in length around 3000-4500 base pairs (Mitchell *et al*., 1995). The IGS separates the different repeat units; the ETS lies between the 18 S and 28 S coding regions. The ITS can be further sub-divided in ITS1 and ITS2 in fungi. ITS is usually considered to be low functional constraint, and it is, therefore, often treated as a typical nonfunctional spacer sequence.

There are some examples, where taxonomy has been ambiguous or there is a compelling need to develop rapid identification tools due to their commercial value or to understand the evolutionary trends among the species of the genus. These problems have been solved by using molecular tools. Taxonomy of some of these genera, like *Phoma, Rhizopus*, endoparasitic species of Erysiphaceae (*Leveillula, Phyllactinia* and *Pleochaeta*) has been discussed here. The genus *Phoma* would be dealt under sub-head 'integrated approach' in this review.

The genus *Rhizopus* has high commercial value as the strains of the genus are used in fermented food in East and Southeast Asia (Hesseltine, 1983) and hence it was desirable to develop rapid identification tools for different species of *Rhizopus*. On the basis of morphological and physiological characteristics many species of *Rhizopus* have been created by the researchers, and hence, Schipper (1984) reclassified the genus and proposed a new classification based on morphological characteristics. The authors classified all the species of the genus into three broad groups: (i) the *Rh. stolonifer*-group, (ii) *Rh. oryzae,* and (iii) *Rh. microsporus*-group. Currently, there are 13 species belonging to this genus. Furthermore, considering the economic value of *Rhizopus* and its species Abe *et al.* (2006) studied the molecular phylogeny of the genus *Rhizopus* and compared it with current classification given by Schipper (1984). The authors analyzed the sequences of rDNA 18S, ITS, and 28S D1/D2 of all the species of the genus and reported that the results of molecular phylogeny were similar to the morphological groups, except for *Rh. schipperae* and *Rh. stolonifer* var. *lyococcus (=Rh. lyococcus*). They identified three major clusters, A, B and C corresponding to the *Rh. microsporus*-group, *Rh. oryzae* and *Rh. stolonifer*-group respectively.

Another example of successful application of ITS sequences in phylogeny is Erysiphaceae. The family Erysiphaceae includes obligate parasitic fungi that cause powdery mildew diseases. The family is represented by 15 genera, out of which 12 genera are ectoparasitic and three genera (*Leveillula, Phyllactinia* and *Pleochaeta*) show partial or complete endoparasitism. This family show two entirely different mode of parasitism. The parasitism, whether endo- or ecto- has long been a matter of debate. According to one school of thought, the genus *Leveilulla* Arnaud is the most ancestral genus in Erysiphaceae (Katumoto, 1973). On the other hand, Braun (1987) claimed Erysiphae as the most ancestral genus. These all arguments have been based on morphological nature of the members of Erysiphales.

However, molecular phylogenic studies have given a new insight to taxonomy and evolutionary trends in Erysiphaceae. Mori and collaborators in (2000), studied the nucleotide sequence of the 18S, 5.8S, and 28S rDNA and its ITS regions for 33 Erysiphaceae taxa belonging to 15 genera and reported five major lineages in Erysiphaceae. Each lineage has been recognized as a formal taxonomic unit (tribe) of Erysiphaceae. The authors further argued that the endoparasitism was diverged from ectoparasitism. Recently, Takamatsu *et al.* (2008) constructed phylogenic trees of *Phyllactinia* and related genera based on 120 nucleotide sequences of 28S rDNA and ITS regions to evaluate their phylogenic relationships. The analyses of the Erysiphales confirmed the monophyletic origin of endoparasitic genera (*Leveillula, Phyllactinia* and *Pleochaeta*). The authors suggested that evolution in this group of fungi occurred from partial endoparasitism to obligate endoparasitism.

Arbuscular Mycorrhizal (AM) fungi are unique example, where molecular tools have played a significant role in giving new dimension to glomalean taxonomy. These are important fungi living in symbiosis of plants (Smith and Read, 1997). Their involvement in nutrient uptake, particularly in phosphate metabolism is well realized. Van der Heijden *et al.* (1998) reported that diversity of AM fungi directly effects diversity and interactions among plants. Thus, identification and delimitation of AM fungi is essential in ecological studies (Helgason *et al*., 1998).

Traditionally, the identification of glomalean fungi is based on morphology of the spores. Six genera in three families have been defined by their mode of spore formation (Morton and Benny, 1990). Molecular systematic studies by Simon *et al*. (1993) supported this basic three family structure. The order Glomales was placed in Zygomycota, but molecular data (Gehrig *et al.,* 1996) reveal that the closest non-mycorrhiza relative of AM fungi is *Geosiphon pyriforme* (endosymbiont of cyanobacteria). Phylogenic analyses of Glomales by Redecker *et al.* (1997) indicate that evolutionary patterns in the Glomales are much more complex. Previously, it was thought that the earlier glomalean fungi were *Glomus*-like and that formation of such spores is a primitive trait based on fossil record (Taylor *et al*., 1995). In fact, fossil record does not provide the internal structure or other morphological details of the spores. Based on molecular studies, Redecker *et al.* (2000) opined that the

production of both *Acaulospora* and *Glomus* spore types is an ancestral character within the Glomales, and one or other spore type was lost independently during the evolution of various clades.

Fatehi *et al.* (2003) used ITS sequences to determine the genetic (besides integrate morphological pathological and molecular characters) relatedness between isolates described as *Ascochyta* and *Phoma* species (*P. medicaginis* var. *pinodella* /= *P. pinodella*/, *A. pisi* /teleomorph: *Didymella pisi*/ and *A. pinodes* /teleomorph: *D. pinodes*/) associated with blight of peas. The molecular and morphological data show that the isolates are all very closely related and were confirmed by the sequencing of the representative isolates. Also the ITS sequences were used for delimitation the teleomorph stages of *Leptosphaeria maculans-L. biglobosa* species complex (anamorph *Phoma lingam*) (Mendes-Pereira *et al*., 2003), moreover *Phoma tracheiphila* isolates (Balmas *et al.*, 2005). After the lately published *Phyllosticta* (Aa and Vanev, 2002) and *Phoma* monographs based on traditional morphological features (Boerema *et al.* 2004), a new trend seems to be developing in molecular taxonomy of *Phoma*-like fungi (Irinyi *et al.*, 2009, Gruyter *et al.*, 2009). Some proposal was suggested for reclassification of *Phoma sojicola* as synonym of *Phoma pinodella,* and *Phyllosticta sojicola* as synonym of *Phoma exigua* var. *exigua* (Irinyi *et al*., 2009). As *Phoma* anamorphs represent a polyphyletic group, toward a reclassification of the *Phoma* complex, nine *Phoma* sections and represenative strains of 39 allied anamorph genera, including *Ascochyta, Coniothyrium, Deuterophoma, Microsphaeropsis, Pleurophoma, Pyrenochaeta*, moreover 11 teleomorph genera were compared using sequences of ITS (SSU and LSU) regions. Five out of nine *Phoma* sections proved to be related with the teleomorph *Didymella*. Because of molecular evidences the introduction of Didymellaceae Gruyter, Aveskamp and Verkley *familia nova* was proposed (Gruyter *et al.*, 2009).

The highest sequence variation in rDNA exists within the IGS region (also known as the non-transcribed spacer or NTS region). The size of the IGS region may vary from 2 kb upwards and it is not unusual to find hyper variability for this region. Several published molecular phylogenies have demonstrated the utility of ITS regions or rDNA genes in fungal taxonomy but there is less work on IGS regions. Most filamentous ascomycetes have a single uninterrupted IGS region: IGS I and IGS II are found between the end of the LSU and start of the next SSU sequence (White *et al.*, 1990). Variations in rDNA among closely related taxa are found in IGS (Fernández *et al.*, 1994). IGS sequences might be good candidates for the differentiation of strains at the intraspecific level (Hillis and Dixon, 1991; Edel *et al.*, 1995; Mishra *et al.*, 2002) presumably due to relative lack of selective constrains, at least in a large part of its sequence. There are several successful applications of IGS to infer phylogenetic relationships among fungal taxa. Within the *Fusarium* genus Llorens *et al.* (2006) characterized morphologically, physiologically and genetically 44 *Fusarium* spp. isolates as well as Schmidt *et al.* (2004) successfully used IGS regions to clarify the taxonomical position and relationship of *Fusarium langsethiae* to other taxa within the *Fusarium* section *Sporotrichiella*. Hinojo *et al.* (2004) carried out molecular characterization of *Gibberella fujikuroi* isolates analyzing the IGS region by PCR-RFLP. Their results proved that there are differences at molecular level in *G. fujikuroi* isolates (morphologically identified as *F. moniliforme*) from *Zea mays*, *Musa sapientum* and *Pinus pinea.*

Protein-encoding genes

Other genes, or their products, can be used to infer phylogenetic relationships among fungal taxons. Protein coding genes have some advaneges over rRNA genes and spacers int hat the alignement of the sequences is less problematic (Bruns *et al*., 1991). Protein sequences also lend themselves to differential weighting of bases by codon position, and third position sites can provide a relatively good estimate of the neutral substitution rate.

Tubulins (α and β)

It is well known that tubulin proteins play a crucial role in eukaryotic cellular process (Einax and Voigt, 2003). They represent major components of the cytoskeleton including mitotic spindles. They are main component of microtubules. According to McKean *et al.* (2001) the tubulins represent a protein family with α (alpha), β (beta), γ (gamma), δ (delta), ε (epsilon), ζ (zeta) and η (eta). α and β-tubulins are the most abundant in eukaryotic cell as their heterodimers are the primary constituents of the microtubules. Tubulin proteins play an important role as all eukaryotic cells contain microtubules. Stressing upon the importance of tubulins, Tarkka (2006) stated that tubulins are the promising tool for molecular phylogeny of fungi. α- and β-tubulins particularly their N-terminal peptides are remarkably conserved (Little *et al.*, 1981). Sequence identitities of 65-70% among all eukaryotic organisms contribute to a high level of evolutionary conservation of both proteins (Baldauf *et al*., 2000).

α-tubulin plays an important role in molecular phylogeny. For example, α-tubulins have been used for phylogenetic analyses of Microsporidia and Glomeromycota (Keeling, 2003; Corradi *et al.,* 2004). It has been shown that usage of multigene datasets including α-tubulins can increase the resolution of molecular phylogenetic analyses in higher fungi (Lutzoni *et al*., 2004; Binder *et al.,* 2005; Tarkka *et al.,* 2006).

Phylogenetic studies of three protein-encoding genes, mating type (MAT), actin, and β-tubulins as well as concatenated sequences yielded consistent results (Voight *et al*., 2005).

The authors studied twenty-eight isolates of *Leptosphaeria maculans*, which parasitizes on *Brassica napus*, and compared with 20 other species of Pleosporales. For phylogenetic analyses, the authors utilized the

sequences of mating type MAT1-2 (23), fragments of actin (48) and β-tubulin (45) genes. By using maximum parsimony, distance, maximum likelihood, and bayesian approaches. On the basis of these approaches using single gene confirmed that *Leptosphaeria maculans* formed a monophyletic group separate from *L. biglobosa.* These are significant findings because in Europe, both *L. maculans* 'brassicae' and *L. biglobosa* 'brassicae' are usually present on the same *B. napus* plant (West *et al*., 2002).

Emphasizing the importance of *β-tubulin* genes in fungal taxonomy, Keeling (2003) claimed that microsporidia are related to fungi. However, exactly how these groups are related to one another remains unclear. "The only gene that has been sampled sufficiently from both microsporidia and fungi to make any such distinction is *β-tubulin*", Keeling (2003) remarked. Phylogenies provide evidence that microsporidia evolved from within the fungi and they are related to either Ascomycetes or Zygomycetes, but fail to identify a more specific position (Keeling *et al*., 2000). There are a number of studies which supports the use of *β-tubulins* in fungal taxonomy, for example, in case of Basidiomycota analyses have been concentrated on *β-tubulins* (Begerow *et al*., 2004; Juuti *et al.,* 2005; Shi and Perlin, 2001).

Voigt *et al*. (2005) used *β-tubulin* gene among other genes to analyze *Leptosphaeria maculans* (anamorph: *Phoma lingam*) species complex as well as Fatehi *et al*. (2003) to refer molecular relatedness within the '*Ascochyta pinodes*-complex'. Partial *β-tubulin* amino-acid sequences were used by Landvik *et al*. (2001) to assess higher-level phylogenetic relationships in the Ascomycetes, but their results suggest it is less suitable than other genes at this level. According to Hansen *et al*. (2004) *β-tubulin* gene appears less useful at intergeneric level.

Actins

Actins are highly conserved structural proteins, found in all eukaryotes. They are necessary for the transformation of chemical into mechanical energy in eukaryotic organisms for cell growth, division and morphogenesis (Sheterline and Sparrow, 1994). Actin also plays a role in morphogenesis of fungi and the actin cytoskeleton is necessary for the establishment of polarized growth of the hyphae and for nuclear and cellular division. Cytoplasmic actin genes are among the most heavily transcribed genes (Lloyd and Sharp, 1992). The act-1 genes consist of a coding region of about 1200bp and intervening sequences. In contrast to those found in other eukaryotic species, fungal act-1 genes are found as a single copy in each haploid genome. The actin encoding gene *act* has been found to be single copy in the majority of fungi tested (Tarkka *et al*., 2000). These genes have been used to study evolutionary relationships among a number of eukaryotes (Bhattacharya *et al*., 1991; Bhattacharya and Ehlting, 1995; Drouin *et al*., 1995; Bouget *et al*., 1995; Baldauf *et al*., 2000), including groups of fungi and especially the Zygomycota (Cox *et al*., 1995; Wery *et al*., 1996; O'Donell *et al*., 2001). Voigt and Wöstemeyer (2001) successfully determined evolutionary relatedness with analysis of actin and translation elongation factor EF-1α genes among 82 Zygomycetes representing all 54 currently recognized genera from the two zygomycetous orders Mucorales and Mortierellales. Their results indicated deep, ancient and distinct dichotomy of the orders Mucorales and Mortierellales and proved that a complete revision of zygomycete natural systematics is necessary.

Translation elongation factor (tef)

It is well known that ribosomes are the site of protein synthesis, and for proper function, they require the activity of several initiation, elongation, and termination factors. Translation is the final step of the conversion of genetic information into proteins (Moreira *et al*., 2002), and therefore, translation elongation factor plays a crucial role in determination of phylogeny of an individual. According to Baldauf and Doolittle (1997) elongation factor 1 alpha (EF-1α) appears to be well-suited for deep-level phylogeny due to its slow rate of sequence evolution.

Translation elongation factor 1 subunit alpha (EF1=*tef1*) is part of the cytosolic EF1 complex, whose primary function is to promote the binding of aminoacyl-tRNA to the ribosome in a GTP-dependent process (Moldave, 1985). It is an essential component of the protein synthesis process in eukaryotes and archeabacteria. Complexed with GTP, it carries the aminoacyl-tRNA to the A site of the ribosome-mRNA-peptidyl-tRNA complex; upon hydrolysis of GTP it leaves the ribosome as EF-1-GDP.

Simultaneously, elongation factor 1 (EF-1) is a highly conserved ubiquitous protein that has been suggested to have desirable properties for phylogenetic inference (Roger *et al*., 1999). EF-1 is well-suited for determining phylogenetic relationships, due to its universal occurrence, presence typically as a single copy within the genome and slow rate of sequence evolution (Baldauf and Doolittle, 1997). It has been proven to be a useful gene to resolve phylogenetic relationships at species level as well as in deeper divergences where amino acid substitutions provide phylogenetic resolution (Roger *et al*. 1999; Druzhinina and Kubicek, 2005). Knutsen *et al*. (2004) used *tef1* gene for phylogenetic analysis of *Fusarium poae*, *F. sporotrichoides* and *F. langsethiae* species complex as well as Skovgaard *et al*. (2002) to assess genetic relatedness of *F. oxysporum* complex isolated from pea.

An excellent example of use of translation elongation factor is in determination of identity of *Pneumocystis carinii* and its inclusion in fungi. *Pneumocystis carinii* is very important fungus because it is a causal organism

of pneumonia in AIDS patient, and eventually responsible for mortality and morbidity. Previously, *Pneumocystis carinii* was placed in Protozoa. However, interestingly the molecular study using translation elongation factor 3 (EF-3) provided evidence that *P. carinii* should be classified as a fungus. Translation elongation factor 3 is an essential, soluble translation component which is unique to fungal protein synthesis and is not required for protein synthesis in other eukaryotes. Ypma-Wong *et al.* (1992) isolated a gene for EF-3 from *P. carinii*, which provides evidence to include *P. carinii* in fungus.

Van't Klooster *et al*. (2000) studied *tef1* gene in *Phytophthora infestans* and reported that it is a single copy gene *tef1* is constitutively expressed in all the stages of the asexual life cycle of *P. infestans.* Phylogeny analysis of EF-1α sequences from a variety of organisms demonstrates that oomycetes evolved completely independently from the true fungi, and thus there is no evolutionary relationship between oomycetes and fungi. The closest homologue is EF-1α.

A further example for utility of *tef1* gene in population genetic studies is carried out by Váczy *et al.* (2008) analyzing the genetic variability within the Hungarian population of *B. cinerea*. The authors analyzing *tef1* among other genes found that the wine-pathogenic population of *B. cinerea* exhibits significant recombination.

Baldauf and Doolittle (1997) studied the origin and evolution of slime molds (Mycetozoa) and found that molecular phylogenies of rRNA genes show little or no support for a coherent Mycetozoa. These analyses demonstrate *Physarum* as arising early in the tree. On the otherhand, actin and β-tubulin trees place *Physarum* and *Dictyostelium* together. The authors sequenced the EF-1α encoding (*tef1*) gene from *Physarum polycephalum*, *Dictyostelium discoideum* and an amoeboflagellate protostelid *Planoprotostelium aurantium*. Molecular phylogenetic analyses of these sequences supported that Mycetozoa is a monophyletic group.

Irinyi *et al*. (2006a, 2006b, 2007, 2009) examined partial *tef1* gene to infer phylogenetic relationship within taxonomically uncertain *Phoma*-like species and found that the highest resolution could be revealed with the analysis of the *tef1* gene, while sequences of ITS and *β-tubulin* showed the lowest polymorphism among the examined *Phoma* species.

Chitin synthase

Chitin, the structural component that provides rigidity to the cell wall of fungi is the product of chitin synthases (*chs*). These enzymes are not restricted to fungi, but are amply distributed in four of the five eukaryotic 'crown kingdoms'. In most of true fungi the structural microfibrillar component responsible for cell wall rigidity is chitin, a polysaccharide made of N-acetylglucosamine (GlcNAc) units joined through β-1-4 glycosidic linkages and it is not known to occur in plants or bacteria (Ruiz-Herrera *et al.*, 2002). In many species of yeasts, chitin is used to maintain the structure of the junction between the mother cell and the bud, whereas in filamentous fungi chitin is usually the major supporting component of the cell wall (Georgopapadakou and Tkacz, 1995).

Chitin is the product of chitin synthases (*chs*), enzymes from which we know more about the genes that encode them (*chs* genes), than from the proteins themselves (Bulawa, 1993). Chitin synthases have been classified into five classes based on differences in regions of high sequence conservation (and their homologues have been amplified from a large number of fungi and the deduced amino acid sequences aligned to reconstruct phylogenies.

Several study proved the utility of *chs* genes in the estimation of fungal biodiversity. Mandel et *al.*, 2006 described the identification and isolation of six *chs* genes of *Coccidioides posadasii* strain Silveira and they identified an additional *chs* gene in *C. posadasii*. *C. posadasii* contains a single member of each of the seven phylogenetically distinct class of fungal chitin synthases. Matute *et al.* (2007) investigated chitin synthase II gene (*chs2*) of *Paracoccidioides brasiliensis*, in an attempt to determine the evolutionary forces affecting the chitin synthesis metabolic pathway and determine the causes of the patterns of polymorphisms found in the *chs2* gene in *Paracoccidioides brasiliensis* species complex.

Integrated approach

The application of integrated approach for identification and determination of phylogeny in fungi seems to be more beneficial, because sometimes it may not be possible to obtain accuracy in results by using one gene particularly when ITS are used because of its repetitive nature and high intraspecific mutation rate among multiple copies. A recent example of integrated application of morphological characters, ITS, *β-tubulin* genes and *tef1* for identification and uderstanding phylogeny is *Phoma* (Irinyi *et al*., 2009). Moreover, the molecular tools are the supplement to the morphological characters for deciding the authentic identification of the genus or species. Unfortunately, it is a matter of great concern that without identifying the fungal strains morphologically they are sequenced, and it seems to have a common practice (Hyde and Soytong, 2007).

The genus *Phoma* Sacc. occurs as parasite on plants, human beings and animals or as saprophyte or freeliving in air (Rai, 1998). The speciation in the genus *Phoma* was based on host-alone and later on, the trend was to study different species of *Phoma* in pure culture. On the basis of morphological studies on different culture media several species were found to be identical. These studies have prompted many investigators to carry out morphological and cultural studies. The morphological criteria, such as diameter and color of the

colony, shape and size of pycnidia and pycnidiospores, formation of chlamydospores and pigmentation were considered for identification and differentiation of the species (e.g. Kövics *et al*., 1999). However, these criteria are not always reliable and therefore other methods, including molecular studies were carried out. There were attempt to introduce isozyme profile analyzis, viz. α-esterase enzymes in *Phoma* delimitation (Kövics - de Gruyter, 1995), but later on more effective molecular markers like RAPD and ITS-rDNA sequences were used for delimitation for species of *Phoma* and to understand the evolutionary relationship among the species (Irinyi *et al*., 2009).

In 2005, Balmas and collaborators studied thirty six isolates of *Phoma tracheiphila* because it is a causal agent of "mal secco" disease on citrus. The study was based on RAPD analyses and sequencing of internal transcribed spacer (ITS) region of the nuclear rRNA genes. They found that the populations of *P. tracheiphila* in Italy are genetically homogenous, and a close relationship exist between *P. tracheiphila* and *Leptosphaeria congesta*. The molecular assay developed in his study can be used to differentiate *P. tracheiphila* from other *Phoma* and other species/genera parasitizing on citrus.

An integrated approach (multigene approach): *Phoma* as a case study

Very recently, Irinyi *et al*. (2009) obtained DNA sequences from ITS, β-tubulin and translation elongation factor coding genes to resolve phylogenetic relationships among several *Phoma* species, since it has been shown that useage of multigene datasets can increase the resolution of molecular phylogenetic analyses. The analysis of multiple protein-encoding genes with the GCPSR is proposed as a more robust way of determining and recognising species than analysis based on morphology or mating behaviour (Taylor *et al*., 2000).

In their study the authors obtained a region of nuclear rDNA, containing the internal transcribed spacer regions (ITS) 1 and 2, and the 5.8S rDNA (*Figure 1*) as well as they amplified a part of the of *β-tubulin* gene (Glass and Donaldson, 1995; O'Donnell and Cigelnik, 1997) (*Figure 2*) and the large intron of *tef1* gene (Druzhinina and Kubicek, 2005) (*Figure 3*).

Twenty-two isolates of nine *Phoma*-like species were tested for phylogenetic analyses in this present study. All the isolates were identified morphologically according to Boerema *et al*. (2004) based on physiological and morphological characteristics.

Amplifications of 50µl PCR reaction mixture contained 25µl 2X PCR Master Mix (Fermentas, #K0171, Burlington, Canada), 40-40pmol each primer, 20-40ng DNA and nuclease free water were run out. Primers used to amplify cca 520 bp of the ITS region containing the ITS regions 1 and 2, moreover the 5.8S rDNA are based on published composite sequences, SR6R and LR1 (White *et al*., 1990) with the following amplification protocol: 3 min initial denaturing at 95°C, followed by five cycles of 1 min at 95°C, 1 min annealing at 50°C, 1 min at 72°C, and 25 cycles of 1 min at 90°C, 1 min annealing at 50°C, 1 min at 72°C, and 15 min final extension at 72°C. The large intron (cca 300 bp) of the *tef1* gene was amplified by the EF1-728F and EF1-986R primer pair (Druzhinina and Kubicek, 2005) according to the previously described protocol with a temperature of 56°C rather than 50°C. Primers Bt2a and Bt2b (Glass and Donaldson, 1995; O'Donnell and Cigelnik, 1997) were used to amplify a fragment (cca 300 bp) of the β-tubulin gene, and PCR conditions were carried out as described above with an annealing temperature of 58°C. PCR was performed in a Primus thermocycler (MWG Biotech, Martinsried, Germany). Amplification products were subjected to electrophoresis in a 0.7 % agarose gel containing EtBr and visualized by UV illumination. The PCR products were purified by using YM-100 Microcon Centrifugal Filter Devices (Millipore, Billerica, USA). Purified amplification products were sequenced by MWG Biotech, Germany. The obtained DNA sequences were aligned first with ClustalX (Thompson *et al*., 1997) and manually adjusted using Genedoc (Nicholas *et al*., 1997). Single gaps were treated either as missing data or as the fifth base and multistate characters were treated as uncertain. Phylogenetic analyses were performed in PAUP*4.0b (Swofford, 2002). The following settings were used: heuristic search with tree bisection-reconnection (TBR), with random addition of sequences with 1000 replicates. Stability of clades was assessed with 1000 bootstrap replications.

RESULTS

ITS sequences

The PCR amplification resulted in single fragments approx. 520bp of the total ITS region. There was no size variation observed among amplified rDNA fragments. ITS sequences were edited to 454bp to aid alignment with sequences downloaded from GenBank.

The difference between the different *Phoma* and *Ascochyta*, *Leptosphaeria* and *Didymella* species was not significant. Parsimony analysis of ITS revealed 32 sites were considered as informative sites, 5 polymorphic sites, and 417 sites are constant among all isolates. The topology of phylogenetic tree (*Figure 1*) based on ITS sequences is very similar to that of *tef1* sequences. The tree is well resolved and groups are highly supported.

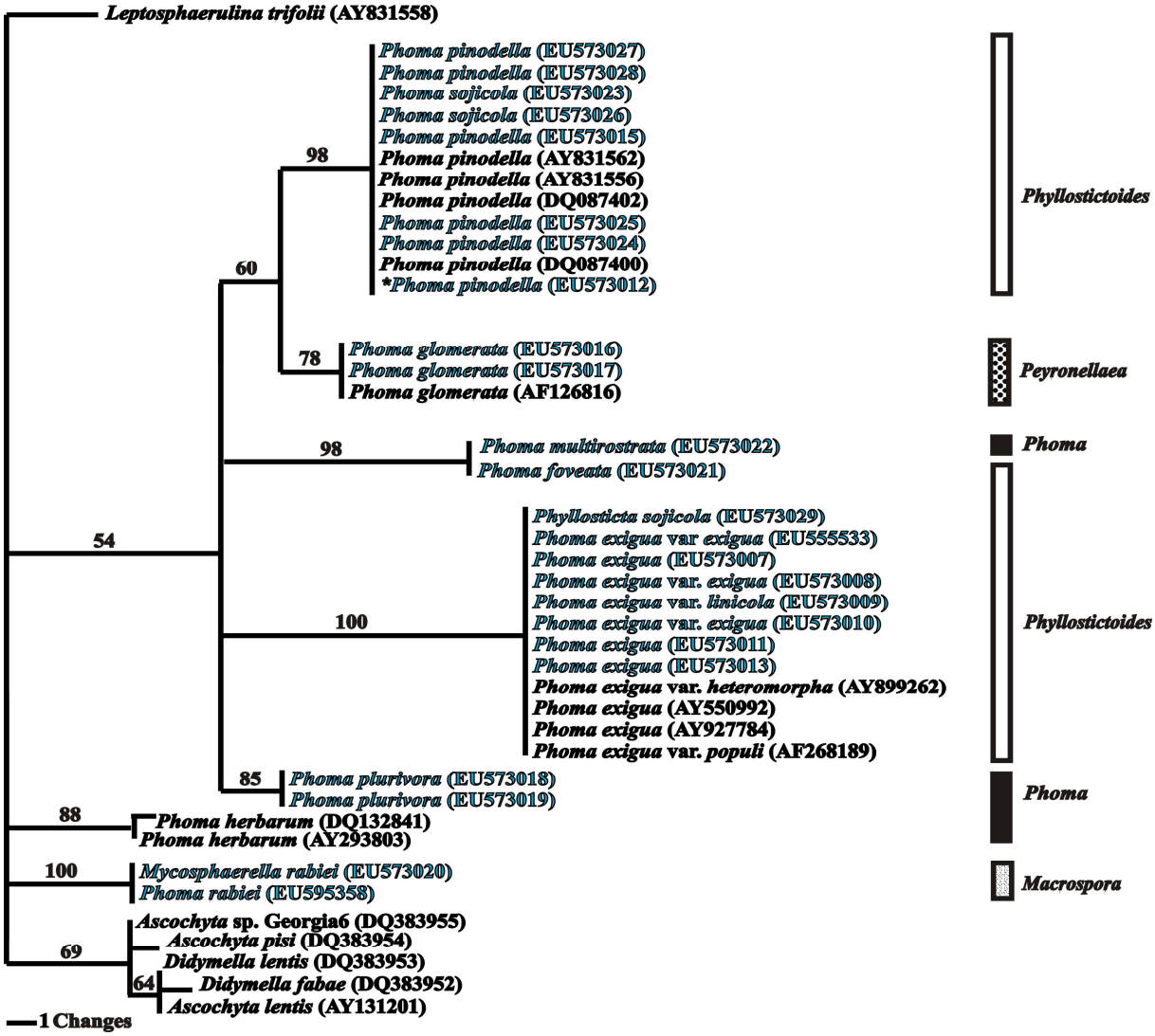
We can state from the parsimony tree that species represented with more than one isolates are placed in the same clade, as we were able to state from the analysis of *tef1* sequences.

The *Phoma pinodella* clade contained the *P. sojicola* sequences (100% BS support), and *Phyllosticta sojicola* grouped together with *Phoma exigua* var. *exigua* (98% BS support).

Ribosomal DNA (rDNA) has long been used as a potential marker for phylogenetic studies (reviewed in Avise, 2004; White *et al*., 1990). Many fungal taxonomy studies have applied ITS regions for resolving relationships at genus and species level (Gottlieb and Lichtwardt, 2001; Nugent and Saville, 2004; Yli-Mattila *et al*., 2004; Voglmayr and Yule, 2006; Morocko and Fatehi, 2007; Pademsee *et al*., 2008; Takamatsu *et al*. 2008). Mendes-Pereira *et al*. (2003) used ITS sequences for studying the molecular phylogeny of *Leptosphaeria maculans–L. biglobosa* species complex. Balmas *et al*. (2005) inferred phylogenetic relationships among isolates of *Phoma tracheiphila* on the basis of ITS sequences as well as Fatehi *et al*. (2003) in *Ascochyta pinodes* complex.

In this study ITS fragments were used to resolve phylogenetic relationships within *Phoma* genus at higher taxonomic levels.

Figure 1: **Phylogenetic relationships of *Phoma* strains inferred by the Parsimony analysis of ITS sequences. Numbers above lines indicate the bootstrap values from 1000 bootstrap samples in Parsimony analysis. The columns on the right side represent the *Phoma* section based on morphological characterization. **P. pinodella* (D/063) misidentified as '*P. exigua* var. *exigua***



Tubulin sequences

β-tubulin sequences were edited to 298bp to aid alignment with sequences downloaded from GenBank. Parsimony analysis of *β-tubulin* (298bp) revealed 49 parsimony informative sites, 20 polymorphic sites and 229 sites were constant among all isolates, when GenBank data were included. The obtained phylogenetic tree (*Figure 2*) is well-resolved with similar clades recovered by *tef1* and ITS analysis.

Similarly to the *tef1* and ITS analysis, species represented with more than one isolates, moreover *Phoma pinodella* clade contained the *P. sojicola* sequences (100% BS support), and *Phyllosticta sojicola* grouped together with *Phoma exigua* var. *exigua* (98% BS support).

Several studies proved that *β-tubulin* at the nucleotide level can be suitable for phylogenetic studies at low taxonomic levels within Ascomycetes (O'Donnell *et al*., 1998; Jong *et al*., 2001; Schoch *et al*., 2001). Voigt *et al*. (2005) used *β-tubulin* gene among other genes to analyze *Leptosphaeria maculans* (anamorph: *Phoma lingam*) species complex, as well as Fatehi *et al*. (2003) to refer molecular relatedness within *Ascochyta pinodes* complex. Partial β-tubulin amino-acid sequences were used by Landvik *et al*. (2001) to assess higher-level phylogenetic relationships in the Ascomycetes, but their results suggest it is less suitable than other genes at this level.

In this study we have used β-tubulin fragments to resolve phylogenetic relationships within *Phoma* genus at higher taxonomic levels.

The phylogenetic analysis of multiple protein-encoding genes with the Genealogical Concordance Phylogenetic Species Concept (GCPSR) is proposed as a more robust way of determining and recognising species than analysis based on morphology or mating behaviour (Taylor *et al*., 2000). Phylogenetic analysis of the two protein-encoding genes, *tef1* and *β-tubulin* together with the complete ITS sequences yielded consensus results. *P. sojicola* isolates always formed one clade with all the examined *P. pinodella* isolates, while representative isolate of *Phyllosticta sojicola* grouped together with *P. exigua* var. *exigua*. The highest resolution could be revealed with the analysis of the *tef1* gene, while sequences of *β-tubulin* showed the lowest polymorphism among the examined *Phoma* species.

Figure 2: **Phylogenetic relationships of *Phoma* strains inferred by the Parsimony analysis of *β-tubulin* sequences. Numbers above lines indicate the bootstrap values from 1000 bootstrap samples in Parsimony analysis. The columns on the right side represent the *Phoma* section based on morphological characterization. **P. pinodella* (D/063) misidentified as '*P. exigua* var. *exigua*'**

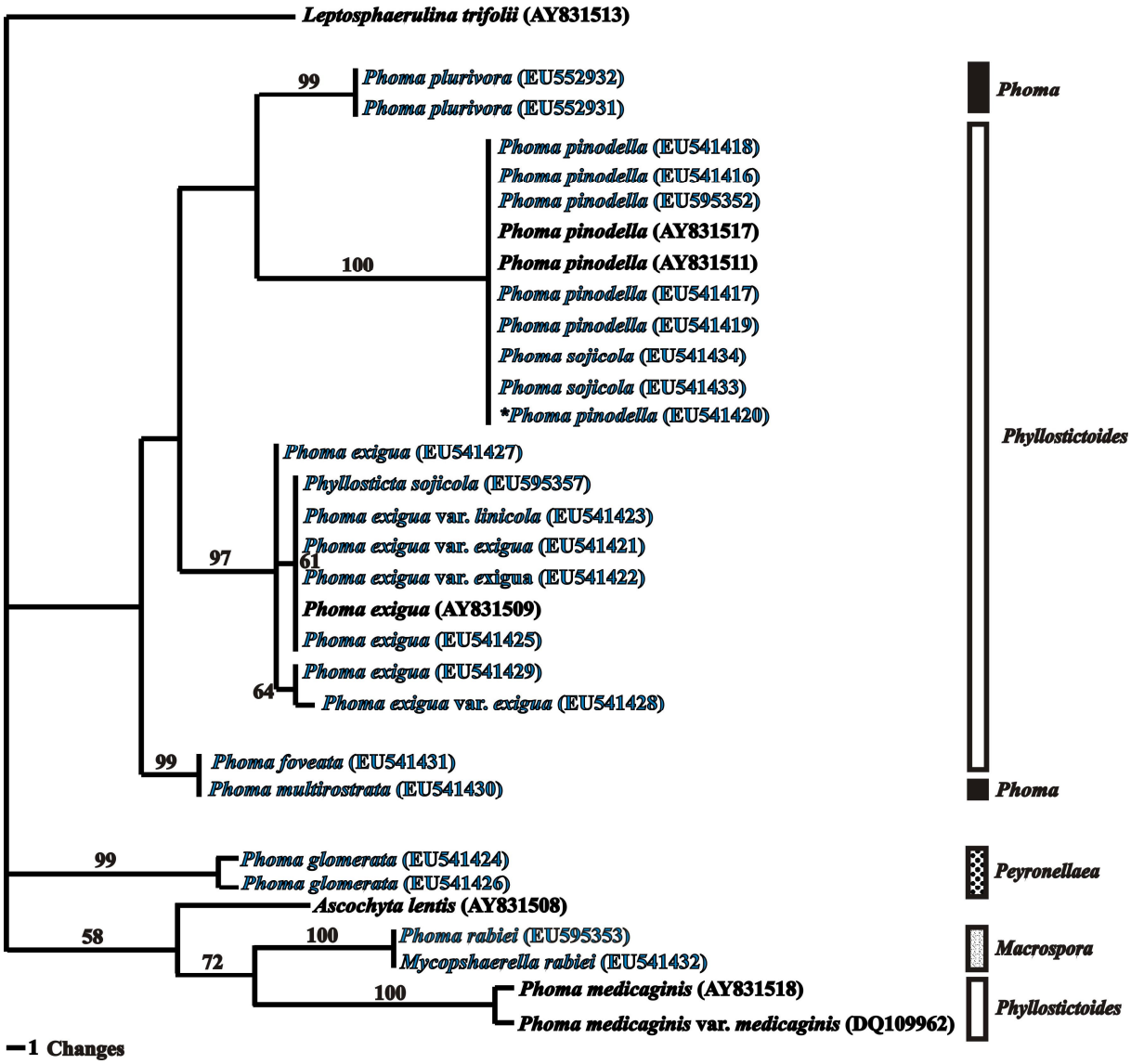
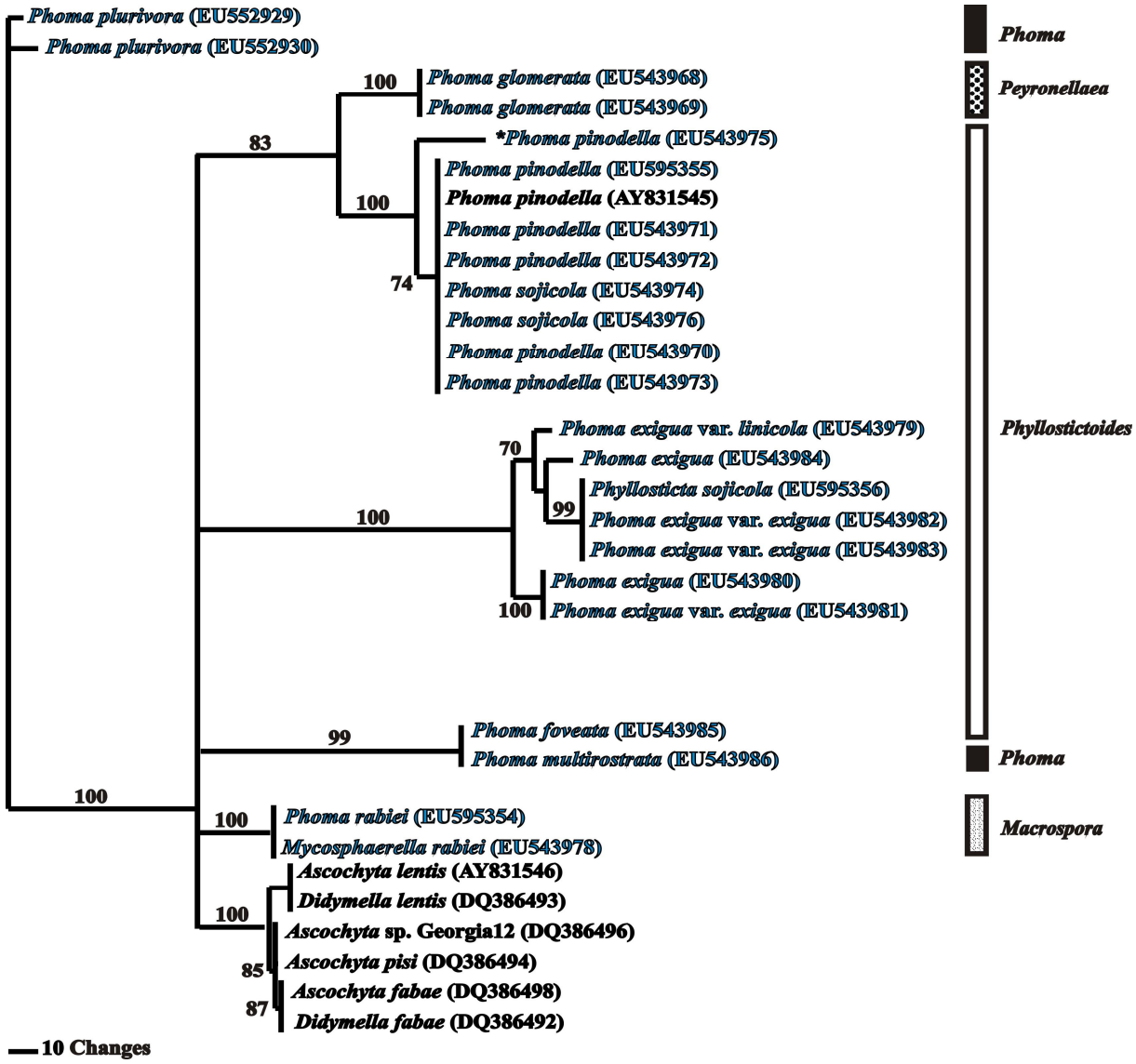


Figure 3: **Phylogenetic relationships of *Phoma* strains inferred by the Parsimony analysis of *tef1* sequences. Numbers above lines indicate the bootstrap values from 1000 bootstrap samples in Parsimony analysis. The columns on the right side represent the *Phoma* section based on morphological characterization. **P. pinodella* (D/063) misidentified as '*P. exigua* var. *exigua***



Translation elongation factor

0.3 kb fragment of the large intron of the *tef1* gene from *Phoma* isolates were amplified sequences and subjected it to a parsimony analysis with PAUP.

Parsimony analysis of *tef1* revealed 173 parsimony informative sites, 16 polymorphic sites and 121 sites are constant among all isolates, when GenBank data were included. The topology of the most parsimonious is shown on the *Figure 3*.

Species represented with more than one isolates are placed in the same clade. The support of both the *P. pinodella* group and the *P. exigua* vars. group was 100%.

Ascochyta isolates formed a separate clade with 100% bootstrap values from the examined *Phoma* species. On the basis of our investigation, carried out with *tef1* sequences all *Phoma* species form a well distinguishable group from the *Ascochyta* species, which proves the monophyletic origin of *Phoma* genus.

The support of both the *P. pinodella* group containing the *P. sojicola* isolated, and the *P. exigua* vars. *exigua* group containing the *Phyllosticta sojicola* isolate was 100%.

Translation elongation factor 1 subunit alpha (EF1α) encoding gene (*tef1*) has been proved to be a useful gene to resolve phylogenetic relationships at species level as well as in deeper divergences of fungi (Roger *et al*., 1999; Druzhinina and Kubicek, 2005). Knutsen *et al*. (2004) used *tef1* gene for phylogenetic analysis of *Fusarium poae*, *F. sporotrichoides* and *F. langsethiae* species complex, as well as Skovgaard *et al*. (2002) to

assess genetic relatedness of *F. oxysporum* complex isolated from pea. However, this gene has not been used for the genetic analysis of the *Phoma* genus yet.

CONCLUSIONS

After refutation of identity of fungi on 'host-specificity' on host-alone, taxonomists very much relied on the morphological charcteristics, both macroscopic and microscopic, which has long been a tradition. However, morphological and cultural studies gave rise to several identical species when studied *in vitro* (e.g. species of *Phoma, Phyllosticta* and *Ascochyta*). Unfortunately, the major drawback of fungal identity based on morphological characteristics is that usually such species comprise more than one species when diagnosed by molecular markers. Therefore, the molecular analyses can be used as supplement to the morphological characteristics. Ribosomal DNA has been used as a potential marker in many genera and species and solved the taxonomic problem partially or fully. But due to high intraspecific rate of mutation sometimes they do not yield much accuracy as required for identity and delimitation of species. The protein encoding genes (*tubulins* and *tef*) being less variable with little chance for mutation, are the better answer for understanding the molecular analyses and phylogeny of fungi. Finally, the best way would be to apply integrated approach of morphological characteristics coupled with rDNA approach and protein encoding genes to revealed the mysteries of identity and phylogeny of fungi with ambiguous taxonomy.

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The results of phytopathological evaluation of woody plants in National Cemetery in Martin in Slovakia

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SUMMARY

Results of phytopathological evaluation of health state and vitality of woody plants in National Cemetery in Martin are listed in this work. Four hundred eighty six trees and shrubs from 25 genera were evaluated. Ninety-four microscopic and wood-decay fungi from 52 genera were detected on hosts. The total health state and degree of damage were evaluated by 6-point scale, which was supplied with damage cause and suggestion for treatment to improve the evaluation of present health state of woody plants. No visible symptoms of damage were on 195 woody plants, they were assessed as healthy. Nineteen woody plants were devaluated by high damage degree. The life expectancy of woody plants was assessed. Thirty one trees had low vitality, 142 woody plants had life expectancy more than 30 or 40 years. After total evaluation of damage degree 37 woody plants (31 trees, stumps and 3 shrubs) were suggested to cut down.

Keywords: fungi, health state, insects, methods of evaluation

INTRODUCTION

The National Cemetery is divided into six sectors A, B, C, D, E, and F. Lindens more than 140 years old border pathways (Bednárik 1972; Rózová and Halajová 2002; Feriancová 2003). The maximal attention must be given to treatment of woody plants in cemeteries. Kolařík *et al.* (2003) and Shigo (1991, 1993, 1994) are engaged in problems of woody plants in urban greenery. Gáper (2005); Tkáčová (2005); Hrubík and Tkáčová (2004a, b); Juhásová et al. (2005) are evaluated originators of the woody plants damage. High-quality greenery must become the unembarrassed part of our life. The urban greenery must have mainly the sufficient health state if it can carry out its role. The aim of this article was to evaluate the health and condition state of woody plants in National Cemetery in Martin.

MATERIAL AND METHODS

Samples were taken from damaged trees and they were processed using common phytopathological methods. Methods according to were used to identify the originators of diseases. The nomenclature of fungal pathogens rules according to Index Fungorum (http://www.indexfungorum.org./). The health and condition state and presumptive lifetime were evaluated at all woody plants. The following signs were evaluated on the trees: woody plant species, circumference of trunk $d_{1.3}$ (in cm), the total health and condition state (degree of damage), damage cause, suggestion for treatment. The total health state and degree of damage were evaluated by 6-point scale:

0- healthy – without symptoms of damage

1. degree – originators of the disease occurred sporadically on evaluated trees

2. degree – occurrence of microscopic and wood-decay fungi cause particular withering of tree, hollows of small size occur in stem, stability is not disturbed

3. degree – in 1/3 size of crown branches dry as the result of infection with fungi or of pest damage. Medium size hollows caused by wood-decay fungi, wood-decay insects, mechanical damage or climatic factors are in stem. The trees are suitable to next cultivation after treatment.

4. degree – drying of branches in ½ size of crown caused by fungal diseases, pests or abiotic agents, dominance of damage on main and structural branches, large hollows occur in the stem, the tree stability is reduced as result of wood-decay caused by fungi. Treatment of hollows, disinfection or to leave to spend the rest of tree life is recommended.

5. degree – totally dry or withering tree in more than 2/3 size of crown. Large rots in stem, stability is markedly disturbed, hew down of tree is recommended.

Based on evaluation of health state of woody plants their vitality was assessed in particular conditions in National Cemetery in Martin. The vigor of trees was expressed by points 0 - 4.

Vigor 0 - must be removed from growth in the shortest time.

Vigor 1 – woody plant will carry out your role circa to 10 years.

Vigor 2 – woody plant will carry out your role more than 20 years.

Vigor 3 – woody plant will carry out your role more than 30 years.

Vigor 4 – woody plant will carry out your role more than 40 years.

RESULTS AND DISCUSSION

Based on our long-time practice we certified that this 6 point scale of damage degree is not sufficient for objective formulation of woody plant state. Therefore degree of damage was supplemented with new method to improve the evaluation of health state of woody plants. Damage of degree was supplied with two other marks: damage cause and suggestion for treatment. Damage cause is expressed by numbers from 1 to 83 and suggestion for treatment by number from 1 to 39. Directly in the field are these numbers entered in table to each evaluated woody plant. The results of phytopathological evaluation of woody plants in National Cemetery in Martin are summarized in *Table 1*. There is a list of microscopic and wood-decay fungi detected on evaluated host woody plants. Ninety four microscopic and wood-decay fungi from 52 genera were detected on evaluated host woody plants. The results of the evaluation of total health state and degree of damage are summarized in *Table 2*. No visible symptoms of damage are on 195 woody plants, they are assessed as healthy. 267 woody plants that are evaluated from damage degree 1 to damage degree 3 are suggested for further cultivation after treatment. 19 woody plants were devalued by high damage degree, 4 and 5. To present all results of the evaluation of health of each woody plant will be very extensive (a total of 486 woody plants). Therefore an example how the total evaluation looks is given in Appendices (the presented woody plants were selected randomly from Sector C). After total evaluation of damage degree and horticultural value 37 woody plants (31 trees, stumps and 3 shrubs) were suggested to cut down.

Table 1.

Phytopathological evaluation of woody plants in National Cemetery in Martin

No.	Tree species	Circumference (in cm)	Damage degree	Life	Damage cause	Suggestion for treatment
0	*Ligustrum vulgare* L.		0	1		0
1	*Thuja occidentalis* L.	15	0	2		0
2	*Tilia platyphyllos* Scop.	417	2	4	1,2	1,2
3	*Tilia platyphyllos* Scop.	178	3	3	1,2,5a,13a,14	1,2,6
4	*Tilia platyphyllos* Scop.	180	3	3	1,2,5a,13a,14	1,2,6
5	*Ulmus glabra* Huds.	207	3	2	1,2,9a(80x40x20),31, 74b(5cm),79	1,2,4,36b

Table2.

The purport of numbers of additional mark damage cause used in evaluation of health state of woody plants.

No	Purport
1	dry thin branches
1a	lateral branches shielded with other trees
1b	inside the tree crown
1c	lower shielded branches
2	dry structural branches
2a	lateral branches shielded with other trees
2b	inside the tree crown
2c	lower shielded branches
5a	wound on branches
5b	wound on stem
5c	wound on the basis of stem
9	hollow in basis of stem
9a	open hollow (in cm)
9b	close hollow (in cm)
9c	callusing margins
9d	continuous hollow

No	Purport
13	Broken branches
13a	broken branches hang on the crown of the trees, the safety of people is threaten
13b	branches broken by wind
14	Simple break of the branch
31	Pests
74	The tree grows in the immediate vicinity
74a	Of the house
74b	Of the brick fence
74c	Of the chain link fence
74d	of the headstone in cemetery
74e	of the pathway
74f	of the thoroughfare
74g	of the bench at headstone
74h	electrics over the tree crown
79	To review the health state during reconstruction

No	Purport
1	To prune dry and attacked branches
2	To prune structural branches
4	To treat hollows

6	To treat wounds after break branches
36	To make thinning of the thick growth
36a	of trees
36b	of shrubs

According to Garton and Tankersley (2008) the condition of tree is determined by evaluating its structure and state of health. The health state is determined with % of damage (minor or major insect and disease problems) (Radócz, 2002). No detailed data about the health state of evaluated tree are available from this rating. More methodologies are devised for tree condition rating, but from them no detailed data about health state of tree are available. Our method of phytopathological evaluation gives a complete picture of woody plant and it is a part of a total tree condition rating.

Table 3

Results of phytopathological evaluation of woody plants in National Cemetery in Martin

Name of woody plant	Name of fungus
Acer platanoides L.	*Ascochyta acericola* Massa
	Cercosporella acerina (Hartig.) Arn.
	Cylindrosporium acerinum Tracy & Earle
	Gloeosporium acericola Allesch.
	Marssonina truncatella (Sacc.) Magn.
	Mycosphaerella aceris Woron.
	Mycosphaerella latebrosa (Cke.) Schröet.
	Phyllosticta aceris Sacc.
	Rhytisma acerinum (Pers. ex St. Amans)
	Rigidoporus populinus (Schum. ex Fr.) Pouzar
	Sawadea bicornis (Wallr. ex Fr.)Fr.
	Septoria acerinum Pk.
	Trametes unicolor (Bull. ex Fr.) Pilát.
	Verticillium alboatrum (Hartig) Arn.
Aesculus hippocastanum L.	*Asteromella aesculi* (Sacc.) Petr.
	Cytospora ambiens Sacc.
	Ganoderma resinaceum Boud.
	Guignardia aesculi (Peck) Stewart.,
	Mycosphaerella aesculi (Cocc. & Morini) Tomilin
	Nectria cinnabarina (Tode ex Fr.)
	Phellinus pomaceus (Pers.) Maire
	Phyllosticta sphaeropsidae Ell. et Ev.
	Septoria aesculicola (Fr.) Sacc.
	Valsa ambiens Sacc.
	Verticillium alboatrum (Hartig) Arn.
	Vuilleminia comedens (Nees.) Maire
Aucuba japonica Thunb.	*Phomopsis aucubae* Grove
Betula alba L.	*Cytospora betulicola* Fautr.
	Gloeosporium betulae Fckl.
	Libertella betulina Desm.
	Marssonina betulae (Lib.) Magn.
	Phlyctaena albocincta (Mont.) Desm.
	Phyllactinia guttata (Wallr. ex Schlecht.) Lév.
	Quaternaria quaternata (Pers. et Fr.) Schröet.
Berberis vulgaris ´Atropurpurea´	*Microsphaera berberidis* (DC. ex Mérat) Lév.
	Mycosphaerella berberidis (Auersw.) Lindau
	Phoma berberidicola Vestergr..
	Septoria berberidis Niessl.
Caragana arborescens Lam.	*Microsphaera palczewski* Jacz.
Forsythia suspenza (Thub.)Vahl.	*Ascochyta forsythiae* (Sacc.) Höhn.
	Phyllosticta forsythiae (Sacc.) Höhn.
Fraxinus excelsior L.	*Phyllactinia guttata* (Wallr. ex Schlecht.) Lév.
Fraxinus ornus L.	*Cercospora fraxini* (DC.) Sacc.
	Fusarium lateriticum Nees.
	Mycosphaerella fraxini (Niessl.) Mig.
	Nectria cinnabarina (Tode ex Fr.) Fr.

Name of woody plant	Name of fungus
	Phyllosticta fraxinicola (Curr.) Sacc.
Hedera helix L.	*Colletotrichum hedericola* Laub.
	Gloeosporium hedericolum Maubl.
	Phoma hedericola (Dur. et Mont.) Boerema
Ilex aquifolium L.	*Cercospora ilicis Ellis*
	Coniothyrium ilicis A.L. Sm. & Ramsb.
	Phacidium aquifolii (DC.) J.C. Schmidt & Kunze
	Phytophthora sp.
Larix decidua Mill.	*Cytospora* sp.
	Lachnellula willkommii (Hartig) Dennis
Ligustrum vulgare L.	*Mycosphaerella ligustri* Lind.
	Phomopsis brachyceras Grove.
	Septoria ligustri (Desm.) Fckl.
	Verticillium dahliae Kleb.
Mahonia aquifolium (Pursch.) Nutt.	*Cumminsiella sanguinea* (Peck) Arthur
	Microsphaera berberidis Dietr.
Picea pungens Engelm.	*Armillaria ostoyae* (Romagn.) Herink
	Botrytis cinerea Pers.
	Heterobasidion annosum (Fr.) Bref.
Pinus sylvestris L. *Pinus mugo* Turra	*Brunchorstia pinea* var. *pinea* (P.Karst.) Höhn. (anamorpha *Gremmeniella abietina* var. *abietina* (Lagerb.) M.)
	Diplodia pinastri Grove
	Lophodermium pinastri (Schrad.) Chevall.
Prunus laurocerasus L.	*Coryneum beyerinckii* Oudem.
	Trochila laurocerasi (Desm.) Fr.
Rosa sp.	*Marssonina rosae (Lieb.) Died.*
	Phragmidium subcorticium (Schrank) G. Winter
	Sphaerotheca pannosa (Wallr.) Lév.
Thuja occidentalis L.	*Didymascella thujina* (E.J. Durand) Maire (syn. *Keithia thujina* E. J. Durand)
Thuja occidentalis 'Ericoides'	
Thuja occidentalis 'Aurea'	
Thuja orientalis L.	
Thuja plicata D. Don.	
Tilia cordata Mill. *T. tomentosa* Moench *T. platyphyllos* Acop.	*Alternaria tenuis* Nees
	Cladosporium cladosporioides (Fresen.) G.A. de Vries
	Gloeosporium tiliae Oudem.
	Gnomonia tiliae Rehm
	Mycosphaerella millegrana Desm.
	Pullularia pullulans (de Bay et Loew) Berkl.
Yucca filamentosa L.	*Coniothyrium concentricum* (Desm.) Sacc.

The results of evaluation of the life expectancy of woody plants are summarized in *Table 3*. Based on obtained results 31 trees have vigor 0. There is no presupposition to improve their health state after treatment. 163 woody plants have also low vigor (point 1) (up to 10 years). There were assigned almost over aged shrubs that must be changed gradually. After treatment 146 trees have the life expectancy 20 years. 142 woody plants have life expectancy more than 30 or 40 years. Some health trees had low life expectancy. These trees were planted near to headstones and in short time can threaten them. Insufficient attention was paid to treatment of woody plants in National Cemetery in Martin in previous years. Dry thin and thick branches are in the tree crowns, wounds and hollows in all size occur on branches and stems. Branches are broken, some of breaks are simple, and some of them reach to the stem. The reason of poor health state of evaluated woody plants is interventions during maintenance of access paths, walkways to headstones and memorials. The roots were damaged during these activities. The mighty trees were planted close to memorials and headstones. The native role of roots is mechanical (to fasten the plant in soil), sorptive (to take water and soluble mineral matter from soil) and conductive (water and soluble mineral matter distribution into over ground parts of plant). Root capillaries which have sorptive role are very important. Damaged roots were disturbed physiological balance of trees, which were weakened. It was the reason of withering of thin and thick constructional branches in the tree crowns (Tarcali and Radócz, 2007). All trees assigned to 2 and 3 damage degree must be treated. Trees assigned to 4 and 5 damage degree were meant to cut down because they threat the safety and health of visitors and there

is a danger of material damage on headstones after unexpected breaking of branches or whole tree crown (*Table 4*).

Fungi from *Mycosphaerella* genus were often occurred on evaluated locality (on *Acer, Aesculus, Berberis, Fraxinus, Hedera, Ilex, Ligustrum, Tilia).* Their anamorph states cause small, round spots with light yellow border on leaves. Ascopores cause the initial infection, conidia and pycnospores cause secondary infection during the growing season (Tarcali, 2007). Fungi from genera *Microsphaera, Erysiphe, Phyllactinia, Podosphaera* reduce aesthetical value of woody plants and cause intense floury coat on leaves and shoots (of *Caragana, Acer, Berberis*, *Mahonia*). Saprophytic fungi causing blackness on species from genus *Tilia* reduce aesthetical and decorative value, simultaneously excessively damage memorials and headstones that cover with black slimy coat (*Table 5*).

Table 4

The results of evaluation of damage degree of woody plants in National Cemetery in Martin.

Sector	Damage degree							
	0	1	2	3	4	5	stump	total
A	45	37	23	19	5	2	0	131
B	47	28	12	14	4	0	0	105
C	47	31	19	10	2	0	0	109
D	27	18	2	6	4	0	0	58
E	20	14	3	2	0	0	1	40
F	9	16	8	5	2	0	3	43
Total	195	144	67	56	17	2	4	486

Table 5

Results of evaluation of the expected life of woody plants in National Cemetery in Martin

Sector	Woody plants life						
	0	1	2	3	4	stump	total
A	9	48	40	29	5	0	131
B	5	54	29	14	3	0	105
C	0	29	34	29	17	0	109
D	15	5	25	9	4	0	58
E	1	12	12	12	2	1	40
F	1	14	6	12	6	3	42
Total	31	163	146	105	37	4	486

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Common smut disease of maize and its development by downy mildew infection

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SUMMARY

A positive relation was obtained between downy mildew and common smut disease of maize. Downy mildew disease played an important role in spreading common smut disease by increasing the plant regulator IAA content in the plant which helps the infection by U. maydis. Spraying of maize plants twice by growth regulators gibberellic acid (GA) at 25, 50 and 100 ppm and naphthy acetic acid (NAA) at 10 and 20 ppm increased significantly infection (%) and disease index of common smut disease.

Keywords: maize, common smut, downy mildew, *Ustilago maydis*

INTRODUCTION

Interactions between downy mildew disease caused by *Prenosclerospora sorghi* and common smut disease incited by *U. maydis* were studied by Tolba and El-Sayed (2005). They reviewed that, the disease incidence % of maize common smut disease was much higher in maize plants infected by downy mildew (DM) disease especially on tassel. The production and role of auxin in plant disease have been studied in fungal and bacterial diseases of plants. Most of these studies demonstrated that indole acetic acid (IAA) was involved in the development of disease symptoms, such as corn smut induced by *Ustilago maydis*, club root induced by *Plasmodiophora brassica* or crown gall induced by *Agrobacterium tumefaciens* (Agrios ,1994, Ludwing-Muller and Hilgenberg, 1990 and Schmidt *et al.*,1996). Moreover, Couvreur *et al.* (1991), Couvreur and Herman (1993) and Merrien (1994) found that, growth regulators (parlay C, tri-apenthenol and ethephon) increased the disease incidence and susceptibility to Sclerotinia on oil seed rape, barley and sunflower leading to heavy yield losses. In addition, Ouf *et al.* (1994) reported that, infection of susceptible maize cv. Balady and resistant cv. Pioneer-514 with virulent strain of *U. maydis* increased the IAA content in plant tissue. Also, Saxena (1983) and Khangura and Sokhi (1995) reported that, a higher level of gibberellic acid, zeatin, abscic acid and a lower level of IAA had been found in *Brassica* and *Eruca sativa* infected with *Albugo* and *Albugo-Peronospora,* respectively, than healthy ones.

The objective of the present work is to study the relationship between common smut and downy mildew of maize.

MATERIALS AND METHODS

Preparation of sporodial suspension

Smut galls were collected from maize plants infected by *U. maydis* grown in different governorates in Egypt during the growing season 2002. Galls were transported to the lab. where galls were air dried and stored at room temperature. To isolate the haploid sporidial stage, teliospores were treated with steam of chloroform for 1 min. to activate the germination, then rinsed in 2% copper sulfate solution for 24-48 hrs, it was plated at low densities on PDA (Day and Anagnostakis, 1971). Plates were incubated for 2-5 days at 27^{o}C causing teliospores to undergo meiosis and produce probasidia and sporidia. Pure monosporidial cultures of each teliospore were picked and kept on PDA slants at 4^{o}C for further work. Ten monosporidial isolates were selected from each governorate.

Preparation of inocula

Ten isolates of *U. maydis* (represent different governorates) each consists of mixture of eight isolates of monosporidial stage which isolated as mentioned above. For all tested isolates, inocula were prepared by using PD broth medium. Glass bottles of 500 ml containing 100 ml medium were autoclaved for 15 min., then inoculated by 2 ml of 10^6/ml sporidial suspension of *U. maydis*.isolates Glass bottles were incubated at 27^{o}C for 10 days. Inoculum was prepared by flooding the sporidia cultures of each isolate with sucrose solution 5%.contaning 0.1 ml Tween 20 per 100 ml. Germination of sporidia spores of *U. maydis* were stimulated by sucrose (Tseng, 1988). The suspension was filtrated through two layers of cheese cloth, sporidia were counted by hemacytometer and suspension was adjusted to 10^6 sporidia/ml.

Field experiment

Seeds of maize cvs., single crosses (SC) 10, 11, 12, 13, 14, 122, 123, 124, 129, 155; three way crosses (TWC) 326, 327, Double crosses (DC) 2010, 3040, 3080; sweet corn , open pollinations Giza 2 (G_2) and forage genotypes, *Teissent maxicana* were planted. Randomized complete block design with three replicates was used.

Each plot included two rows 3 m long at 70 cm distance and 20 hills sown by 3-5 seeds/hill, thinned to one plant/hill after three weeks. The experiment was carried out during two successive seasons 2004 and 2005 under artificial inoculation. The plants were inoculated by sporidial suspension as aforementioned after 21 days from planting. All cultural practices were applied at the proper time. Disease was recorded as infection percentage and disease index (DI).

Disease assessment

After 30 days from inoculation, the disease readings were estimated and expressed as percentage of diseased plants (infection %) according to the equation adopted by Khalil (1973) as follows:

$$\text{Infection \%} = \frac{\text{No. of infcted plants}}{\text{Total no. of plants (infected + healthy)}} \times 100$$

Diseased plants were classified into eight classes according to the size of gall in order to have the disease severity expressed as disease index (DI) as adopted by Khalil (1973) as follows:

$$\text{DI} = \frac{\sum(\text{NPC} \times \text{CR})}{\text{NIP} \times \text{MSC}} \times 100$$

where: NPC = No. of plants in class rate, CR = Class rate, NIP = No. of inoculated plants and MSC = Maximum number of class rate

The following is the suggested class rates:

0 = no infection.
1 = galls less than 1 cm in diameter.
2 = galls more than 1 cm and less than 2 cm in diameter.
3 = galls more than 2 cm and less than 3 cm in diameter
4 = galls more than 3 cm and less than 4 cm in diameter
5 = galls more than 4 cm and less than 5 cm in diameter
6 = galls more than 5 cm and less than 6 cm in diameter
7= galls more than 6 cm and less than 7 cm in diameter
8 = galls more than 7 cm and less than 8 cm in diameter

Interaction between downy mildew and common smut

a. Pathological relation

The aim of this experiment was to study the effect of downy mildew infection on development of common smut.

Balady maize cv. and Single Cross 129 which have been known to be highly susceptible to common smut and resistant against downy mildew were used in this study. The untreated and treated seeds by the fungicide apron (2 gm,/kg seeds, as recommended) against downy mildew disease were sown in field under artificial inoculation condition of downy mildew using Giza 2 sorghum cv. as a spreader.

Randomized complete block design with three replicates was used. Each plot was included tow rows 6 m long at 70 cm distance. After 25 days from planting (symptoms of downy mildew were observed), healthy and infected plants by downy mildew disease were inoculated by suspension of *U. maydis* isolate no.3. The experiment was carried out during two successive seasons, 2004 and 2005. Disease readings of downy mildew were recorded as percentage of infection. Disease readings of common smut disease for plants which were infected or uninfected by downy mildew were also recorded as mentioned before. All cultural practices were applied at proper time.

b. Phytohermone content relation

Four samples of Balady maize cv. were collected from the above mentioned experiment to analysis of the phytohermone content of indole acetic acid (IAA). The samples were as follows: leaves of maize plants infected by downy mildew (DM), leaves of infected plants by both DM and common smut, leaves of infected plants by only common smut and leaves of healthy plants as control. To extract IAA, 20 g fresh green leaves were macerated in pre chilled methanol at 4^{o}C and left for 24 hrs. This was filtrated and the filtrate was evaporated at 45^{o}C to a small volume and refiltrated through celite. This was then fractionated by extraction into ether at pH 3.0. These fractions were concentrated, tested to IAA and bioassay as described by Witham *et al.* (1971) to measure the IAA content. The standard procedure was to study the effect of IAA concentrations on growth of avena coleoptile sections. Coleoptiles (5 mm), cut from avena seedlings grown in red light, were first washed with basal medium (2% sucrose on 0.01 M KH_2PO_4 buffer pH 4.5) to remove endogenous auxin and were then treated for 10 hrs with basal medium containing different strengths of the isolated IAA material. Increased lengths of sections over control sections can be related to the auxin conc. The fraction of IAA was used in four conc. i.e. 20, 40, 60 and 80% with 10 sections of avena coleoptile for each treatment.

c. Effect of specific growth regulators on common smut disease development

Growth regulator substances of oras [2-naphthy acetic acid (NAA) at 1.57% + 2- naphthoxy acetic acid at 0.95% + 4B at 0.1%] at concentrations 10, 20 and 40 ppm of active ingredient NAA and perlix [gibberellic acid (GA) 10%] at 25, 50 and 100 ppm of active ingredient GA were used. These substances were sprayed once at 15 days and twice at 15 and 25 days from planting date of maize plants cv. Balady. Tween 20 was used as wetting agent at 0.5%. Control treatment was sprayed with water containing only the Tween 20. All treatments were inoculated by *U. maydis* after 30 days form planting. The experiment was performed twice in greenhouse during season 2006. Maize seeds were sown in pots 30 cm diameter, containing 8 kg soil and 12 seeds. Three pots were used for each treatment, in complete randomized design. Inoculum preparation was prepared as mentioned before. Disease grades, length of plants and plant fresh weight were recorded after 50 days from planting.

RESULTS

Interaction between downy mildew and common smut disease

a. Pathological relation

Data presented in *Table 1* showed that, a positive relationship between downy mildew (DM) disease caused by *Perenosclrospora sorghi* and common smut disease caused by *U. maydis* (isolate no.3). The infection (%) of DM which was estimated 62.21% in maize plants Balady cultivar (highly susceptible) resulted to high disease incidence of common smut of 38.68% and disease severity (disease index) of 26.91 under field artificial inoculation by both diseases in season (2004). On the other hand, plots treated with fungicides against DM disease showed a significant decrease of common smut infection % In general the highly susceptible maize cv. Balady by downy mildew disease had high infection (%) by common smut disease and disease index. Concerning the use of resistant maize cv. Single Cross 129 against downy mildew and smut, a relationship between both diseases was also observed. When, the infection (%) of downy mildew in the field was 8.66%, an increase of infection (%) of 37.07 and Disease index (DI) of 28.7 by common smut was reported. On the other hand, the treated plots against DM which were inoculated by common smut disease had infection (%) of 10.30 and 10.04 and disease index (DI) of 8.60 and 9.7 % in seasons 2004 and 2005 respectively. Data summarized that infection by downy mildew disease in the susceptible and resistant maize cvs led to significant increase in both infection% and disease index of common smut disease.

Galls of common smut appeared in DM infected plants 2-3 days earlier compared DM free plants and infected by common smut. Maximum size of galls was observed in plants infected by DM after 8-10 days from the inoculation date by *U. maydis* and after 20-30 days in plants inoculated by common smut and free from downy mildew.

Table 1

Effect of downy mildew on infection development of maize common smut disease, on Balady cv. and Single Cross 129 during seasons 2004 and 2005

Cultivar and genotype	2004 season			2005 season		
	DM %	CM %	DI	DM %	CM %	DI
Balady (susceptible)	62.21* c 0.0** a	38.68 c 21.61 b	26.91 d 22.57 c	79.16 d 0.01 b	68.58 d 21.46 b	47.79 d 23.68 b
SC129 (resistant)	7.05* b 0.00** a	20.23 b 10.30 a	14.86 b 8.60 a	8.66 c 0.00 a	37.07 c 10.04 a	28.70 c 9.70 a

*Seeds non treated by apron.

** Seeds treated by apron.

DM: Downy mildew disease (*Perenosclerospora sorghi*)

CM: Common smut disease.

In the same column, means followed by the same letters are not significantly different according to DMRT at 5% level of signficancy.

b. Phytohermone content relation

Data in *Table 2* show that IAA extracted from maize plants infected by common smut only (at concentrations from 20, 60 and 80%), from maize plants infected by both DM and smut (at concentrations of 40 and 80%) and from maize plants infected by downy mildew alone (at concentrations of 20, 60 and 80) had a positive effect on increasing the length of avenae coleoptile cuttings comparing to healthy plants (control).

Table 2

Effect of four concentration of IAA extracted from maize plants infected by downy mildew, downy mildew and common smut and common smut on avenae coleoptile length (mm).

Type of infection	IAA extract concentrations			
	20%	40%	60%	80%
	Avenae coleoptile length (mm)			
Downy mildew (DM)	5.70 c	5.74 b	5.64 c	5.44 c
DM + smut	5.84 d	5.92 c	5.95 d	5.28 b
Smut	5.56 b	5.96 d	5.42 b	6.04 d
Control	5.48 a	4.68 a	5.31 a	5.24 a

c. Effect of specific growth regulators on common smut disease development

The results in *Table 3* show that, spray application of Per lix (commercial product of gibberellic acid at 10%) once at 50 ppm and twice at 25, 50 and 100 ppm as well as spray application of Oras (NAA), (commercial product of 2-naphthy acetic acid at 1.57% and 2-naphthoxy acetic acid at 0.95%) twice at 10 and 20 ppm increased significantly the infection percentage of common smut disease. The infection percentage ranged from 43.75 to 51.60% compared to 35.0% with maize plants untreated by growth regulators (control). The other tested concentrations of growth regulators had no effect in increasing of infection percentages.

As for disease index, treatment by exoheromone GA and NAA had a positive effect in increase of common smut diseases and led to an increase in disease index. The least effect was recorded with spray application once and twice of NAA at 40 ppm. The most effective treatment was spray application of exophermone GA once at 100 ppm, which recorded disease index 35.70 followed spray application of GA once at 50 ppm.

Table 3

Effect of exohermones treatment on common smut disease development and plant growth characters of Balady maize cv. in greenhouse experiment

Exoheromones	Conc. ppm	Times of spray application	Infection %	Disease index	Plant length (cm)	Fresh weight (g)
Per lix* (GA)						
	25		33.81 ab	18.5 c	66.6 de	202.22 d
	50		51.60 f	25.5 e	63.13 cde	143.27 b
	100		36.10 abc	35.7 f	87.4 h	1111.00 a
		Once				
Oras** (NAA)	10		30.66 a	14.48 a	59.23 bcd	192.13 cd
	20		41.66 b-e	23.21 d	57.0 bc	170.22 c
	40		37.47 abc	17.46 b	52.07 ab	212.23 de
Per lix* (GA)						
	25		49.62 ef	26.0 e	78.83 g	211.44 de
	50		46.75 def	23.32 d	97.56 i	259.30 fg
	100		43.75 c-f	25.33 e	74.76 fg	174.19 c
		Twice				
Oras** (NAA)	10		49.64 ef	23.16 d	69.07 ef	237.37 ef
	20		46.69 def	18.73 c	88.78 h	273.00 g
	40		39.28 bcd	16.53 b	99.33 i	286.56 g
Control			35.0 ab	16.53 b	48.80 a	262.66 fg

*: Per lix, commercial product of gibberellic acid at 10%.

**: Oras, commercial product of 2-naphthy acetic acid at 1.57% and 2-naphthoxy acetic acid at 0.95%.

In the same column, means followed by the same letters are not significantly different according to DMRT at 5% level of signficancy.

DISCUSSION

In relation to pathological relation of downy mildew (DM) and common smut diseases, the obtained results confirmed that infected corn plant by DM in resistant (Single Cross 129) and susceptible (Balady) cultivars led to significant increase of infection % and disease index of common smut disease compared to corn plants which were not infect by DM and inoculated by *U. maydis* in the field. The obtained data showed also early appearance of smut gall and great increase in gall size (DI) in corn plants which were infected by DM and inoculated by *U. maydis* compared to other ones, which were not infected by DM and inoculated by *U. maydis*. Similar results were obtained by Tolba and El-Sayed (2005) who found that maize common smut disease was spread in plants which infected by downy mildew disease especially on tassels of susceptible maize genotypes. They added also that infection by (DM) significantly increased total and free phenols, total and reduced sugar, protein, ash, free fatty acids and acidic value in the infected tassels by DM, so it became very suitable to be invaded by *U. maydis*.

A positive relationship had been shown between two tested diseases i.e. downy mildew and common smut. Downy mildew plays a real role in spread common smut disease by increasing the plant phytohormone (IAA) which led to increase the length of avenae coleoptile (mm). The obtained results showed also infection by common smut alone and/or downy mildew increased IAA content compared to healthy corn plants. The obtained results were supported by the findings of Ludwing-Muller and Hilgemberg (1990) and Schmidt *et al.*(1996), who reported that IAA was involved in the development of disease symptoms such as corn common smut induced by *U. maydis*, club root induced by *P. brassica* or crown gall induced by *A. tumefaciens*. Also, Saxena (1983) and Khangura and Sokhi (1995) reported that high levels of gibberellic acid, zeatin, abscic acid and lower level of IAA had been reported in *Brasica* and *Eruca sativa* infected with *Albugo* and *Albugo-Poronospora*, respectively, than healthy one.

As for the effect of growth regulators on common smut disease development, the results showed that treatment of spray application twice with plant growth regulators GA at 25, 50 and 100 ppm and NAA at 10 and 20 ppm significantly increased the infection % and disease index of common smut disease. The obtained results were in agreement with those reported by Couvreur *et al.* (1991), Couvreur and Herman (1993) and Merrien (1994), who found that growth regulators (parlany C, triapenthenol and ethephon) increased the disease incidence and susceptibility to Sclerotinia on oil seed rape, barley and sunflower leading to heavy yield losses. In contrast, the present results are in disagreement with Zaky *et al.* (2002) who showed that IAA and Kinetin induced the resistance in spearmint plants against rust disease (*Puccinia menthae*).The obtained results were in agreement with those reported by Mendez Moran *et al* (2005) and Sosa Morales *et al.* (1997) who found that IAA is synthezed by *U. maydis in vitro*. They added that an increasing symptom of the infected Arabidopisis plants induced by an excess of IAA production by *U. maydis* or by the plant through stimulation by the fungus and transported by the plant. The obtained results were in disagreement with Ueno *et al.* (2004), who reported that barley leaves pretreated with IAA solutions and inoculated with *M. gersea* after 24 hrs pretreatment led to inhibit both blast lesion and infection hyphae formation significantly.

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Studies on yearly variations of the dominance of cereal viruses in winter barley breeding lines of Kompolt

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SUMMARY

During 2006-2009 the incidence of Barley yellow dwarf viruses (BYDV-MAV, BYDV-PAV, BYDV-RMV, BYDV-SGV), Cereal yellow dwarf virus (CYDV-RPV) and Wheat dwarf virus (WDV) was studied in cereal species for the determination of virus dominance. Surveys were carried out at Kompolt, on winter barley breeding lines showing leaf yellowing and stunting symptoms. In 2006 490 winter barley samples were tested. In 2007 the number of samples collected were 500 from winter barley. In 2008 500 winter barley samples were tested. In 2009, 100 winter barley samples were collected for virus testing. Virus diagnosis was carried out using DAS-ELISA for the detection of Barley yellow dwarf viruses (BYDV-MAV, BYDV-PAV, BYDV-RMV, BYDV -SGV), Cereal yellow dwarf virus (CYDV-RPV) and Wheat dwarf virus (WDV). The results obtained for barley breeding line showed that virus dominance varied from year to year.

The yearly dominance of BYDV-MAV, BYDV-PAV, BYDV-RMV, BYDV-SGV, CYDV-RPV and WDV in symptom-showing samples of winter barley breeding lines of Kompolt varied, a contrasting tendency can be observed between the incidence rates of WDV and BYDVs. With arise of incidence in the WDV, the proportion of BYDV decreased and vice-versa.

Keywords: winter barley, yearly variations of the dominance, cereal viruses, BYDV, CYDV, WDV

INTRODUCTION

In recent years viral diseases have become more frequent on cereals in Hungary. Approximately 90 different viruses of *Poaceae* are described of which about 60 are present in Europe. In Hungary almost 13 viruses of cereals and grasses are known which impose a threat to cereals, of which only some are of economic importance. The most frequent of these are *Barley yellow dwarf viruses* (BYDV-MAV, BYDV-PAV, BYDV-RMV, BYDV-SGV, *Cereal yellow dwarf virus-RPV* (CYDV-RPV) and *Wheat dwarf virus* (WDV), which are thus able to cause the greatest quantitative and qualitative damages. BYDVs and CYDV-RPV are the most economically important and widespread virus pathogens of cereal crops in the world.

Several strains or serotypes of BYDV have been differentiated on the basis of vector specificity, the efficiency of transmission by aphids, serological and molecular biological properties. Rochow (1969) differentiated four strains of BYDV by their relative vector specificity in transmission to the oat, variety Coast Black and in virulence on the host plant.

These strains were named according to their predominant aphid vectors. One strain (RPV) was specifically transmitted by *Rhopalosiphum padi*, the second strain (MAV) specifically by *Macrosiphum avenae* and the third strain (RMV) specifically by *Rhopalosiphum maidis.* The fourth strain (PAV) was transmitted non-specifically by both *Rhopalosiphum padi* and *Macrosiphum avenae.* Gill (1969) described a fifth strain of BYDV in Manitoba, which was specifically transmitted by *Schizaphis graminum.* This vector-specific strain was weakly virulent in the oat variety Clintland 64. There are many reports concerning the strain incidence and dominance of BYDV strains in different cereal species in the United States. In Illinois, Azzam and D'Arcy (1989) reported that PAV was the prevailing strain, while the RPV and MAV strains occurring at low frequency. In Idaho, Foster *et al.*(1990) reported that the SGV strain appeared to play a significant role in BYDV epidemiology during 1977 and 1985. Hewings and Eastman (1995) reported that surveys on the incidence of BYDV strains throughout North America suggested that PAV was the dominant strain in most areas but that the incidence of the strain alone and in mixed infections differed from region to region and year to year. In areas where the PAV strain usually dominated, other strains occurred occasionally in epidemic proportions. Haber (1990) stated that a PAV-like serotype was also the most common BYDV strain identified in surveys in west-central Canada in recent years. It should be mentioned that BYDV was first identified in Europe, in the Netherlands by Oswald (1951) and was confirmed in the UK (Watson and Mulligan, 1957). In Spain, the first surveys on BYDV incidence in the main cereal growing areas were carried out in 1985 and 1986 based on ELISA for PAV, MAV and RPV serotypes. The PAV serotype was the most common, followed by RMV. The MAV serotype was only found in the central regions (Fereres *et al.* 1989). Subsequent surveys (1987-1989), using only antisera of PAV and RPV, confirmed the presence of the PAV and RPV strains. RPV was particularly frequent in wild grasses. The frequency of PAV strain in winter cereals was variable from year to year (Moriones and Garcia-Arenal, 1990).

An intensive survey carried out from 1987 to1990 in Spain, using ELISA to test for the PAV, MAV and RPV strains, showed that PAV and MAV were predominant in cereals (Comas *et al.* 1995).

The first report on the occurrence of BYDV in winter barley in Hungary was made by Szirmai (1967). It was based on visual observations and confirmed by aphid transmission tests. In 1982 a very severe epidemic occurred in the Hungarian barley growing areas, with yield losses caused by BYDV ranging from 27 to 100% in the

different barley varieties (Pocsai and Kobza, 1983). Systematic work on the frequency of BYDV strains in cereals in Hungary has been in progress since 1994. Pocsai *et al.* (1995) reported that all the five BYDV strains were present in Hungary. They demonstrated that, among the BYDV strains, the PAV strain was dominant in cereals.

The taxonomy of BYDV strains has been modified several times since the first classification based on vector specificity (Rochow, 1969). This classification has proved very useful but the aphids on which this classification was based were not endemic worldwide, thus in some areas other aphids and strains occurred. It has become obvious that more than one characteristic should be used to classify BYDV strains.

Pringle (1998) summarized the new taxonomic proposals approved by the Executive Committee of the International Committee on the Taxonomy of Viruses, which included proposals for the family of *Luteoviridae*. Fauquet and Mayo (1999) gave a list of virus names and their abbreviations and assigned the family and genus to which the given virus belonged. According to this list, BYDVs are consist of five viruses, BYDV-GPV, BYDV-MAV, BYDV-PAV, BYDV-RMV and BYDV- SGV, belonging to the family *Luteoviridae*. Among these, BYDV-MAV and BYDV-PAV belong to the genus *Luteovirus*. The remaining three viruses were classified as unassigned within the family *Luteoviridae*. The name of the BYDV-RPV strain was changed to *Cereal yellow dwarf virus (*CYDV-RPV) which belongs to the genus *Polerovirus* within the family *Luteoviridae*.

Pocsai *et al.* (2001) reported that in Kompolt (North Hungary), the virus dominance in winter barley changed from year to year. In 1996 RMV was the most prevalent, followed by the MAV. In 1997, *Cereal yellow dwarf virus* (RPV) was dominant, followed by RMV. In 1998, the PAV occurred at the highest rate, and in 1999 the *Cereal yellow dwarf virus*. In 2000, MAV was dominant. The virus dominance in different cereal species also changed from year to year in Martonvásár (Middle-Hungary). In 1996, PAV was dominant, while SGV occurred with the highest frequency in 1997. In 1998, the PAV was dominant again, while MAV was the most prevalent in 1999. The *Cereal yellow dwarf virus* (RPV) was found to be dominant in 2000. In South Hungary (Szeged), the dominance of the PAV was stable in winter wheat and durum wheat from 1996 till 1999. In 2000, the MAV and the *Cereal yellow dwarf virus* (RPV) occurred at the same rate in winter wheat. In West Hungary (Táplánszentkereszt), the *Cereal yellow dwarf virus* (RPV) was dominant in winter barley in 1999, while the PAV was the most prevalent in spring barley. In 2000, only MAV occurred in winter barley, while in spring barley the *Cereal yellow dwarf virus* (RPV) was dominant. In many countries where BYDV has been studied, efforts have focused on reducing yield losses, and very little is known about the incidence and dominance of BYDVs or the role of particular aphid vector species. WDV was first described by Vacke (1961). The virions are geminate, not enveloped, 18 nm in diameter, 30 nm in length, angular in profile without a conspicuous capsomer arrangement (Bisztray and Gáborjányi, 1989, Lindsten *et al.* 1980). In Hungary, its occurrence was reported by Bisztray *et al.* (1988), Pocsai *et al.* (1991), Szunics *et al.* (2000) and Pribék *et al.* (2006).

The epidemic occurrence of WDV was observed in 1960, 1961, 1965, 1968, 1983 and 1986 in different regions of Bohemia, Moravia and Slovakia. WDV chiefly affects winter wheat causing global problems, but it also occurs on winter barley and rye. Yield reductions caused by WDV vary from 5 to 97% WDV was first described in Czechoslovakia by depending on the time of infection (Vacke, 1988). Lindsten and Vacke (1988) found that some isolates of WDV collected in Sweden were unable to infect barley. They also found barley-infecting isolates which did not infect wheat. Bendahmane *et al.* (1995) reported that WDV is a frequently occurring virus disease in several locations of central France. WDV was described in other European countries (Lindsten *et al.* 1970, Lindsten and Lindsten, 1999, Stephanov and Dimov, 1981, Tomenius and Oxelfelt, 1981, Pocsai 2001, Pocsai *et al.* 1991, 1997, 1998, 1999 a,b, 2001a,b, 2002, 2003, Pocsai and Murányi 2001, Szunics *et al.* 1997, 2000, Bakardjeva and Habekuss 1998; Huth 1998, Commandeur and Huth 1998; Ilbagi *et al.* 2003, Mehner *et al.* 2003, Bukvayova *et al.* 2006).

In all European countries, the species *Psammotettix alienus* plays the main role in the epidemiology of this virus disease. A large population of this vector is often present on cereal growing areas of Hungary as well.

The aim of our study was to determine the yearly variation of the dominance of cereal viruses in winter barley breeding lines of Kompolt.

MATERIALS AND METHODS

During 2006-2009 the incidence of *Barley yellow dwarf viruses* (BYDV-MAV, BYDV-PAV, BYDV-RMV, BYDV-SGV), *Cereal yellow dwarf virus* (CYDV-RPV) and *Wheat dwarf virus* (WDV) were studied in cereal species for the determination of virus dominance. Surveys were carried out at Kompolt, in winter barley breeding lines showing leaf yellowing and stunting symptoms. The following samples were collected for different years:

In 2006, 490 winter barley samples were tested. In 2007, the number of samples collected were 500 from winter barley. In 2008, 500 winter barley samples were tested. In 2009, 100 winter barley samples were collected for virus testing.

The leaf samples collected were homogenized using a leaf pressing machine with the addition of ELISA sample buffer solution at a ratio of 1:10. Virus diagnosis was carried out using DAS-ELISA for the detection of *Barley yellow dwarf viruses* (BYDV-MAV, BYDV-PAV, BYDV-RMV, BYDV-SGV), *Cereal yellow dwarf virus* (CYDV-RPV) and *Wheat dwarf virus* (WDV) from leaf samples exhibiting symptoms. In 2009, virus

diagnosis was done only for the detection of BYDV-MAV, BYDV-PAV, CYDV-RPV and WDV. The diagnostic materials used for *Barley yellow dwarf virus*es and *Cereal yellow dwarf virus-RPV* were made by Agdia and Bioreba, while those used for *Wheat dwarf virus* was produced by Biorad and Sediag. The serological reactions were evaluated using a Labsystems Multiskan Plus photometer at 405 nm.

RESULTS AND DISCUSSION

The results of ELISA on incidence of BYDV-MAV, BYDV-PAV, BYDV-RMV, BYDV-SGV, CYDV-RPV and WDV in symptom-showing samples of winter barley breeding lines of Kompolt collected during 2006-2009 shown in *Table 1*.

372 of 490 samples were found to be infected with cereal viruses in 2006. From the 372 infected samples 235 samples were positive for BYDV-MAV. CYDV-RPV was present only in one sample. WDV was detected in 147 samples.

In 2007, 433 of 500 samples were proved to be infected with cereal viruses. 424 of 433 barley samples collected were found to be infected by WDV according to the results of the ELISA. The incidence of Bids and CYDV-RPV was relatively low.

In 2008, BYDV-PAV was the dominant virus. It was present in 195 of 226 infected samples.

The incidence of WDV was surprisingly low, only 10 of 226 samples were found to be infected by WDV.

In 2009, WDV was the most prevalent. 85 of 100 samples collected were proved to be infected with WDV. BYDV-MAV and BYDV-PAV occurred in 4 and 8 samples.

Table 1

Incidence of cereal viruses in winter barley breeding lines of Kompolt during 2006-2009.

Years	No. of samples tested	No. of samples infected by Bids CYDV and WDV	BYDV - MAV	BYDV - PAV	BYDV - RMV	BYDV - SGV	CYDV - RPV	WDV
2006	490	372	235	168	65	69	1	147
2007	500	433	11	14	3	11	2	424
2008	500	226	16	195	9	2	2	10
2009	100	90	4	8	*nt	*nt	0	85

*nt= not tested

Yearly variation of the dominance of BYDV-MAV, BYDV-PAV, BYDV-RMV, BYDV-SGV, CYDV-RPV and WDV in symptom-showing samples of winter barley collected during 2006-2009 is illustrated in *Figure 1.*

In 2006, all four BYDV-s, CYDV-RPV and WDV were present in symptom-showing winter barley samples. Among the viruses occurring in samples infected by BYDVs, CYDV-RPV and WDV the BYDV-MAV was the most prevalent (63.1%), followed by BYDV-PAV (45.1%).

In 2007, WDV was the dominant virus (97.9%), BYDV-MAV, BYDV-PAV, BYDV-RMV, BYDV-SGV and CYDV-RPV were present only at a low frequency.

In 2008, three BYDVs (BYDV-MAV, BYDV-PAV and BYDV-RMV) occurred, of which BYDV-PAV (86.2%) was prevalent in the BYDV-infected samples of winter barley.

In 2009, only two BYDV-s (BYDV-MAV and BYDV-PAV) and WDV occurred and WDV was found at the highest rate (94.4%).

In spite of the fact that the frequency of BYDV-MAV and CYDV-PAV changed from year to year, WDV was the most prevalent virus in winter barley during the four year test, followed by the BYDV-PAV. CYDV-RPV occurred in 2006, 2007 and 2008 at a low frequency (0.4 -1.7%).

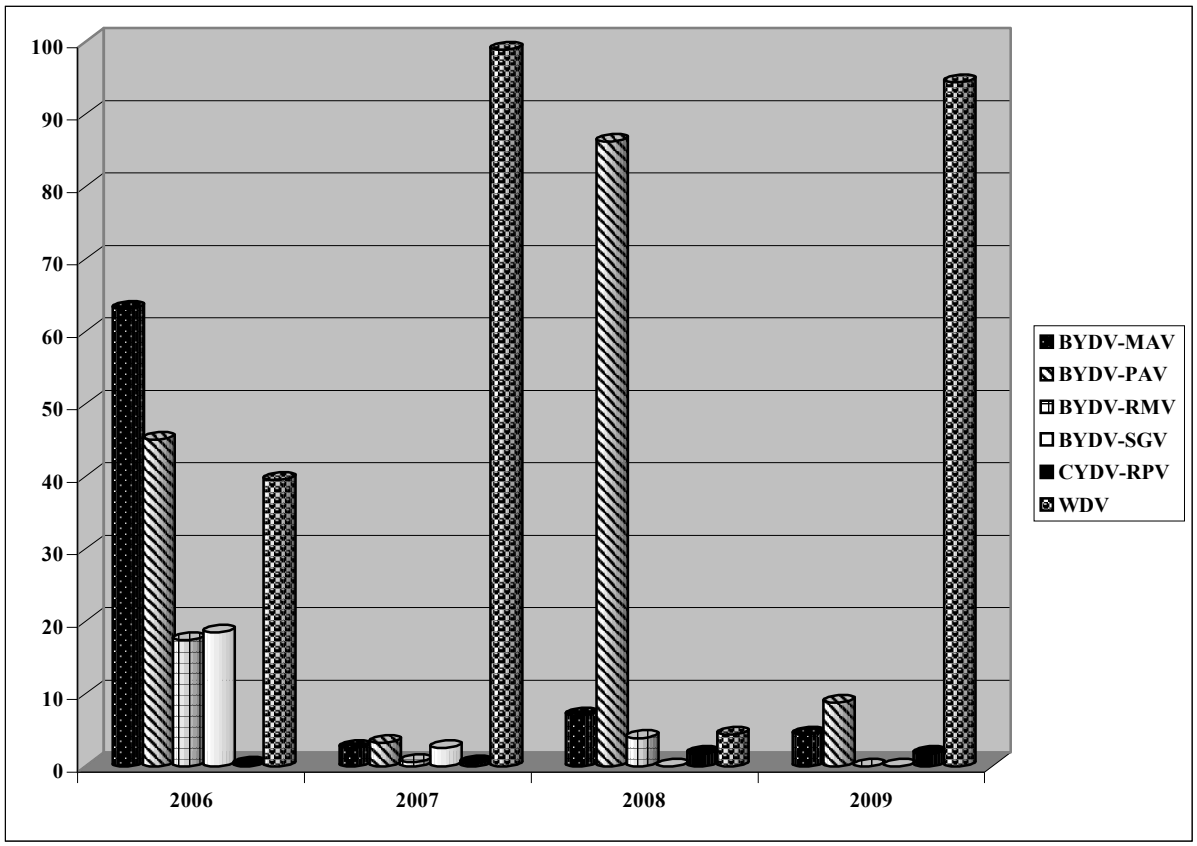
CONCLUSIONS

The results obtained for barley breeding line show that virus dominance varied from year to year. The yearly dominance of BYDV-MAV, BYDV-PAV, BYDV-RMV, BYDV-SGV, CYDV-RPV and WDV in symptom-showing samples of winter barley breeding lines varied and influenced by many factors on Kompolt. A contrasting tendency can be observed between the incidence rates of WDV and BYDV. With arise of incidence in the WDV, the proportion of BYDV decreased, and vice-versa.

From the data of the four-year survey, it can be concluded that the importance of WDV is increasing in cereal-producing regions of Hungary. The virus dominance is complex and influenced by many biotic and abiotic factors. Changes in predominating viruses could be an important factor in epidemiology. The explanation for such changes probably involves a complex interaction between the different vector species, the host plant for both vector and virus, and weather conditions. There is no reason to think that this situation occurred only in Hungary. Similar results were observed in other regions of Europe (Lindsten and Vacke, 1991; Lindsten and Lindsten, 1999; Huth and Lesemann, 1994; Bendahmane *et al.* 1995; Szunics *et al.* 2000; Bukvayova *et al.*

2006). The incidence of CYDV-RPV in cereal species is variable, but it is present in almost every year in a low ratio. The interaction between dominant viruses and vectors has a significant impact on virus epidemiology.

Figure 1: **Yearly variations of the dominance of cereal viruses in winter barley breeding materials of Kompolt during 2006-2009**



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Study approaches on the resistance of Chinese chestnut cultivars to *cryphonectria parasitica*

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SUMMARY

The chestnut is widely cultivated in Asia. China is currently one of the most important chestnut producing countries in the world, with 130.000 hectares harvested chestnut trees .The Chinese chestnut (Castanea mollissima Blume) is an important tree species in world chestnut production and the best source of resistance to Cryphonectria parasitica (Murr.) Barr, the casual agent of chestnut blight. In China, Cryphonectria parasitica, the casual agent of chestnut blight was first observed and identified at near San-tun-ying, Chili Province, 1st. of June, 1913. The incidence of C. parasitica in China decreased gradually from north to south. Goal of our examination was to study the resistance of Castanea mollissima to the chestnut blight fungus. The susceptibility of Chinese chestnut and European chestnut to Cryphonectria parasitica were compared based on the results of the field examinations in China and in Hungary.

In China, field examinations on Chinese chestnut stands showed that there are C. parasitica infections on every examined sites. Blight symptoms were detected on the Chinese chestnut trees, but there was not high degree of destruction. The result showed the great resistance of Castanea mollissima to the chestnut blight disease. European chestnut has not resistance to C. parasitica, and application of hipovirulent strains is one of the efficient protection method against this parasite. Resistance breeding programs are very important and it may be another method to prevent serious Cryphonectria parasitica damages.

Keywords: chestnut blight, *Cryphonectria parasitica*, Chinese chestnut, *Castanea mollissima*, *Castanea sativa,* European chestnut, resistance

INTRODUCTION

Chestnuts belong to the family *Fagaceae* and subfamily *Castanea*, about 10 species of chestnuts is known in the World. There are 3 native species of chestnut in China including Chinese chestnut (*C. mollissima* Bl.), pearl chestnut (*C. henryi* Rehd. et Wils.) and Seguin chinquapin (*C. seguinii* Dode.). The two introduced species are Japanese chestnut (*C. crenata* Sdieb. et Zucc.) and European chestnut (*C. sativa* Mill) also planted there. Chinese chestnut readily cross-pollinates with them to form hybrids.

The Chinese chestnut (*Castanea mollissima* Blume) (*Figure 1*) is an important species in World chestnut production and the best source of resistance to *Cryphonectria parasitica* (Murr.) Barr (Graves, 1950). This fungus was introduced into North America from the East-Asia at the end of the XIX. th. century and spread within the next five decades throughout all the main chestnut areas. *C. parasitica* was identified first in the USA in 1904, that had been introduced from either China or Japan, when lesions and necrosis were observed on trunk and branches of the American chestnut [*Castanea dentata* (Marsh) Borkh] leading to the destruction of this species (Anagnostakis, 1987). In 1938, the pathogen was first discovered in Europe near Genova, Italy (Biraghi, 1946). The fungus spread rapidly and at the end of the last century most parts of Europe were affected by the pathogen, including Austria (Donaubauer, 1964) and Hungary where chestnut blight symptoms were first identified in 1969 (Körtvély, 1970). Then symptoms of the fungus were also detected in the other parts of the Carpathian-basin, including Slovakia (Juhasova, 1976), Romania (Florea and Popa, 1989) and Ukraine (Radócz, 2001).

In China, *Cryphonectria parasitica*, the casual agent of chestnut bligh was first observed and identified at near San-tun-ying, Chili Province, 1st of June, 1913 (Fairchild, 1913; Anagnostakis, 1992). The incidence of *C. parasitica* in China decreased gradually from north to south. The disease mainly occurred at the valley of Yellow River and the Yangtze River, including Beijing, Hebei, Shandong, Jiangsu, Anhui, Zhejiang, Henan, Hunan, Shaanxi, Sichuan and Hubei (*Figure 2*). Symptoms of the fungus had different degree occurrence in different areas of chestnut (Liu et al, 2002).

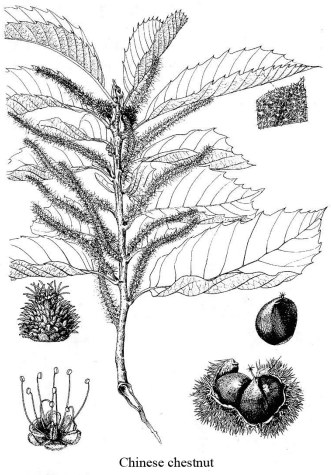
Although *C. parasitica* is endemic in all major chestnut-growing areas of China (Zhang et al, 1987), considerable variation in blight resistance has been reported (Headland et al, 1976; Zhao, 1980). Zhao (1980) surveyed 24 southern cultivars in the Nanjing area of China and found blight incidence to range from 0 to 63 %. Most infected trees remained productive despite of the infection by *C. parasitica*, and yields were reduced more by poor orchard management, nutrient deficiency, and other disease and insect problems (Zhao, 1980; Zhang et al, 1987). Artificial inoculations and field observations of 12 *Castanea* species over a 30-year period showed the Chinese chestnut (*Castanea mollissima*) to be the most resistant to the chestnut blight (Graves, 1950). The disease incidence and severity increase with the aging of chestnut trees (Zhou, 1993; Liu, 2002) and it is important to use the best source of resistance in breeding programs.

The history of Chinese chestnut cultivation could be as far as dated as 1,000 D.C up to the Han Dynasty (206 B. C.-A.D. 220). It became an important kind of economic trees at that time. The production of chestnut fruit has

been gradually restored and developed since the founding of the People's Republic of China. Nowadays, chestnut has been a mainstay industry in many villages and towns of producing areas, and it will be a vast prospect in making rational use of land resources and promoting economical development in hilly and mountainous areas of the country. The chestnut is widely cultivated in China (*Figure 3-5*). The natural range of the Chinese chestnut extends from the far North of Jilin province is (north 40°26′) to the tropical region of Hainan province (18°30′) in China (Zhang et al, 1987). The main producing areas of Chinese chestnut are concentrated at the Yellow River valley and the Yangtze River valley.

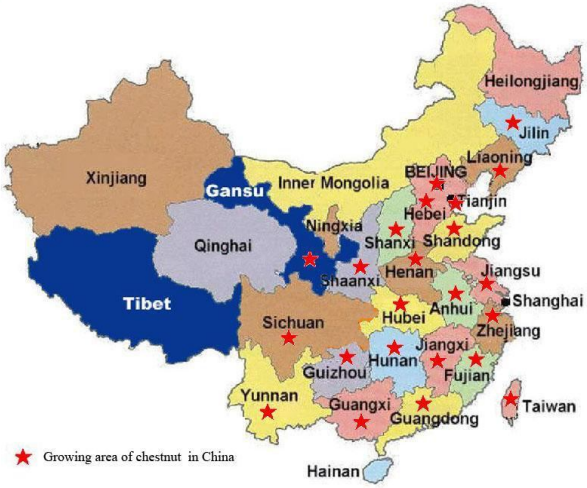
China is currently one of the most important chestnut producing countries in the World, with 130.000 hectares harvested chestnut trees. In 2007, the nut productions was 925.000 tons, took the top place in the World. In Beijing region, there was 41.667 hectares chestnut growing land in 2006.

Figure1: **Chinese chestnut** (*C. mollissima* Bl.)



Chinese chestnut

Figure 2: **Chestnut geogrophical distribution in China**



MATERIALS AND METHODS

Field investigations were done on 7 test sites in 5 chestnut growing areas of Beijing region, China, on 26-29 of August, 2009. Visual investigations were done to measure of damages caused by *C. parasitica* on Chinese chestnut (*Castanea mollissima*). Infection ratio (I%) were measured in the examined chestnut populations and infection index (Ii) were calculated according to a classification system (*Table 1*). The 5 examined areas were the follows: Huairou chestnut orchard, Miyun chestnut orchard, Changping chestnut orchard, Pinggu chestnut orchard and Yanqing chestnut orchard.

During the field examinations bark samples for laboratory identifications and further examinations were collected from the infected trees with a disinfected sharp scalpel. In the laboratory PDA (potato-dextrose-agar) media were used for examinations. Surface sterilized bark samples were cultivated on PDA media and the isolates were incubated for 7 days in a climate chamber. Then vegetative compatibility tests were done. First isolates were paired to study their compatibility. Than pure cultures of the isolates were paired to classify their Vegetative Compatibility Groups (VCG-s) according to the method of classification was referenced by Anagnostakis (1988). Isolates which formed a visible barrage zone at the edge of the growing mycelia were incompatible with each other and those were classified into different VCG-s.

Our goal was to study the resistance of *Castanea mollissima* to chestnut blight. The susceptibility of Chinese and European chestnut to *Cryphonectria parasitica* were compared based on the results of field examinations in China and in Hungary.

Table 1

Classification system (Ii = 1 - 5) degrees on chestnut (according to Radócz, 1998)

Infection degree	Damage of leaves (%)	Damage of bark tissue (%)
Healthy tree	0 %	0 %
I.	< 10 %	Max. 10 %
II.	11-25 %	Max. 25 %
III.	26-50 %	Max. 50 %
IV.	51-99 %	Max. 99 %
V.	100 %	Dead tree or dead tree with spear growing

RESULTS AND DISCUSSION

Previous results in China

The infection of *Cryphonectria parasitica* fungus is the main plant pathological problem for chestnut growing areas in Beijing, Hebei, Liaoning, Shandong, Jiangsu, Anhui, Zejiang, Henan, Hunan, Guangdong, Guangxi, Shanaxi, Hubei, Fujian, Sichuan. The incidence of disease in 7 locations form Shanaxi, Hebei, Beijing and Hubei were from 21,2% - 33,3%. In China, 131 VCG-s from 219 isolates of *C. parasitica* fungus were identified (Wang et al., 1991).

The spatial structure of genetic diversity among 17 populations of *Cryphonectria parasitica* in China was investigated using RAPD markers with the spatial autocorrelation analysis. The result revealed a lack that genetic variations of the most polymorphic loci were randomly distributed. However, cline depression, lump, or double cline structures of the genetic variation were found at some RAPD loci with significant Moran's *I* in several distance classes. The spatial patterns of genetic differentiation in populations appeared to be a combining result of long-distance gene flow, human activities, local effects of geographic isolation and reproduction behaviour of *C. parasitica*, and it speculated that Southwest China could be a possible center of *C.parasitica* origin in China based on the cline pattern at some loci (Yan et al., 2003).

The efficiency of dsRNA transmission in *Cryphonectria parasitca* was tested by co-culture the dsRNA donor and recipient, and virulence of dsRNA-containing isolates was determined by inoculation in both *in vitro* and *in vivo*. Results showed that the transmission rate ranged from 0 to 100% within different combinations. The data of relative virulence tested by inoculation trials indicated that the virulence of 6 transmitted isolates, 12 donors, 6 pairs of non-transmitted isolates, and 6 pairs of transmitted isolatse and wild type of the parent were extremely lower than that of recipient and wild type of donor. They were hypovirulent or mid-virulent and no significant difference was observed among them. The wild type of donor possesses comparative virulence to recipient which exhibited high virulence. The results suggested that the potentiality of the biological control for chestnut blight was higher than our expectation based on the assay data (Liu et al., 2005).

Results of field examinations in China and in Hungary

Field examinations on Chinese chestnut stands showed that there are *C. parasitica* infections on every examined sites. Blight symptoms (*Figure 7-8*) were detected on the Chinese chestnut trees, but there were not a high degree of destruction. Infection ratio (I%) and infection (Ii) index were measured on each test sites. The infection ratio (I%) ranged between 14–34 % on the 7 examined chestnut stands (*Table 2*). Most of the symptoms were in the I. infection degree (it is the less heavy infection), but there were some in the II. and in the III. infection degree, which represented more serious infections. 3 chestnut trees were found on the 7 test sites with the infection degree IV. These trees were infected very seriously. There were not found any killed Chinese chestnut (*Castanea mollissima*) trees because of *Cryphonectria parasitica* on the examined chestnut fields in the Beijing region. The result showed the great resistance level of *Castanea mollissima* to the chestnut blight disease.

Table 2

Results of field examinations on Chinese chestnut (*Castanea mollissima*) in Beijing region, China

Chestnut growing areas (test sites)	Time of field examinations	Number of examined trees	Infection degree						Ii	I%
			Healthy tree	I.	II.	III.	IV.	V.		
HUAIROU chestnut orchard I.	26.08.2009.	100	66	12	14	7	1	-	1,91	34
HUAIROU chestnut orchard II.	26.08.2009.	100	68	13	11	8	-	-	1,84	32
MIYUN Chestnut orchard I.	04.09.2009.	100	80	6	11	4	-	-	2,00	20
MIYUN Chestnut orchard II.	04.09.2009.	100	74	11	8	7	-	-	1,85	26
CHANGPING chestnut orchard	28.08.2009.	100	78	5	12	12	2	-	2,35	31
PINGGU Chestnut orchard	02.09.2009.	100	86	5	5	4	-	-	1,93	14
YANQING chestnut orchard	23.09.2009.	100	76	13	8	3	-	-	1,58	24

On the other hand the results in Hungary (and moreover results from other growing areas of the Carpathian-Basin) showed that there are more significant *C. parasitica* destructions on European chestnut (*Castanea sativa*). In the Carpathian-Basin higher infection rate (F%) with higher degrees of damages (Ii) can be found in *Table 3*. Also several chestnut trees were killed by the *C. parasitica fungus* there. European chestnut has not resistance to

chestnut blight disease. This comparison of field results showed the differences between the resistance level of *Castanea molissima* and *Castanea sativa* to *Cryphonectria parasitica* fungus.

Although applying hipovirulent strains is the one efficient protection method against chestnut blight in Europe, resistant breeding programs are very important and it may be another way to prevent serious *Cryphonectria parasitica* damages.

Table 3

Results of field examinations on European chestnut (*Castanea sativa*) in the Carpathian-Basin

Chestnut growing areas (test sites)	Time of field examinations	Number of examined trees	Infection degree						Ii	I%
			Healthy tree	I.	II.	III.	IV.	V.		
Cák (HU)	20.05.1994	100	55	15	11	10	4	5	2,40	45
Csepreg (HU)	19.04.1994.	100	32	14	13	12	14	15	3,04	68
Velem (HU)	20.04.1994.	100	39	15	17	7	9	13	2,80	61
Nemeshetés (HU)	21.04.1994.	100	22	13	18	17	18	12	2,97	78
Sand (HU)	19.10.1994.	100	31	17	19	10	12	11	2,72	69
Gödöllő (HU)	06.07.1994.	100	40	13	16	16	13	4	2,68	60
Zengővárkony(cemetery)(HU)	24.07.1997.	100	7	12	16	17	21	27	3,38	93
Zengővárkony(Kócsid) (HU)	01.06.1997.	100	34	15	12	17	10	12	2,88	66
Sopron (Fáber-rét) (HU)	29.03.1995.	100	49	13	9	11	9	9	2,84	51
Sopron (Bánfalva) (HU)	28.05.1996.	15	0	-	-	-	-	-	-	100
Fertőszentmiklós (HU)	29.03.1995.	100	11	21	22	16	19	11	2,74	89
Baia Mare-Veresvíz (RO)	08.11.2006.	100	5	5	6	7	8	69	4,47	95
Bobivisce IV. (UA)	27.07.2009.	100	2	6	11	29	22	30	3,60	98

Remarks: HU – Hungary, RO – Romania, UA – Ukraine

Figure 3: **Chinese chestnut orchard in Huairou**

Figure 4: **The nuts of *Castanea mollissima***

Figure 5: **Young chestnut trees in Miyun**

Figure 6: **Healthy chestnut trees**

Figure 7: ***C. parasitica* cankers on the bark of a Chinese chestnut tree**

Figure8: **Symptoms of the blight disease in the chestnut foliage**

(Photos: G. Tarcali, 2009)

CONCLUSIONS

In China, *Cryphonectria parasitica* is an endemic parasite on Chinese chestnut, and blight symptoms were detected on the examined growing areas. However there are not so high degree of destruction and the *Castanea mollissima* trees show great resistance to the chestnut blight disease. European chestnut has not resistance to *C. parasitica* fungus. In Europe, application of hipovirulent strains is the one efficient protection method against this blight fungus on *Castanea sativa*. However resistant breeding programs are very important and it may be another way to prevent serious *Cryphonectria parasitica* damages (*Figure 3-6*).

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Fruit melanotic ringspot – a new disease of pepper (*Capsicum annuum* L.)

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SUMMARY

An apparently new disease called fruit melanotic ringspot (FMRS) has been observed affecting pepper (Capsicum annuum L.) in Hungary. Besides the first symptomathological description of FMRS, this paper demonstrates a TSWV like plant virus as the putative causal agent. Based on symptomathological surveys it could also be concluded, that FMRS should be a special reaction of some pepper genotypes to viral infection.

Key words: pepper, Capsicum, fruit disease, melanotic ringspot tospovirus

INTRODUCTION

Fruit (pod) of pepper (*Capsicum* spp.) can be attacked by a great number of pathogens and affected by a range of physiological diseases and physical injuries. Disease symptoms caused by bacteria (víz. *Xanthomas* sp., *Pseudomonas* sp., *Erwinia* sp.). and fungi (*Phytophtora*, *Alternaria*, *Colletotrichum* spp.) are widely known (Black et al., 1991; Goldberg, 1995; Pernezny et al. 2003). Early viral infections induce systemic symptoms in the foliage and often in the fruits (e.g. necrotic spots, rings or streaks, deformations) as well. In many cases of late viral infections symptoms may appear only in the fruits (cf. Salamon 2008). Physiological disorders like blossom-end rot (the consequence of Ca deficiency) or toxic effects of chemicals as well as physical injuries including sunburn and sandstorms cause defects of fruit quality. Fruit damages are also caused by insects. In Hungary, epidermal brown scars in the pods induced by western flower thrips (*Frankliniella occidentalis*) are of special interest because these „cosmetic" malformations make the fruit of forced white „Cecei" peppers unmarketable.

In the autumn of 2008 and in the spring and summer of 2009 a previously unkown fruit disease called here as „fruit melanotic ringspot (FMRS)" has been observed occurring in experimental forced pepper (*Capsicum annuum* L.) lots in Kecskemét and Gödöllő, Hungary. The disease attacked exclusively the fruits and up to now it has been detected in white conical „Cecei type" peppers. This paper gives the first description of the distinctive symptoms of FMRS. Data on symptomathological surveys and pathological tests strongly suggest, that FMRS should be an infectious disease closely associated with high density of thrips (*Frankliniella occidentalis*), a thrips transmitted plant virus and the genotype of pepper.

MATERIALS AND METHODS

Symptomathological surveys were carried out from October to the late November of 2008 and in the spring of 2009 at Kecskemét where in both seasons about 3000 plants representing a wide range of pepper types (e.g. conical white „Cecei", apple shape, blocky, etc.) were grown in a plastic house. In early summer of 2009 eight white Cecei pepper genotypes marked 1-8 were evaluated at Gödöllő under plastic tunnel. Each plant populations were assessed visually for all diseases and damages known to be caused by pathogens or insects.

Fruits of different ages showing typical symptoms of FMRS were collected and stored either in room temperature or in cool chamber at 10 ^{0}C. Tests for isolation of pathogenic bacteria were made by Dr. Maria Hevesi (Corvinus University, Budapest) and Dr. Ferenc Olasz (Agricultural Biotechnological Center, Gödöllő). The presence of pathogenic and saprophytic fungi were evaluated visually and microscopically after incubation the diseased fruits in wet chamber. For isolation of viruses pieces of symptomatic fruits were washed with water then homogenized in sterile mortal adding phospate buffer (1/15M, pH = 7.0). Test plants (*Nicotiana benthamiana, N. clevelandii, N. glutinosa* and *N. tabacum cv.* Xanthi-nc) were mechanically inoculated using cellite as an abrasive.

RESULTS

Occurrence and symptoms of pepper fruit melanotic ringspot disease

The disease FMRS has been first observed in the autumn of 2008 in Kecskemét. 1-4 fruits (characteristically the 2nd-5th fruit sets) of pepper plants having apparently healthy foliage showed distinct dark blackish-brown spots in the white skin (*Figs. 1, 3*). These spots were usually ring-shaped or ovoid and measured 5-20 mm in diameter (*Fig. 2*). In the center of each of these dark spots a defined light brown pin-point lesion of ca. 1-2 mm in diameter developed (*Fig. 1.*). The dark spots appeared in any parts of the fruit body (*Figs. 2, 4*) showing a tendency to concentrate to the fruit shoulder. In a single pod usually 2-10 spots (rarely more) were observed. As these spots enlarged, they often coalesced causing browning of largest area in the fruit (*Fig. 2*). In the coalesced large spots the central „light brown eye" of each of the individual spots could be clearly visualized. The surface

of dark spots was initially bright and slick, then it became more or less knobby with regular or irregular edges. The brown spots usually showed an inner pattern of pigmentation consisted of light and dark brown rings. In the young fruits the pericarp often crushed near the spots. The spots were felt always hard. During ripening (either in the plants or in harvested fruits stored in room temperature) the spots lost their brightness and became leathery wizened. The flesh of fruit became black or dark brown under the discolored epidermis. Around the spots brown pigmented infiltration were often observed, especially in fruits stored at room temperature. These peripheral infiltrated (discolored) areas became more or less soften and shriveled during ripening and storage of fruits. The central spots and the infiltrated areas around them never became rotted. Because of the structure and characteristic pigmentation of the spots described above the distinctive name „fruit melanotic ringspot (FMRS)" is proposed to denote the new fruit disease of pepper.

In Kecskemét, FMRS has been found in 3-5 % of the individuals of pepper populations consisted in several genotypes. The plants affected by FMRS were found exclusively in groups of some white fruited „Cecei" lines suggesting the focal distribution of a „putative" pathogen or the extreme susceptibility of some genotypes to this „putative" agent. In Gödöllő FMRS appeared in 20-30 % of individuals of five white cecei genotypes marked 1, 2, 3 and 5, 6.

Both in Kecskemét and Gödöllő, a high density of western flower thrips (*Franckliniella occidentalis*) was observed and the great majority of pepper fruits showed thrips induced brown scars. Besides them, leaf and fruit symptoms characteristic to tomato spotted wilt virus (TSWV) infection (eg. leaf yellowing, chlorotic-necrotic rings in leaves and fruits, sometimes top necrosis and severe stunting) could be recorded in about 30 % of plants. It is of special importance to note, that pepper plants showing FMRS always had healthy foliage, while the individuals affected by viral systemic leaf symptoms did not express melanotic ringspots in the fruits. For example, in Gödöllő, 20-30 % of plants of the genotypes 4, 7 and 8 showed foliage and fruit symptoms typical of TSWV infection but they remain free of FMRS. However, no leaf symptoms were detected in the genotypes marked 1-3 and 5-6, the individuals of which were affected up to 30 % by FMRS.

Figure 1: **Characteristic symptoms of pepper fruit melanotic ringspot disease**

Figure 2: **Pepper fruits affected by melanotic ringspot disease**

Figure 3: **Melanotic ringspots in a fruit of forced pepper having healthy foliage**

Figure 4: **Melanotic ringspots in pepper fruits at the time of collection in Gödöllő**

Detection of pathogens

No pathogenic or saprophytic fungi were detected in the malanotic ringspots even the diseased fruits were stored for weeks at room temperature or in wet chamber for days. Similarly, no pathogenic bacteria were isolated. However, in each cases (six samples from Kecskemét and 4 samples collected in Gödöllő) a mechanically transmitted plant virus was isolated from the symptomatic fruit flesh. These virus isolates caused chlorotic-necrotic local spots and severe systemic necrotic pattern in *N. tabacum* cv. Xanthi-nc, *N. clevelandii* and *N. glutinosa* as well as severe vein clearing and yellowing in *N. benthaniamana*. The systemically infected test plants often died 3-4 weeks post inoculation.

CONCLUSIONS

To our best knowledges, disease symptoms characteristic to FMRS have not yet been described in pepper. The growing of melanotic ringspots from a central tiny spot and their concentric pattern suggested the presence of a pathogen. Infections with pathogenic bacteria or fungi could be excluded, while virological tests were always positive. Based on the above results, viral origin of FMRS can be assumed. The susceptibility and reactions of test plants strongly suggest the infection of fruits with TSWV or a *Tospovirus* related to TSWV. It is of special interest, that in spite of the TSWV epidemy in the plastic houses investigated, plants affected by FMRS never expressed foliage or fruit symptoms known to be caused by TSWV or other pepper pathogenic tospoviruses. So, it may be hypothesized, that FMRS can be a special reaction of the fruits of some pepper genotypes to TSWV or other tospoviruses. To clear the etiology of FMRS, identification of the isolated viruses and back inoculation experiments to different pepper genotypes are under investigations.

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Fusarium stalk rot of maize genotypes in Martonvásár

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SUMMARY

Stalk rot of maize (Zea mays L.) is of world wild significance. Stalk rot is a disease complex caused by several different species of Fusarium and Macrophomina. Yield and grain quality are reduced. The degree of infection of three hybrids and their parental lines to fusarium stalk rot was tested by artificial inoculation with two Fusarium graminearum isolates (FG36, FGH4) over three years (2006–2008). The cellulase activity in artificially infected maize stalk tissue was measured in 2006, and the correlation between stalk rot caused by maize Fusarium and the level of cellulase activity was determined. The greatest level of infection was recorded in 2007, and FGH4 proved to be the more pathogenic isolate. When testing for resistance to fusarium stalk rot it is not sufficient to observe only natural infection levels. The genotypes examined had different levels of resistance to fusarium stalk rot. The regression analysis showed that cellulase activity of the Fusarium isolates is in close positive correlation with the aggressiveness of Fusarium isolates.

Keywords: *Fusarium spp*., maize, corn stalk rot, enzyme activity

INTRODUCTION

Stalk rot is probably the most important and destructive disease of maize (Zea mays L.) in the world (Christensen & Wilcoxson, 1966, McGee 1988, De Leon & Pandey, 1989). Stalk rot involves a complex process where bacteria and fungi participate in tissue degradation (Koehler 1960, Dodd, 1980, Byrnes & Carroll, 1986, Kizmus et al., 2000). The disease cause premature plant death and contribute to stalk lodging (Smith and White, 1988). The mass appearance of stalk rot begins when the ears reach physiological maturity and is highly influenced by low moisture stress (Dodd, 1980; Schneider & Pendery, 1983). Stalk rot leads to dry rot in the stalk, caused mainly by enzymes produced by the *Fusarium* pathogen, which decompose the cell-wall (Szécsi, 1985; Riou et al., 1991; Lorenzo et al., 1997, Juge, 2006). The correlation between stalk rot and cellulase production was significant in maize (Chambers, 1987). The level of infection is greatly influenced by environmental factors, the genotype × environment interaction and the pathogen resistance of the given maize genotype (Christensen & Wilcoxson, 1966). Considerable differences in *Fusarium* stalk rot resistance have been observed between maize hybrids and inbred lines (Kommedahl and Windels 1981; Mesterházy, 1983; Kovács et al., 1988; Todd and Kommedahl 1994, Palaversic et al. 2007, Szőke et al., 2009).

The dry weather of 2007 again drew attention to the importance of maize stalk strength. The present paper deals with fusarium stalk rot, a disease which has a considerable influence on stalk strength.

MATERIAL AND METHODS

Three single-cross hybrids and their six parental lines were inoculated with two *Fusarium graminearum* isolates (FG36, FGH4) in 2006–2008. The two isolates were selected on the basis of previous pathogenicity tests in the phytotron, where they proved to be the most aggressive. The genotypes were sown in a split-plot design in four replications, with the maize genotypes in the main plots and the treatments (FG36, FGH4, sterile, natural infection) in the subplots. Inoculation was carried out on the 12th day after flowering by placing infected wheat grains in the second internode from the roots on six plants per plot. The grains were sterilised in a 60°C water bath for 2×5 min, after which they were placed in test tubes with 2 ml of a 10^6 conidia/ml suspension of the above isolates at 27°C for 14 days. Sterile wheat grains were placed in the maize stalks as a control, and natural infection was scored on the fourth plot. The collection and processing of samples was begun on October 10th. The stalk samples were cut in half lengthwise and all the samples were photographed with a digital camera to determine the area of the lesions on the pith using the Colim 4.0 image analysing program. Percentage values were calculated from the complete area of the internode and the infected area. The enzyme activity in the stalk tissue extracts was determined using the modified cup-plate method in 2006. The assay medium contained 1740 mg dipotassium phosphate, 840 mg citric acid, 1500 mg agar (Sigma), 100 mg AZCL-HE-Cellulose (Megazyme), 50 mg Na azide (Sigma) in 100 ml water, pH 5.0. Wells (5mm diameter) were made in the assay medium (5mm thick), and filled with 0.2 ml of enzyme preparate. Plates were incubated at 37 ^{o}C for 24 hrs. The cellulase activity was determined from the diameter of the activity rings using the Colim 4.0 image analysing program followed by the calculation of the ring area in mm². The data were evaluated using analysis of variance (Sváb 1981).

RESULTS AND DISCUSSION

It can be seen from the data in *Table 1* that both the hybrids and the lines suffered a substantial level of infection after artificial inoculation with both of the *Fusarium graminearum* isolates. In the first two years (2006, 2007), FGH4 was the more pathogenic of the isolates at the $LSD_{5\%}$ level in the case of both hybrids and lines, while in 2008 FG36 was more pathogenic, significantly so at the $LSD_{5\%}$ level for the lines. The lines became considerably more infected than the hybrids. This difference was more than 10% in 2006 and 2008, while in 2007 the level of infection was similar for hybrids and lines. The treatment with sterile wheat grains, used as a control, gave a relatively high level of infection for the genotypes tested. This could be explained by the fact that the experiment was set up on an area used as a pathological nursery for several decades (until the appearance of the western corn rootworm), so the soil may have a high level of pollution with conidia and chlamydospores. In addition, considerable damage caused by corn borers was recorded in the experimental years, especially in 2007. The extent of natural infection was only 1.33%, averaged over the years, with no significant difference between the years. The highest level of natural infection was recorded for the lines in 2007.

Of the three years, the hybrids suffered the greatest infection (63.55%) in 2007, when the weather was extremely hot and dry at flowering, while rainfall quantities in September and October were close to the long-term mean. Due to the heat and drought stress at flowering there was a reduction in the carbohydrate content of the stalk tissues, which were destroyed to a great extent by the cell wall-decomposing enzymes of the *Fusarium* species playing a major role in the course of the disease (Szécsi 1985).

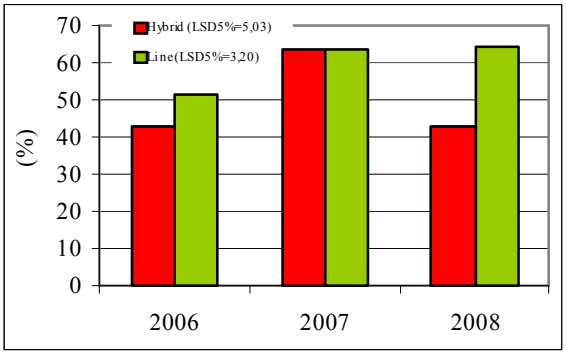
Table 1

Effect of treatments on hybrids and lines, 2006–2008

Treatment / Year	Hybrid			Line			Hybrid	Line
	FG36	FGH4	STER	FG36	FGH4	STER	Natural infection	
2006	46.46	58.31	23.26	50.85	66.69	36.74	0.76	1.66
2007	62.60	77.97	50.09	64.44	79.66	47.60	0.42	2.08
2008	52.80	45.83	29.37	78.16	69.47	45.78	1.55	1.56
$LSD_{5\%}$	**8.72**			**5.54**			**1.78**	**1.79**

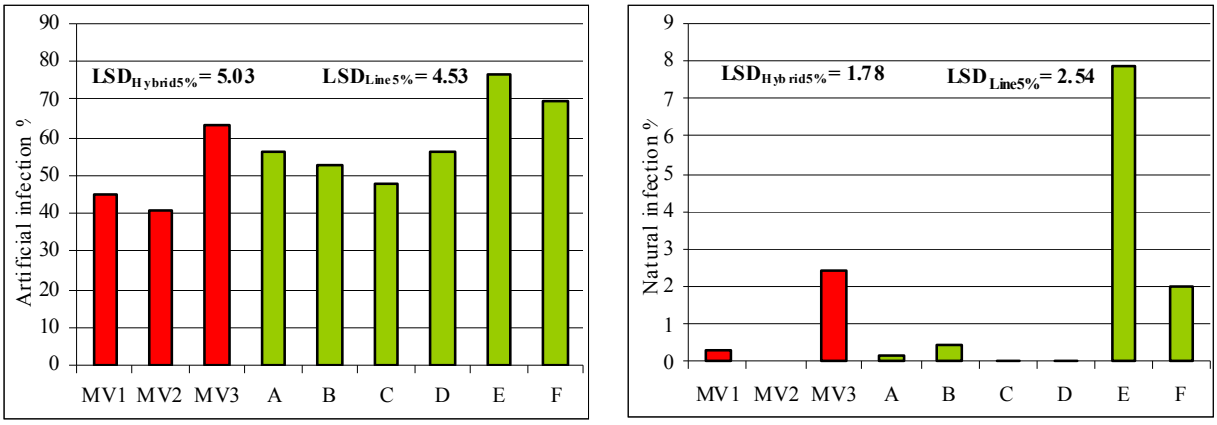
In 2006 and 2008 the hybrids exhibited similar extents of infection (42.68%). The year effects were greater for the lines than for the hybrids, with infection levels of around 65% in 2007 and 2008 and 51.42% in 2006 (*Figure 1*).

Figure 1: **Degree of infection of hybrids and lines, 2006–2008**



An analysis of the infection levels in the hybrids and their parental lines (*Figure 2*) revealed that, averaged over the treatments, the hybrid MV3 was most severely infected, followed by MV1 and MV2, both of which had a significantly lower level of infection than MV3. Among the lines, the differences in infection levels were more pronounced.

Figure 2: **Level of infection of hybrids (MV1-MV3) and their parental (A-F) components averaged over treatments (left) and natural (right) infection.**



Line E was the female partner and line F the male partner in this cross, and these lines had the highest infection level in all three years. Line A and Line B exhibited a moderate level of infection after artificial inoculation, compared with the other genotypes. Line D was the third most sensitive line to infection. Line C was the least infected of all the lines.

A comparison of natural infection and artificial inoculation data (*Figure 2*) indicated that hybrid MV3 and its parental components (lines E and F) also tended to respond similarly to natural infection, though the response to artificial inoculation was of course more intense. Hybrid MV1 also suffered considerable infection, but the natural infection was little and in this case the parental components (lines A and B) involved had different levels of infection under natural and artificial conditions. For hybrid MV2 significant differences were found between the hybrid and the parental components (lines C and D) after artificial inoculation, while in the case of natural infection there was very little difference between the three genotypes. These data draw attention to the fact that exact data on the resistance or susceptibility of a given population to stalk rot can only be obtained after artificial inoculation.

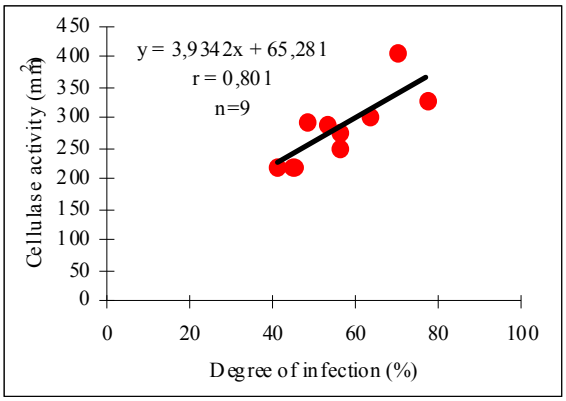
Table 2

Level of activity of the cellulase enzyme averaged over genotypes and treatments, 2006.

Hybrid				Line			
Factor	df	MS		Factor	df	MS	
Genotype (A)	2	13535.96*		Genotype (A)	5	17763.23*	
Infection (B)	2	41927.33**		Infection (B)	2	94810.06***	
AxB	4	12493.73*		AxB	10	4642.49	
Residual	8	2897.99		Residual	17	4921.09	
Genotype (A)	mm²	Infection (B)	mm²	Genotype (A)	mm²	Infection (B)	mm²
MV1	221.00	FG36	252.95	A	252.35	FG36	330.33
MV2	219.57	FGH4	328.56	B	288.93	FGH4	382.72
MV3	302.55	STER	161.62	C	293.13	STER	209.41
$LSD_{5\%}$	**57.80**	**$LSD_{5\%}$**	**57.80**	D	274.85	**$LSD_{5\%}$**	**49.82**
				E	330.00		
*P=5%				F	405.67		
**P=1%				**$LSD_{5\%}$**	**70.46**		
***P=0,1%							

The activity of the cellulase enzyme was recorded in infected stalk tissue in 2006. Different enzyme activities were observed for the two *Fusarium graminearum* isolates, that of isolate FGH4 being considerably greater (*Table 2*). The greater cellulase activity isolate (FGH4) caused a higher level of infection. Stalks treated with sterile wheat grains had significantly lower enzyme activity than those infected by either of the isolates. This suggests that the *Fusarium* strains found in the given environment were less aggressive, as confirmed by the low level of stalk rot recorded in response to natural infection in 2006, when the degree of infection was also lowest in plants treated with sterile grains. Significant differences in cellulase activity were also observed between the genotypes (*Table 2*). The enzyme activity was lower in the hybrids than in the lines, while in the field susceptible genotypes had greater cellulase activity than resistant ones. Regression was calculated between the level of infection (aggressiveness of isolate) induced by artificial inoculation in the field and the activity of the cellulase enzyme, and a close positive correlation was found between the two factors (*Figure 3)*.

Figure 3: **Relationship between the infection caused by artificial inoculation in the field and the cellulase enzyme activity in infected stalk tissue extract (2006)**



CONCLUSIONS

Due to the increasing frequency of extreme weather conditions, renewed attention must be paid to the development of maize lines and hybrids with resistance to stalk rot. The present results indicated that both natural infection and artificial inoculation produced the greatest level of infection in 2007, among the years tested. This year was ideal for the development of stalk rot (little rainfall at flowering, wet weather in the autumn months). Of the two *F. graminearum* isolates used for the artificial inoculation, although FG36 caused significantly greater infection to the lines in 2008, in 2006 and 2007 the FGH4 isolate was more pathogenic to both hybrids and lines. The high infection rate observed in the sterile grain treatment draws attention to the fact that the development of fusarium stalk rot is greatly facilitated by any type of injury to the stalk (pests, cultivation tools, hail), so the mechanical parameters of the stalk (thickness, strong outer layer) should also be considered in the course of selection. Selection for resistance to stalk rot is not possible on the basis of natural infection data alone, as natural infection does not cause a great enough level of infection to distinguish between the tested genotypes. The genotypes (3 hybrids and their 6 parental lines) examined had different levels of resistance to fusarium stalk rot. Among the isolates tested, a higher level of cellulase enzyme activity was recorded for FGH4, which was more pathogenic according to the data of field experiments. The data revealed a close correlation between the susceptibility or resistance of a given genotype and the cellulase enzyme activity of the infected stalk tissue extract.

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New data of *Cryphonectria parasitica* (Murr.) Barr occurrence in Sub-Carpathia

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SUMMARY

Cryphonectria parasitica (Murrill) Barr [syn: Endothia parasitica (Murr.) P.J. Anderson and H.W. Anderson] causes big damages in chestnut stands throughout the World. The pathogen was transferred into Europe from the USA in the middle of the last century, and infected the European chestnut (Castanea sativa) populations in Western-Europe. Then the disease spread towards to Central Europe, and arrived to the Carpathian-Basin. Chestnut blight symptoms were reported first on chestnut in Hungary in 1969. Until 1998 the fungus was only detected on chestnut in the Carpathian-Basin. Then blight symptoms were also detected on some young sessile oak (Quercus petraea) trees in South-Transdanubie. Main goals of our studies were to estimate damages caused by Cryphonectria parasitica in Ukraine on chestnut and on oaks. Our field examinations in Ukraine were started in 2001. The symptoms of C. parasitica on chestnut trees were detected on two examined stands (in Seredne and in Bobovisce). It was the first report of the appearance of this fungus in Ukraine. Our new field examinations were done on 27. 07. 2009. We found that three chestnut growing areas were infected by the fungus Cryphonectria parasitica (those populations what were infected earlier). The infection ratios were the highest in 2009 (I% -35-98). The infection indexes of the examined sites were also higher (Ii -2,00 – 4,20). According to the laboratory examinations all of the isolates cultivated from the Ukrainian bark samples were virulent. 2 different VC types (EU-12 and EU-13) of the fungus were identified in the vegetative compatibility examinations. Since 2004. oak trees were also examined in Ukraine. Infected oak trees by Cryphonectria parasitica were not found until now.

Keywords: *Cryphonectria parasitic*a, blight symptoms, VCG-s, *Castanea sativa, Quercus spp.*

INTRODUCTION

*Cryphonectria parasitic*a (Murrill) Barr [syn.: *Endothia parasitica* (Murr.) P.J. Anderson and H.W. Anderson] is one of the most important pathogen for *Castanea* species. At the beginning of the XX-th. century, this fungus appeared in the USA and destroyed almost the whole American chestnut (*Castanea dentata*) populations (Anagnostakis, 1987). It was also reported first in Europe, in 1938 near Genova on European chestnut (*Castanea sativa*) (Biraghi, 1946). Than *C. parasitica* spread rapidly throughout the most important chestnut growing areas of Europe. Symptoms of the fungus were detected also in the Carpathian-Basin, including Hungary (Körtvély, 1970), Austria (Donaubauer, 1964), Slovakia (Juhasova, 1976), Romania (Florea and Popa, 1989) and Ukraine (Radócz, 2001).

At the second part of the last century, typical blight symptoms were observed on some oak trees in the USA (Torsello et al., 1994), in Switzerland (Bissegger and Heiniger, 1991) and in South-Italy (Dallavalle and Zambonelli, 1999). In 1998 typical blight symptoms were first detected on some young *Quercus petrea* trees in Hungary in a mixed chestnut-oak forest at Zengővárkony (Radócz and Holb, 2002). Than chestnut blight symptoms were reported on oaks in the eastern part of the Carpathian Basin, near Baia-Mare, in Romania (Tarcali and Radócz, 2006) (*Figure 1*). Although symptoms were not so serious on *Quercus petraea* than on *Castanea sativa,* it seems that *Cryphonectria parasitica* became a new serious pathogen for young oak trees in the Central-European countries, mainly in heavily infected chestnut forests (Tarcali, 2007).

MATERIALS AND METHODS

Our field investigations in Ukraine were started in 2001 when we first detected chestnut blight symptoms on *Castanea satina* in two populations. Then field works on the examamined areas were repeated year by year. Last investigation were done on 07. 27. 2009. Main goals of our studies were the followings:

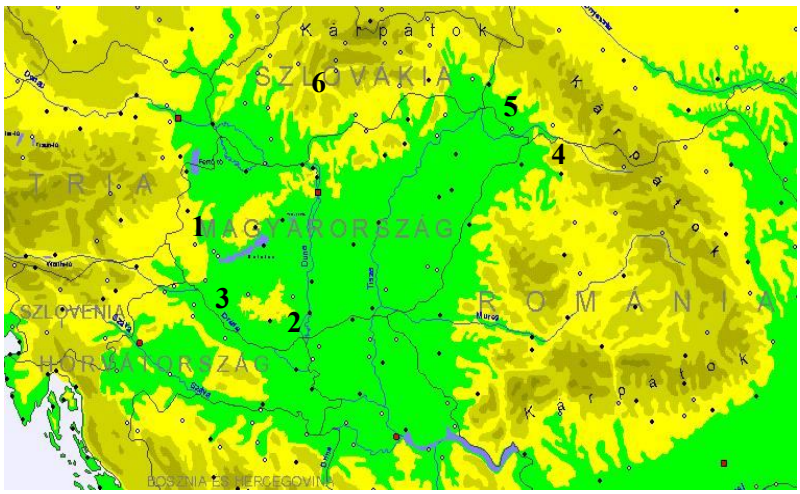
- visual investigations of damages caused by *C. parasitica* on chestnut and on oak trees in several Ukrainian test sites (*Figure 1*),
- collecting of bark samples from infected or „suspected looking" plant tissues on European chestnut and on oak species for further laboratory investigations,
- analysis of the collected samples and testing the isolates in laboratory.

Infection ratio (I%) and infection index (Ii) were measured in 10 chestnut populations near Uzghorod and Munkachevo. During the field examinations bark samples for laboratory identifications and further examinations were collected from the infected or suspect trees with a disinfected sharp scalpel.

In the laboratory PDA (potato-dextrose-agar) media were used for examinations. Surface sterilized bark samples were cultivated on PDA media and the isolates were incubated for 7 days in a climated chamber. In the next step vegetative compatibility tests were done. First isolates were paired to study their compatibility. Than pure cultures of the isolates were paired with EU-tester strains to classify their Vegetative Compatibility Groups

(VCG-s). Isoletes which formed a visible barrage zone at the edge of the growing mycelia were classified into different VCG-s.

Figure 1: **Map of the Carpathian–basin and with test sites**



Remarks:

1 - Kőszeg,
2.-South-Hungarian test sites,
3 - Zengővárkony,
4 - Romanian test sites
5 - Ukrainien test sites
6 - Slovakian test site

RESULTS AND DISCUSSION

Field examinations in Ukraine started in 2001. There were found symptoms of *C. parasitica* in two examined chestnut stands. It was the first report of the appearance of this fungus in Ukraine (Radócz, 2001.). Blight symptoms were detected on chestnut trees at Seredne and in Bobovisce. The ratio of the infection (I%) on the first investigation were not so high (I% - 18 in Seredne, I% - 8-12 in Bobovisce). The infection index (Ii) showed an initial stage of the blight infection (Ii – 1,00-1,17 on the scale between 1-5). Other investigated chestnut stands at Uzghorod, in Gajdos and in Gluboka no infection of *Cryphonectria parasitica* were detected. Results of the first field examinations are detailed in *Table 1*.

In 2004. two new Ukrainien chestnut test sites were involved into our investigations (Rostovjatitsja and Perestiv). The chestnut population at Rostovjatitsja were infected at the ratio 20% and the index of the infection were more serious there (Ii – 2,55). Chestnut trees in the other new examined population (at Perestiv) were free of blight disease in 2004.

Last field examination were done on 27. 07. 2009. Three chestnut growing sites were infected by *Cryphonectria parasitica* (those populations what were infected at the early field investigations). The infection ratios were the highest (I% -35-98). Very serious infection were detected on the Bobovisce IV. test site where 98 chestnut trees were infected from 100 trees (I% -98). The infection indexes of the examined sites were also higher (Ii -2,22-Seredne – 3,60-Bobovisce IV). Some chestnut populations (Bobovisce IV, Bobovisce III) were seriously damaged of the fungus (*Figure 2-4*), and several chestnut trees were killed by *Cryphonectria parasitica* fungus (Ii – 5) (*Table 1*). On the other hand it was found that the pathogen did not spread away from the infected sites.

Laboratory examinations were done on the collected bark samples. Pure cultures of *C. parasitica* were cultivated. Results showed that every Ukrainian isolates were virulent. Hypovirulent isolate was not identified. Results of the vegetative compatibility tests showed that two different fungal strains are on the examined areas in Ukraine. EU-12 strain of the pathogen existed in Seredne and only EU-13 fungal strain was found in Bobovisce and in Rostovjatitsja.

Since 2004. oak trees mixed with chestnuts on the test sites were also examined. Some suspected trees were found on the fields, but laboratory examinations did not confirm chestnut blight infection on them. Therefore infected oak trees by *Cryphonectria parasitica* were not found until today in the Sub-Carpation region of Ukraine.

Table 1

Results of field examinations in Ukraine

Test sites	Time of field examinations	Examined trees	Infection degrees						Ii	I %
			Healthy tree	I.	II.	III.	IV.	V.		
UNG	I. 2001.04.20.	36	36	-	-	-	-	-	-	0
UNG	II. 2002.05.08.	35	35	-	-	-	-	-	-	0
UNG	III. 2003.10.29.	30	36	-	-	-	-	-	-	0
UNG	IV. 2004.11.02.	35	36	-	-	-	-	-	-	0
UNG	V. 2006.03.14.	35	35	-	-	-	-	-	-	0
UNG	VI. 2006.10.23.	35	35	-	-	-	-	-	-	0
UNG	VII. 2009.07.27.	35	35	-	-	-	-	-	-	0
SER	I. 2001.04.20.	100	82	15	3	-	-	-	1,17	18
SER	II. 2002.05.08.	100	94	2	3	1	-	-	1,83	6
SER	III. 2003.10.29.	100	88	6	5	1	-	-	1,58	12
SER	IV. 2004.11.02.	100	85	8	5	2	-	-	1,60	15
SER	V. 2006.03.14.	100	79	7	8	3	2	1	2,14	21
SER	VI. 2006.10.17.	100	76	7	10	2	2	3	2,33	24
SER	VII. 2009.07.27.	100	55	12	23	2	4	4	2,22	45
GAJ	I. 2001.04.21.	100	100	-	-	-	-	-	-	0
GAJ	II. 2002.05.09.	100	100	-	-	-	-	-	-	0
GAJ	III. 2003.10.31.	100	100	-	-	-	-	-	-	0
GAJ	IV. 2004.11.02.	100	100	-	-	-	-	-	-	0
GAJ	V. 2006.03.14.	100	100	-	-	-	-	-	-	0
GAJ	VI. 2009.07.27.	100	100	-	-	-	-	-	-	0
BOB I.	I. 2001.04.21.	100	88	10	2	-	-	-	1,17	12
BOB I.	II. 2002.05.08.	100	87	8	5	-	-	-	1,38	13
BOB I.	III. 2003.10.30.	100	88	7	5	-	-	-	1,42	12
BOB I.	IV. 2004.11.03.	100	86	7	6	1	-	-	1,57	14
BOB I.	V. 2006.03.13.	100	84	6	8	2	-	-	1,75	16
BOB I.	VI. 2006.10.17.	100	82	8	7	3	-	-	1,72	18
BOB I.	VII. 2009.07.27.	100	65	10	4	11	7	3	2,69	35
BOB II.	I. 2001.04.21.	100	92	8	-	-	-	-	1,00	8
BOB II.	II. 2002.05.08.	100	90	7	3	-	-	-	1,30	10
BOB II.	III. 2003.10.30.	100	89	7	4	-	-	-	1,36	11
BOB II.	IV. 2004.11.03.	100	90	4	4	2	-	-	1,80	10
BOB II.	V. 2006.03.13.	100	89	5	3	2	-	1	2,00	11
BOB II.	VI. 2006.10.17.	100	84	7	6	2	-	1	1,88	16
BOB II.	VII. 2009.07.27.	100	55	3	19	20	8	5	3,40	45
BOB III.	II. 2002.05.08.	50	48	2					1,00	4
BOB III.	IV. 2004.11.03.	50	46	4	-	-	-	-	1,00	8
BOB III.	V. 2006.03.13.	50	44	5	1	-	-	-	1,17	12
BOB III.	VI. 2006.10.17.	50	44	4	2	-	-	-	1,33	12
BOB III.	VII. 2009.07.27.	50	10	3	7	8	13	9	3,45	80
BOB IV.	I. 2009.07.27.	100	2	6	11	29	22	30	3,60	98
GLU	I. 2001.04.21.	100	100	-	-	-	-	-	-	0
GLU	II. 2002.05.08.	100	100	-	-	-	-	-	-	0
GLU	III. 2003.10.29.	100	100	-	-	-	-	-	-	0
GLU	IV. 2004.11.02.	100	100	-	-	-	-	-	-	0
GLU	V. 2006.03.13.	100	100	-	-	-	-	-	-	0
GLU	VI. 2006.10.23.	100	100	-	-	-	-	-	-	0
GLU	VII. 2009.07.27.	100	100	-	-	-	-	-	-	0
ROS	IV. 2004.11.03.	100	80	6	5	4	2	3	2,55	20
ROS	V. 2006.03.13.	100	73	9	5	5	2	6	2,66	27
ROS	VI. 2006.10.17.	100	71	10	6	5	1	7	2,62	29
ROS	VII. 2009.07.27.	100	66	6	11	6	3	8	2,88	34
PER	IV. 2004.11.03.	25	25	-	-	-	-	-	-	0
PER	V. 2006.03.13.	25	25	-	-	-	-	-	-	0
PER	VI. 2006.10.23.	25	25	-	-	-	-	-	-	0
PER	VII. 2009.07.27.	25	25	-	-	-	-	-	-	0

Figure 2: **Dead chestnut trees in Bobovisce III. test site**

Figure 3: **Infected chestnut trees in Rostovjatitsja**

Figure 4: **Cankers on the bark of a young chestnut tree**

CONCLUSIONS

Cryphonectria parasitica infection on European chestnut (*Castanea sativa*) on the Sub-Carpatian region of Ukraine is increasing year by year. The pathogen causes big damages on chestnut there. Although *C. parasitica* infected oak trees were not found until today in Ukraine, this pathogen is also a new serious potential danger for local oaks there.

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Occurrence of stone fruit yellows phytoplasma disease in Gönc region, Northern-Hungary

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SUMMARY

Plant diseases caused by phytoplasmas have increasing importance in fruit growing. Phytoplasma diseases occur throughout the world on many crops and responsible for serious losses both in quality and quantity of fruit production. In the long run these diseases cause the destruction of the bearing fruit trees. Apricot phytoplasma disease (Ca. Phytoplasma prunorum) was first reported in Europe in 1924 in France. Than the pathogen spread in all European apricot growing areas. In 1992, the disease has also been observed in Hungary mainly in peach plantations. On the base of growers' signals serious damages of "Candidatus Phytoplasma prunorum" Seemüller and Schneider (2004) (formerly: European stone fruit yellows phytoplasma) could be observed in different stone fruit plantations in the famous apricot-growing area near-by Gönc town, Northern-Hungary. By now, it became one of the most important diseases of apricot. Field examinations were done in early October, 2009 in 9 stone fruit plantations in Borsod-Abaúj-Zemplén County mainly in Gönc region. There is one of the most important apricot growing regions in Hungary, named "Gönc Apricot Growing Area".

Our goal was to diagnose the occurrence of Ca. Phytoplasma prunorum on stone fruits (especially on apricot) on the examined North-Hungarian growing areas by visual investigations. On the base of our observations it is evident that the notable losses caused by Ca. Phytoplasma prunorum is a new plant health problem to manage for fruit growers in Hungary. It is obvious that the phytoplasma is a serious potential danger for almost every kind of stone fruits.

Key words: *Ca. Phytoplasma prunorum,* European stone fruit yellows pytoplasma, ESFY, apricot, peach, sour cherry, stone fruits, *Cacopsylla pruni*

INTRODUCTION

Plant diseases caused by different phytoplasmas have increasing importance for almost all fruit growers. Phytoplasma diseases occur throughout the world on many crops, and cause serious losses both in quality and quantity of fruit production. In the long run these diseases cause the destruction of the bearing fruit trees. Plant diseases known as "yellows diseases" were thought to be caused by viruses. This was because the yellows disease agents had certain characteristics which are common at virus agents. They could not be grown on culture media like most fungal or bacterial pathogens could. In addition, while a yellows disease had characteristic symptoms, there were never signs – obvious structures of a disease organism – on infected plants (Welliver, 1999).

In 1967, Japanese researchers (Doi *et al.*, 1967) found microorganism by electron microscope in yellows diseased plants. This new class of plant disease agent was named a "mycoplasma-like organism", because of its resemblance to mycoplasmas that were saprophytic or that caused disease in humans and animals (Welliver, 1999).

Plant pathogenic MLOs can be broadly classified by the symptoms they produce in plants (reviewed by Kirkpatrick, 1989, 1991). Some MLOs produce symptoms of virescence (greening of floral tissues) and phyllody (leaflike petals and sepals) in their herbaceous hosts (virescence MLOs). Others do not produce virescence and phyllody but rather produce a general decline of infected plants (decline MLOs). However, members of both groups can produce similar symptoms, such as chlorosis, stunting, and shoot proliferation. Thus, classification of MLOs based solely on disease symptoms cannot precisely differentiate all of the MLOs. In addition, because plant host range and vector transmission characteristics are time consuming and often difficult to obtain, these characteristics have not been determined for most of the MLOs. The reliance upon biological and pathological characteristics to classify MLOs has resulted in confusion in naming MLOs and distinguishing between different MLO isolates. Mycoplasma-like organisms are nonculturable, parasitic prokaryotes of the class Mollicutes associated with diseases of several hundred plant species (McCoy *et al.,* 1989). Until recently, differentiation and characterization was mainly based on host range and the symptoms induced in natural hosts and in the experimental host *Catharanthus roseus* (periwinkle) (Marwitz, 1990). The need for more reliable and specific traits to classify MLOs has resulted in the development of MLO specific serological and DNA hybridization assays (Khuske *et al.*, 1991).

In 1992, characterization of the organism associated with yellows diseases had progressed to a point that it became clear they were unique and should be given their own name: phytoplasma (ICSB, 1993).

Phytoplasmas are single-celled organisms that are similar to bacteria but lack a rigid cell wall. Phytoplasmas are obligate parasites. They grow and reproduce in the cytoplasm of host cells, both in insect vectors and in plants. Phytoplasmas are very small agents; they look like amorphous sacks or blobs, ranging from 70-1000 nm in diameter. Phytoplasmas reproduce asexually, by budding. Phytoplasmas reside in the phloem tissues of the plants, and are transmitted by phloem-feeding insect vectors. Thy psyllid *Cacopsylla pruni* Scopoli was

described as vector of *Candidatus* Phytoplasma prunorum (Carraro *et al*., 2001; Fialová *et al.*, 2007). Phytoplasmas cannot be transmitted mechanically.

Candidatus Phytoplasma prunorum (formerly named European stone fruit yellows phytoplasma; ESFY) (Kövics, 2009) is one of the most important fruit tree phytoplasmas in cultivated *Prunus* species in Europe. In many European countries the disease has been identified as one of the most prevalent problems facing apricot trees (Jarausch *et al*., 2001; Navratil *et al.*, 2001; Torres *et al*., 2004). *Ca*. Phytoplasma prunorum causes serious economic losses in cultivated *Prunus* species. Susceptible young apricot trees infected by *Ca*. Phytoplasma prunorum die quickly (within 1-2 years after infection), and the pathogen also causes yield and quality losses on trees older than five years (Németh, 1986). The age of the trees, environmental conditions and the rootstocks influence the severity of symptoms and the progress of the disease. European plum has been determined to be tolerant to the pathogen, whereas Japanese plums are highly susceptible (Carraro *et al.*, 1998; Mona *et al*., 2009).

Apricot phytoplasma disease was reported first in Europe in 1924 in France, than the pathogen spread to all European apricot growing areas. In 1992, the disease was also observed in Hungary (Süle, unpublished), although its symptoms were observed before too. Later on (Viczián *et al.,* 1997; Süle *et al.,* 1997) confirmed the occurrence of the ESFY in Hungary. Symptoms were also observed on dying apricots and on almonds. The symptoms resemble to those of causing fungal (*Cytospora* spp.), bacterial (*Pseudomonas syringae*) pathogens, and abiotic reasons (e.g. frost damage). Up-today, the EFSY has become one of the most important diseases of apricot (Süle *et al*., 2003). The occurrence of phytoplasma was observed in peach (Németh *et al*., 2001) and *Prunus mahaleb* cv. Cemany (Varga *et al*., 2001) as well. Spreading of disease may intimately connect with decreased usage of wide-ranging insecticides which promoted the propagation of vector species.

Apricot is an important fruit in Hungary. Although its yield reduced during the last decades because of the unsettled weather conditions, the ageing of several plantations and the unfavourable economical situation in fruit growing. In Hungary, there are 8 greater apricot growing areas, viz. Balaton, Mecsek, Lake Velencei, Buda, Pest-Gödöllő, Mátra-Bükkalja, Danube-Tisza and Gönc. One of the most important is the Gönc Apricot Growing Area, the latest situated in Borsod-Abaúj-Zemplén County, Northern-Hungary.

MATERIALS AND METHODS

Field observations were made on 2nd October, 2009 in nine stone fruit plantations (4 apricot cultivars, 1 peach population, 3 sour cherry and 1 cherry plantation) in which are excellent growing areas for stone fruits, especially for apricot. Our goal was to diagnose investigations of damages caused by *Ca.* Phytoplasma prunorum on stone fruits (especially on apricot) on the examined Northern-Hungarian growing areas visually.

Infection ratio (1%) and infection index (Ii) [according to a classification system (*Table 1*)] were measured in the fruit populations based on the following visible symptoms of the disease caused by *Ca.* Phytoplasma prunorum:

- on leaves: yellow colour change and rolling of leaves to its abaxial surface,
- on branches: general yellowing or „scalding-like" drying,
- on the barks: striped the bark of tree orange or light brown colour change can visible in the phloem
- on trees: general yellowing on several branches or general drying; withered, dead or fell tree, and there are no secretion of gum,
- in plantation: infections and destruction of trees starting in a circular direction around the infected tree.

100 trees were examined on every sites, 10 trees of a circle were selected randomly for examination, totally 100 trees from 10 circles.

Table 1

Classification system

Degree	Symptoms
I	Healthy tree
II	Initial symptoms on 1 branch
III	Initial symptoms on several branches
IV	1 dead branch
V	Dead or felled tree

RESULTS AND DISCUSSION

Results of field observations and examinations

A 4-year-old new apricot plantation was examined first. Most of the trees were healthy, but there were found a few trees (2%) infected by *Ca.* Phytoplasma prunorum (*Table 2*). According to Süle *et al.* (2003) description, the first symptoms of the pathogen can be observable from the age of 3-4 years of the apricot trees, and this thesis is justified in that visited apricot garden.

Table 2

Phytoplasma infections on fruit plantations in Borsod-Abaúj-Zemplén County (results of field examination)

No.	Time of field examination	Kind of examined fruit trees	Age of trees (years)	Area (ha)	Number of examined trees	Degree of infection					Ii	I%
						I	II	III	IV	V		
1	02.10.2009	Apricot	4	20	100	98	1	1	-	-	1,03	2
2	02.10.2009	Apricot	8-9	5	100	45	4	6	5	40	2,91	55
3	02.10.2009	Apricot	~8	3	100	15	7	7	6	65	3,99	85
4	02.10.2009	Apricot	12-13	10	100	30	6	4	35	25	3,21	70
5	02.10.2009	Peach	~8	6	100	79	7	2	2	10	1,57	21
6	02.10.2009.	Cherry	~10	22	100	70	9	4	6	11	1,79	30
7	02.10.2009.	Sour cherry	8-9	5	100	38	14	10	8	30	2,78	62
8	02.10.2009.	Sour cherry	7	~5	100	91	3	1	1	4	1,24	9
9	02.10.2009.	Sour cherry	~30	8	100	64	6	9	13	8	1,95	36

The second apricot plantation was an 8-9 years old cultivation. At first sight it was visible well that there is a very serious destruction on apricot trees caused by phytoplasma. More than 50 percentages of the trees were infected and 40 percent of apricot trees were dead. Most of the killed trees were felled (about 35%). The owner of the plantation said that one year before only 1-2 trees showed symptoms of disease. The general drying has began in this year from the end of blooming of apricot trees (in the first half of May), and then destruction has progressed fast. There was noticed another interesting fact to observe. Because being a few old plum trees among the apricots in the fruit garden, it is evident the presence of *Cacopsylla pruni* on the plum trees which are the main vector in the transmission of the pathogen (*Figure 1*).

Figure 1: **The vector of *Ca.* Phytoplasma prunorum - *Cacopsylla prunorum*** (Source: Dr Wolfgang Jarausch, Agroscience)

The grown kinds of apricot on the plantations were the follows: Ceglédi Óriás (Cegléd Giant), Ceglédi Arany (Cegléd Gold), and Magyar Kajszi (Hungarian Apricot). The Cegléd varieties are more susceptible to phytoplasma disease than the Hungarian Apricot one.

65% serious destruction was experienced in the third apricot population on 3 hectares. Similar sights were visible on the fourth examined plantation where grown 12-13 year-old apricot trees. More than 20% was the rate of the killed trees, and further 40 percentage of apricot trees were just in the fast destructing run.

Other stone fruit varieties were also examined during the field investigations in fruit gardens in Borsod-Abaúj-Zemplén County. A more moderate infection was experienced in a 12-13-year-old peach cultivar where phytoplasma infection with 21% rate was experienced. There was not so high rate the destruction on peach than it was on apricot, but the problem with *Ca.* Phytoplasma prunorum is evident. Three sour cherry and one cherry plantations were also examined. There were found destructions (in different rate) caused by phytoplasma. On the first examined sour cherry plantation was a very high infection rate (62%) and there were several withered or fell trees. It was observable well that sour cherry and cherry are also endangered by *Ca.* Phytoplasma prunorum infection.

Summarizing the results of the field experiences and the measures of infections we can say that the conditions of stone fruit plantations on the visited areas are rather bad conditions (illustrated by the photos on *Figure 2-10*).

Figure 2: **Dead apricot tree**

Figure 3: **Dried branches on Apricot tree**

Figure 4: **A destructed cherry tree**

Figure 5: **Leaf rolling symptoms on peach**

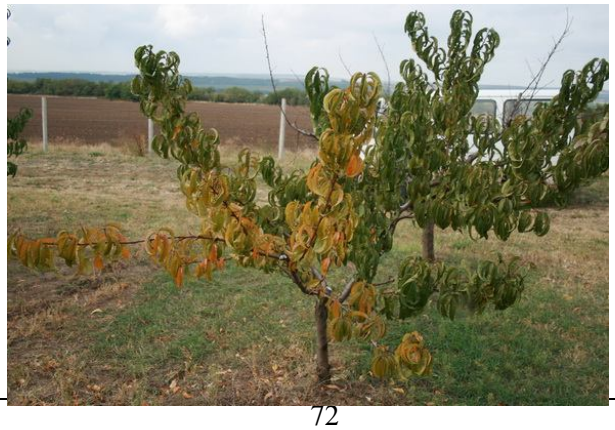
Figure 6: **Dried leaves on apricot trees**

Figure 7: **Yellowing leaves on apricot trees**

Figure 8: **Stumps of destructed and felled apricot trees**

Figure 9: A **dried and felled sour cherry tree**

Figure 10: **Yellowing peach brunches**



Photos: by G. Tarcali

Possible protection methods against phytoplasmas

A promising strategy to avoid phytoplasma disease is the identification or development of resistant plant varieties (Welliver, 1999). But management and control have to focus mainly on the clean stock programs, eliminating sources of the phytoplasma, and controlling vectors as the follows:

- propagate from phytoplasma-free plants,
- eliminate perennial and biennial weed hosts,
- avoid planting susceptible plants next to plant harboring phytoplasma,
- control the vector in the plant and nearby weeds early in the season,
- plant varieties that are more resistant to the disease, if available.

The ecology of phytoplasmas is complex, and affected by the host range and geographic distribution of both phytoplasma and the insects that transmit them, and is strongly affected by weather conditions. As more is learned about relationships among disease organisms, vectors and hosts, surprising ecological niches have been uncovered, and theories of how disease may have evolved have been developed. These suggestions may be important in choosing management strategies for disease, and in forecasting where new disease outbreaks may occur.

CONCLUSIONS

This study was the first accurate examination to evaluate the occurrence of *Ca.* Phytoplasma prunorum (ESFY phytoplasma) on stone fruits in Gönc Apricot Growing Area, situated in Borsod-Abaúj-Zemplén County, Northern-Hungary. The disease caused by that pathogen is an increasing and relatively new problem for fruit growers in Hungary. It can be seen well that the problem is very serious, and *Ca.* Phytoplasma prunorum endanger almost every stone fruit plantations. Experiences relating to the results of our investigation have to pay attention to the increasing phytoplasma problem in stone fruits, and have to develop new effective management strategies.

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Fungicide resistance against botryoticids in Hungarian vineyards

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SUMMARY

In grapevine, the frequent occurrence of B. cinerea prior to harvesting results serious losses of fruits and deterioration of wine quality. B. cinerea has been shown to have several variable genetic and physiological traits. It is able to act as a saprophyte as well as a pathogen, and it has developed resistance to most of the fungicides used to control it. Under examinations the isolates form Eger, Tokaj, Badacsony and Villány wine regions for fenhexamide and dicarboximide fungicides were tested on agar plates and pirimethanil with in vitro test method.

Keywords: Botrytis cinerea, fungicide resistance, fenhexamide, dicarboximide, pirimethanil

INTRODUCTION

Botrytis cinerea (de By.) Pers. (teleomorph: *Botryotinia fuckeliana*, Whetz) is a cosmopolitan ascomycetous fungus that causes grey mould on a great number of plants in the temperate zone worldwide by infecting various tissues. The agricultural producers, using either traditional or integrated or ecological pesticide management, make huge efforts to control the disease, which causes large outgivings for them. In practice, protection usually means the application of chemicals. Because the infection is endemic, and the fungus is extremely variable, the appearance of resistant strains against almost all the fungicides used is a frequent phanomenon. The widespread employment of fungicides increases the risk of environment pollution. In order to maintain the effectivity of chemicals and decrease the risk of environment pollution, it is necessary to determine the general parameters of *B. cinerea* populations and the status of fungicide resistance in the local populations. Reasonable and effective plant protection technologies can be built based on this knowledge.

In grape wine *B. cinerea* is feared by wine growers because of its qualitative and quantitative effects on wine production (Built and Dubos, 1988). Chemical control remains the main way to reduce the incidence of gray mould. Recently several highly active fungicides have been introduced but with some of them failures of disease control have been observed in vineyards. The present study was carried out to test the in vitro sensitivity of *B. cinerea* isolates, obtained from Hungarian vineyards towards various fungicides, they include members of the main botryoticide families.

MATERIALS AND METHODS

Strains of *B. cinerea* were collected from treated Hungarian vineyards located in Badacsony, Eger, Tokaj and Villány. They were isolated from infected berries in 2007 year at the harvest (september - october). Single-spore isolates were prepared for following works. The examined fungicides were the following: fenhexamid (Teldor 500SC, Bayer), dicarboximide (Rovral 50 WP, BASF) and pyrimethanil (Mythos 30SC, BASF).

For iprodione and fenhexamid tests fungicide added agar plates in three concentrations were used. The Petri dishes was inoculated with an inverted 5 mm diameter mycelium plug. Three replicates were used per treatment and incubation took place at 27 ^{0}C in the dark. The mycelical growth rate was evaluated from the diameter of fungal colonies, measured daily for four days. Fungicide sensitivity categories (sensitive, low resistance, resistance) were defined according to the discriminatory doses that differentiate resistant from sensitive isolates by proportion of EC_{50} resistant and EC_{50} sensitive. (Leroux et al, 1999). The isolation is sensitive if EC_{50} < 2 mg/l, has a low resistance if 2 mg/l < EC_{50} < 6 mg/l, resistant if 6 mg/l < EC_{50} in case of dicarboximide and sensitive if EC_{50} < 2 mg/l, has a low resistance if 2 mg/l < EC_{50} < 5 mg/l, resistant if 5 mg/l < EC_{50} in case of fenhexamid.

In pyrimethanil tests the isolates sampled was inoculated on two apples, variety *Golden Delicious*, one treated with pyrimethanil, one nontreated. In each apple, a hole of 8 mm diameter and 3-4 mm depth was made. The control apple is treated with 50 µl sterile water; the test apple is treated with 50 µl of the 20 ppm pyrimethanil solution. Inoculation is carried out 2 hours after treatment at the minimum in order to let the product penetrated in the apples. Then apples were put in dark condition at 18-20°C. Symptom observed was a necrosis of the apple around the inoculation point. 5 days after inoculation, the diameter of the necrosis was measured. Then the percentage of efficacy was calculated according to the following formula: [(Φ control – 8) – (Φ treated – 8)] / (Φ control – 8) x 100 (Forster and Muller, 1996).

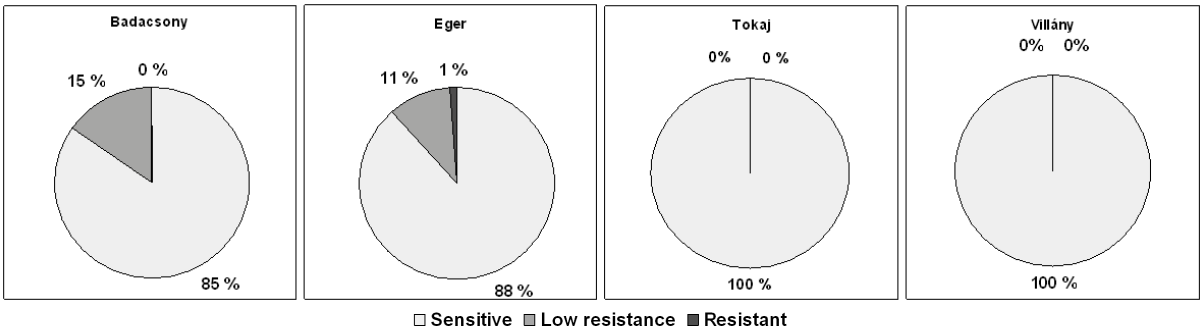
Each isolate showing a percentage of efficacy inferior to 50% are retested at the same concentration, but using 3 replicates. If the percentage of efficacy inferior to 50% was confirmed, the isolate was considered as less sensitive.

RESULTS AND DISCUSSION

More than two hundred and fifty B. *cinerea* isolates were collected from infected berries in within named vineyards in Hungary in 2007.

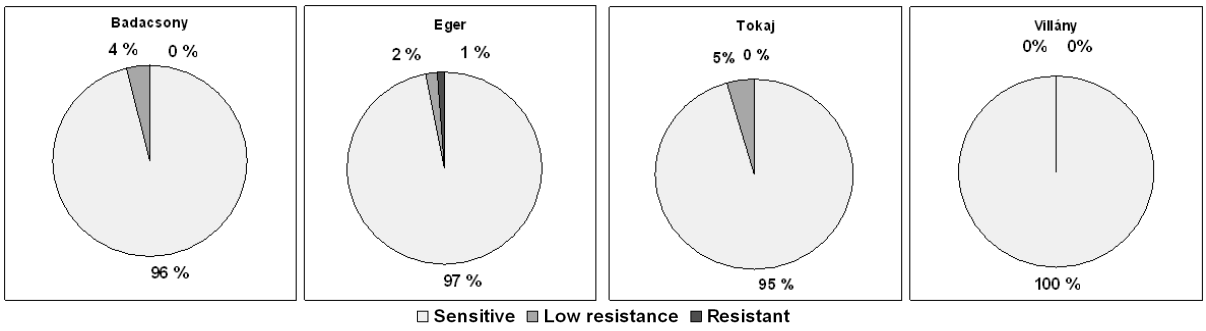
The fenhexamid resistance test shows a great variability against vineyards. Even tough that in Badacsony and Eger there were a noticeable resistance level between the isolates in Tokaj and Villány there were not any resistant isolate (*Figure 1*).

Figure 1: **Distribution percentage of fenhexamid resistance levels in Hungarian vineyards.**



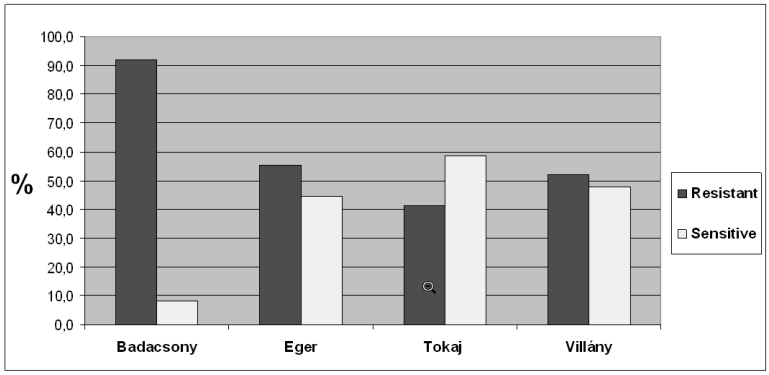
The dicarboximide resistance of isolates was very low in all territories, the resistance was presented in Tokaj and in Villány territories there were not resistant isolates either (*Figure 2*).

Figure 2: **Distribution percentage of dicarboximide resistance levels in Hungarian vineyards.**



In the course of examination the pyrimethanil resistance was found that in average approximately half percentage of isolates were resistant against the fungicide in all territories. The highest was detected in Badacsony , where almost all of the isolates were resistant (*Figure 3*).

Figure 3: **Distribution percentage of pyrimethanil resistance levels in Hungarian vineyards.**



CONCLUSIONS

In summarises the segregation of fungicide resistance do not show a high variability between vineyards in case of fenhexamid and dicarboximide, but there were great difference in case of pyrimethanil.

In the first case isolates had tractable low fungicid resistance towards the fenhexamid and in two vineyards there were not any resistant isolates. The situation with dicarboximide was very similar, the percentage of resistant isolates was low, in one vineyard there was no resistant isolate, and resistant isolates could be detected in one territory.

In the case of pyrimethanil almost half of the isolates showed resistance against the fungicide in all territories, in spite of the fact that this fungicide has been itroduced just some years ago. In one territory almost all of the isolates were resistant against pyrimethanil, but the resistance percentages were about fifty in other territories. Hopefully that was caused just a misguided grape protection technology and not an ordinary tendency, but the fact that the full resistance against this fungicide can speared as quick and efficient forewarn carefulness and circumspection about this fungicide. Due to the well known high genetic variability of *B. cinerea* and in behalf of effective plant protection should not be used the fungicides without due foresight and also with the same mode of action in the sequential years.

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Experiences of raspberry production in "Benedek Fruit Farm" in Hungary

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SUMMARY

The protection against grey mould caused by B. cinerea is one of the crucial points in the raspberry plant protection. The Benedek Fruit Farm has produced berries since 1997 in integrated production system, which fit into the growing area, suitable fruit varieties etc. Control of Botrytis disease concentrated on flowering, spraying in early blossom (10-20% flowering), than weakly repeated until the end of flowering. However there are several questions to discuss with the authorized chemicals.

Keywords: raspberry, *Botrytis cinerea*, fungicide

INTRODUCTION

The Benedek Fruit Farm was founded in 1995. The first fruits were harvested in 1997 from strawberry plantations. Our strawberries were the first fresh fruits awarded with the "Quality Food from Hungary" trademark in 1999.

The three girls of the family have grown up during the passed ten years. They take part in the everyday life of the Benedek Fruit farm and use their special diploma and language skills in the organization of the production and market. Our products also have expanded with autumn raspberry, and red and black currant and sour cherry. Harvesting machines are available for us and the later two fruits are produced in mechanized farming. Integrated production fit into the growing area, suitable fruit varieties etc. The applied cultivation systems considered the demands of the species and the combined, manually and mechanically, harvesting methods in the plantations. Herbicides are used only in narrow inter row area, while mechanical weed killing is applied under the plants. The whole plantation is irrigated with sprinkle irrigation system.

MATERIALS AND METHODS

Raspberry production

The climate of Hungary is favourable for raspberry production but the selection of fruit variety and technology remains a determinant point. Nowadays, the most widespread variety is "Fertődi Zamatos". Its popularity is due to its resistant against diseases, abundant cane production and tasty flavor.

The Benedek Fruit farm chose the "Autumn Bliss" raspberry variety which also bear some fruit on the first-year canes (primocanes) in the late summer and can contribute to the extension of harvesting period which is an important economical point since in autumn the prices are higher and the demand is continuous for fresh fruits. The first-year canes type raspberry bears fruit after the traditional second-year canes type one and the season last till the first freeze.

The special feature of the raspberry production is that the canes are cut and burned right after the harvesting so that the (most important disease, the spur blight caused by *Didymella applanata* (anamorphic stage *Phoma argillacea*) is also preventable.

The advantages of the production on first-year canes are the following: the shrubs are breezy, the fruits are placed high on the outside part of the hedge far away from the ground-level, and the destruction of the canes in autumn reduces the infection sources.

The "Autumn Blissy" variety can be harvested two yields from the shrub, one in summer and one in autumn. This vigorous plant bears large fruits of excellent taste. Special feature technology is to cut the canes after the harvest. In this case there is no summer production but the size of the berries is bigger in autumn. The type is resistant to the root rot caused by *Phytophthora fragariae rubi* W.F. Wilcox & J.M. Duncan but it is sensitive for *Botrytis cinerea* in cold, wet weather conditions.

RESULTS AND DISCUSSION

Control of *Botrytis cinerea*

The protection against grey mould caused by *B. cinerea* is one of the most crucial points in the raspberry plant protection similarly to the other berries. There is an indirect loss in case of fruit infection. The symptoms of grey mould infection can be seen in browning blossom, the decay of unripe berries. Infected ripe berries become soft, watery and light brown with an unpleasant taste (Elad *et al.*, 2004).

In cool, humid weather the mycelium produces large numbers of conidia in the generative parts of the plant. Germinating spores penetrate tissues through wounds but overripe berries also can be infected. Control of *Botrytis* disease must be concentrated on flowering, spraying in early blossom (10-20% flowering), than weakly

until the end of flowering. The infection becomes serious in cool and wet weather. Control of *Botrytis* diseases is aided by the removal of infected berries with early harvest (Jarvis, 1962).

Authorized chemicals against *Botrytis cinerea* in raspberries in Hungary

There are several problems in the protection of ripening berries. One of these is the waiting period for pesticides just before harvest. Moreover, the irrigation is essential for profitable production, but wet conditions are favourable for grey mould infection. The humid autumn weather also increases the risk for infection.

There are more botryocide chemicals are available for the Hungarian raspberry producers than the German ones (*Table 1*). However the chemicals with the latest active ingredients have not been introduced in Hungary because of the costs. The broader spectrum of available chemicals may be advantages for the Hungarian producers, but there is no information about resistance against them. The mode of action of the different ingredients also can be similar to the authorized chemicals in Hungary. Spraying was used in the beginning, in the middle and at the end of blossom till now in our plantation. The spectrum of the applied chemicals is determined by waiting period for harvest (WPH), which results more problem in the first year cane raspberry production.

The efficacy of the spraying depends not only on the way and time of application and the weather conditions, but also on several other factors (Tanovic *et al.*, 2008).

Table 1

Authorized chemicals against *Botrytis cinerea* in raspberry plantations in Hungary and Germany

Commercial name	Active ingredients	Applied doses	Waiting period for harvest (WPH)	Authorized in Hungary	Authorized in Germany
Captan 50 WP	captane 50%	2 kg/ha	10 days	yes	no
Folpan 80 WDG	folpet 80%	1,5 kg/ha	10 days	yes	no
Merpan 50 WP	captane 470 g/kg	2 kg/ha	10 days	yes	no
Merpan 80 WDG	captane 80%	1.25 kg/ha	10 days	yes	no
Mythos 30 SC	pirymethanil 300 g/l	1-2 l/ha	5 days	yes	no
Orthocid 50 WP	captane 50%	2 kg/ha	10 days	yes	no
Quadris	azoxistrobin 250 g/l	0.75-1 l/ha	7 days	yes	no
Rovral 50 SC Aquaflow	iprodione 500 g/l	1 l/ha	14 days	yes	no
Signum*	F 500 6.7% boscalid 26.7%	1.8 kg/ha	3 days	no	yes
Switch**	fludioxonil, cyprodinil	1-2 kg/ha	3 days	no	yes
Teldor 500 SC***	fenhexamid 500 g/l	1-2 l/ha****	7 days	yes	yes
Topsin-M 70 WP	thiophanate-methyl 70%	1 l/ha	7 days	yes	no
Trichodex WP	*Trichoderma harzianum* T-37 strain 20 %	2 kg/ha	1 day	yes	no

*Authorized till 2019; maximum 2 times in a year

** Authorized till 2012.

*** Authorized till 2011 in Germany; maximum 4 times in year.

****Applied doses: 1 l/ha in Hungary, 2 l/ha in Germany

Comparing the chemicals available in Hungary and in Germany, one of the most interesting things is that captane can not be used in Germany; however there are three different chemicals available in Hungary.

The two captan fungicides are authorized only in the apple, the folpet only in grape, but not in raspberry in Germany. Quadris (azoxistrobin) can only be used against *Colletotrichum* in strawberry but not in other berries in Germany. Rovral (iprodione) can not be used in berries in Germany. The fenhexamid containing Teldor can be used in double dose in Germany, than in Hungary. The Signum, which can be used till 2019 in Germany, is authorized only in stone fruits in Hungary.

CONCLUSIONS

There are several problems in the protection of ripening berries. One of the most important fungal pathogen is *Botrytis cinerea* infecting not only blossoms and berries, but also canes and causing grey mould. There are several chemicals are available in Hungary against *Botrytis*. However, the chemicals Signum and Switch have not been introduced in Hungary in strawberry, and there is no information about resistance against the active ingredients. The mode of action of the different ingredients also can be similar to the authorized chemicals in Hungary.

The combination, sub-alternation and alternation of different pesticide would be interesting because we have not had published results in Hungary till now and so that we are not able to take into consideration. The plantation of the new varieties could be a solution but the breeding of first-year canes raspberry is not in progress in Hungary. The plantation of import raspberry varieties could be risky since we do not know too much about how these ones will verify the advantages under Hungarian climate. Getting information for more effective protection can be organized by using internet or taking part on conferences and workshops. However, our results based on direct experiences in our own plantation will be the best to improve our protection methods.

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The experience of development of fungicidal preparations based on mixed salts of imidazolium derivatives

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SUMMARY

The mixed salts of imidazolium derivatives stabilized with beta-glycerophosphoric acid expressed synergetic joint action against benomyl-sensitive Botrytis cinerea. These salts efficiently inhibited benomyl tolerant B. cinerea strain on grape berries, however, the synergy took place only in the case of penconazole+carbendazim mixed salt. The application of carbendazim+chlotrimazole binary salt is particularly interesting, due to low mammalian toxicity of ingredients. The use of β-glycerophosphoric acid has great advantages due to its compatibility to living tissues. Its binary salt of carbendazim and azol-derivatives efficiently can control populations of B. cinerea built up of benomyl-tolerant and benomyl-sensitive strains.

Keywords: imidazolium derivatives, *Botrytis cinerea*

INTRODUCTION

It is well known that imidazol-derivative fungicides react with various mineral acids and the resulted salts inhibit the growth of target phytopathogenic fungi more efficiently than their free bases (Oros and Detre, 2001; Oros and Cserháti, 2009). This phenomenon was utilized and some industrial developments have been protected by patents (HUP 226 358, HUP 226 359). The low stability of these salts as well as the strong corrosive effect of their watery suspensions is the main disadvantages of such preparations (Oros and Detre, 2001). Moreover, the acidity of spray due to dissociation of imidazolium salts may cause fire blight of leaf surface leading to lose of foliage or decreasing the decorative value of ornamental plants.

The imidazole-derivative containing fungicidal preparations exhibit excellent protective and eradicative effects against *Botrytis cinerea*, among them benzimidazole derivatives (*Figure 1*) are of particular importance due to their low mammalian toxicity, and distinctly to other azole fungicides the benzimidazoles in their therapeutic doses either do not alter the organogenesis of plants or such type of effect can be antidoted (Oros, 1997). Unfortunately, like to other phytopathogenic fungi, in *Botrytis* populations rapidly emerge numerous variants of strains tolerant to benzimidazole fungicides pressing the producers to change the pest management technology. This phenomenon was first observed in the population of apple scab fungus acquiring tolerance to benomyl in the first vegetation period next to introduction (Delp, 1980), later was shown that the cross tolerance is complete among this group (Oros, 1981), moreover, the benomyl tolerant strains exhibit tolerance to carbamate type fungicides as well (Dekker, 1987).

The aim of our work was to develop a fungicidal preparation that is useful for simultaneous control populations of *B. cinerea* built up of benomyl-tolerant and benomyl-sensitive strains.

MATERIALS AND METHODS

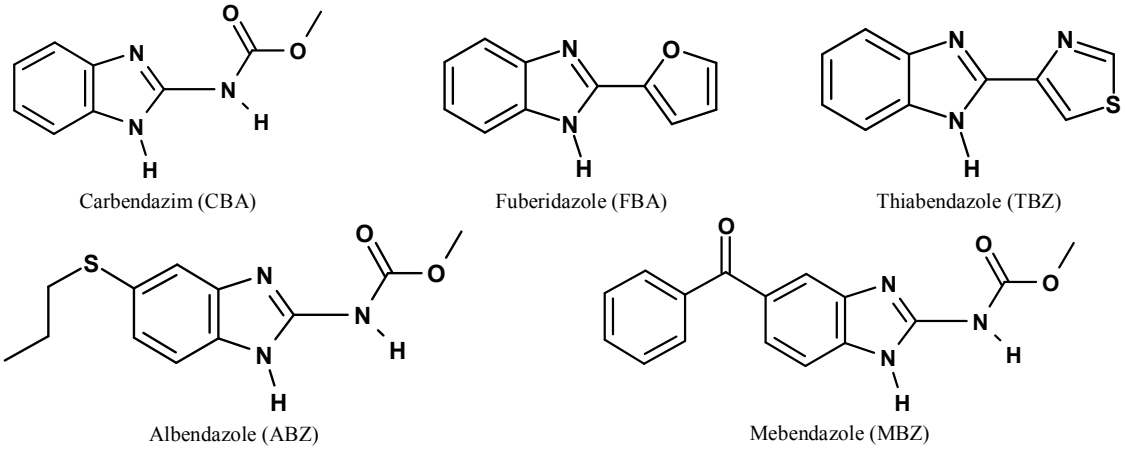
The *Botrytis cinerea* Pers. strains were isolated from grape berries (*Vitis vinifera* L. cv. Chasselas) and maintained in potato dextrose agar slants. Grape berries (cv. Attila) were taken of pesticide free producing orchard. The active ingredients and their formulated fungicidal compositions were made in laboratories of PPI and VetPharma.

***In vitro* determination of fungicidal activity**: The *B. cinerea* conidia were produced on infected grape berries (cv. Attila). Aliquots of the pre-incubated (6 hrs) suspension of conidia (10^5 cell ml^{-1}) in sterile tap water were added to suspensions of test compounds of appropriate molar concentration then incubated further 18 hrs at 24±1°C. The branching of germ tubes was examined in light microscope, and the concentration which completely inhibited the branching of hyphae was taken as effective dose (minimum inhibitory concentration).

Inhibition of gray mould of grape: Single grape berries with stalk were immerged into watery suspension of preparation (50 mg active ingredient per liter, 10 ml per berry) for 20 minutes, and then they were put on plastic lattice to dry up (minimum 1 hour). Using a light needle the skin of berries was perforated centrally (~ 1 mm depth) and 5 µl of conidial suspension was dropped on the wound. The infected berries were incubated in plastic boxes (5×4 berries per box), and after four days the diameter of lesion was measured. The decrease in size of lesion due to treatment as compared to the untreated control was used as a parameter for calculating efficacy of treatment. The inhibitory effect was expressed as a percent.

The significance of difference between efficacies of various treatments was evaluated by Fisher's test. All other details were outlined (Oros, 1993).

Figure 1: **Benzimidazole derivatives**



RESULTS

The branching of hyphae of the benomyl-sensitive *B. cinerea* strain was inhibited by all salts of all benzimidazole derivatives (BDs) more efficiently than their free bases (*Table 1*). Among the organic anions the only beta-glycerophosphate (GPA) increased the efficacy of bases of BDs on the same level as the mineral acids. The benomyl-tolerant strain proved to be tolerant to all benzimidazolium salts with exception of albendazole (ABZ). Its methosulphate partially inhibited the germination of benomyl-tolerant conidia; however, this salt did not caused teratoids on the tips of hyphae unlike to conidia sensitive to benomyl.

The inhibitory effect of organic acids used as counter anion in benzimidazolium salts was examined in form of sodium salts. The methosulphate and p-toluolsulphonate slightly retarded the germination of conidia of both strains, while the other acids proved to be ineffective but beta-glycerophosphate that stimulated the growth of germ tubes. None of organic acids generated micro-morphological alterations, thus their effect were not taken into the consideration at the final evaluation of results.

The infection on grape berries was successful, and about half of their surface was invaded. The two strains did not differ significantly in their agressivity. The free base of carbendazim was less efficient than its glycerophosphorous salt (*Table 2*), and this salt was ineffective against benomyl-tolerant strain either. The reproducibility of treatment was surprisingly high, however, the success against benomyl-tolerant strain varied at higher extent than that of against benomyl-sensitive one (F_{res}=1.15 > F_{sens}=0.18 < $F_{0.1}$=2.44).

Table 1

Inhibitory effect of benzimidazolium salts to *Botrytis cinerea* conidia

No.	Counter anion	Benzimidazole-derivatives[a]				
		CBA	FBA	TBZ	ABZ	MBZ
1	no (free base)	125	500	500	2000	>2000
2	Cl^-	62.5	125	125	500	2000
3	SO_4^{2-}	62.5	125	125	250	1000
4	PO_4^{3-}	62.5	250	125	250	2000
5	NO_3^-	62.5	125	125	250	1000
6	methosulphate	125	250	250	100	>2000
7	laurylsulphate	125	250	125	500	2000
8	dodecylphosphonate	125	250	125	500	2000
9	dodecylsulphosuccinate	125	250	125	250	1000
10	p-toluolsulphonate	125	125	250	500	2000
11	glycerophosphate	62.5	125	125	250	1000
12	acetate	125	250	250	1000	>2000
13	adipate	125	250	250	500	>2000
14	citrate	125	125	250	250	1000

[a]= The labels of compounds corresponds to those in *Figure 1*

The most widely used azol-derivatives inhibited the evolution of infection, however, excepting chlotrimazole and penconazole all proved to be less efficient against benomyl-sensitive strains than carbendazim (*Table 2*).

Table 2

Joint action of carbendazim and azole derivative fungicides

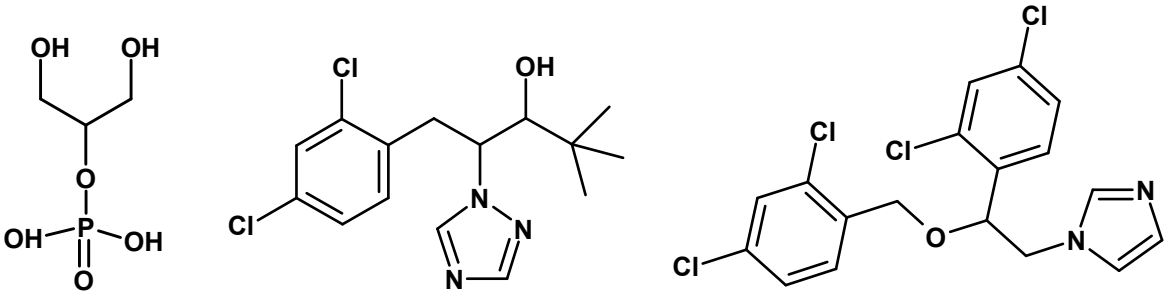
Treatment		Inhibitory effect (%)				Increase of efficacy	
		BCS[a]		BCR[a]			
		X_a	$Y\times[X_a\times X_b]$	X_a	$Y\times[X_a\times X_b]$		
No.	Compounds[b]	A	B	C	D	B-A	D-C
1	Carbendazim	21	32	0	0	+11	0
2	Bitertanol	3	58	7	13	+55	+6
3	Diclobutrazole	6	59	1	2	+53	+1
4	Chlotrimazole	37	41	22	31	+4	+9
5	Myclobutanil	13	53	11	12	+40	+1
6	Miconazole	5	62	4	7	+57	+3
7	Propiconazole	23	53	32	28	+30	-4
8	Penconazole	30	62	15	30	+32	+15
9	Prochloraz	22	35	23	32	+13	+9
10	Triadimefon	23	53	4	7	+30	+3
11	Triadimenol	26	59	10	10	+33	0

[a]BCS = benomyl-sensitive and BCR = benomyl-tolerant *B. cinerea* strains

[b] X_a= any of listed compounds, X_b= carbendazim, Y = beta-glycerophosphoric acid (alone ineffective $LSD_{replication}$=2 (F=0.65), $LSD_{combination}$=7 (F=26.3)

The preparations made of binary salts of carbendazim and most widely used azol-fungicides (HUP 226 358), proved to be stable. All these preparations exhibited good activity against both *B. cinerea* strains. Their improved efficacy against benomyl-sensitive strains was resulted of their synergic joint action, but this synergy manifested only in the case of carbendazim+penconazole against benomyl-tolerant strain.

Figure 2: **The most effective counterparts of carbendazim**



β-glycerophosphoric acid Diclobutrazole Miconazole

DISCUSSION

The mixed salts of imidazolium derivatives stabilized with beta-glycerophosphoric acid expressed synergetic joint action against benomyl sensitive *Botrytis cinerea*. These salts efficiently inhibited benomyl tolerant *B. cinerea* strain on grape berries, however, the synergy took place only in the case of penconazole+carbendazim mixed salt.

The application of carbendazim+chlotrimazole binary salt is particularly interesting, due to low mammalian toxicity of ingredients.

The use of beta-glycerophosphoric acid has great advantages due to its compatibility to living tissues. Its binary salt of carbendazim and azol-derivatives efficiently can control populations of *B. cinerea* built up of benomyl-tolerant and benomyl-sensitive strains.

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Studies on western corn rootworm infestation in relation to the nutrition supply levels of plants and year of production

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SUMMARY

The changes in the corn rootworm populations were examined in long-term fertilization experiments under rainy and droughty weather conditions. The swarming of adult beetles was characterised using traps with floral bait (KLPflor) in unfertilized and intensively fertilized maize plots.

In the rainy production year when maize was grown after winter wheat, the swarming peak occurred in the middle of August. In the droughty year when maize was sown after maize, the first swarming peak was detected on 3rd July. Beside the dates of swarming peak, the maximum values, intensity and duration of swarming also showed significant difference in the two examined years. The number of corn rootworms quickly decreased on the maize plants drying down earlier due to the excessive lack of precipitation. Corn rootworms were last caught in the droughty year on 17th August by traps on unfertilized plants, and 31st August in fertilized maize plots, while in the rainy year the beetles were present until the second decade of October. Weather conditions had less influence on the beetles' activity before the populations reached their swarming peak, i.e. during the stage of intensive population growth than afterwards.

Keywords: western corn rootworm, swarming, fertilization, dry year

INTRODUCTION

In spite of the fact that production risk is increased due to the infestation of corn rootworm (Diabrotica v. virgifera LeConte), maize is often sown for two consecutive years, mainly at production sites enabling intensive plant production techniques and providing higher yields. Due to the reduction in the effectiveness of insecticides from the middle of the last century, the positive effects of agrotechnique, in particular crop rotation became more acknowledged in greatly infested areas (Pike and Gray, 1992). The costs of chemical protection against corn rootworm as part of integrated plant protection in Hungary still mount up to a sum in the order of milliards of forints (Marton et al. 2008). Therefore, it is of great importance to apply every result generated by research in connection with agrotechnique (Széll, 2007), chemical plant protection (Ripka, 2007), and plant breeding (Szőke et al., 2008) to control corn rootworm that can help to reduce the expenditures and loss.

MATERIAL AND METHODS

The changes in the corn rootworm populations were examined in long-term fertilization experiments in Martonvásár in the year 2008 with weather conditions beneficial to maize and in the droughty 2009 (Table 1). The preceding crop grown in 2007 was winter wheat. Examinations were carried out (1) in plots with no fertilization (0 kg/ha N, 0 kg/ha P2O5, 0 kg/ha K2O) and (2) in plots supplied with high levels of fertilizers (120 kg/ha N, 80 kg/ha P2O5, 100 kg/ha K2O).

CSALOMON® KLPflor non-sticky traps with floral bait (Tóth et al., 2006) attracting females and males as well were used in two repetitions. Trapped adults were counted every 3 or 4 days from the beginning of intensive swarming. The populations were detected 23 times from 21st July till 14th October in 2008, and 18 times from 29th June till 23rd September in 2009. Data were analysed in accordance with the guidelines prepared by Sváb (1981). The strength and reliability of correlations between the number of trapped corn rootworms and the meteorological parameters were also analysed. The time intervals before and after the swarming peak were also included in the analysis.

RESULTS AND CONCLUSIONS

In accordance with the criteria defined by Harnos (1993) and on the basis of the amount of precipitation, the year of production at the site of experiments was rainy in 2008 and droughty in 2009 (Table 1). As compared to the 30-year average precipitation data of Martonvásár, the rainfall during the vegetation period of maize in the first year exceeded the average with 170.7 mm (+ 55%), while in the second year it was 135.1 mm (- 43%) less than the average. In the course of the swarming of adult corn rootworms (July-September), the amount of precipitation was 69.9 mm (+ 50%) higher in the first year, and 476 mm (- 34%) less in the second year.

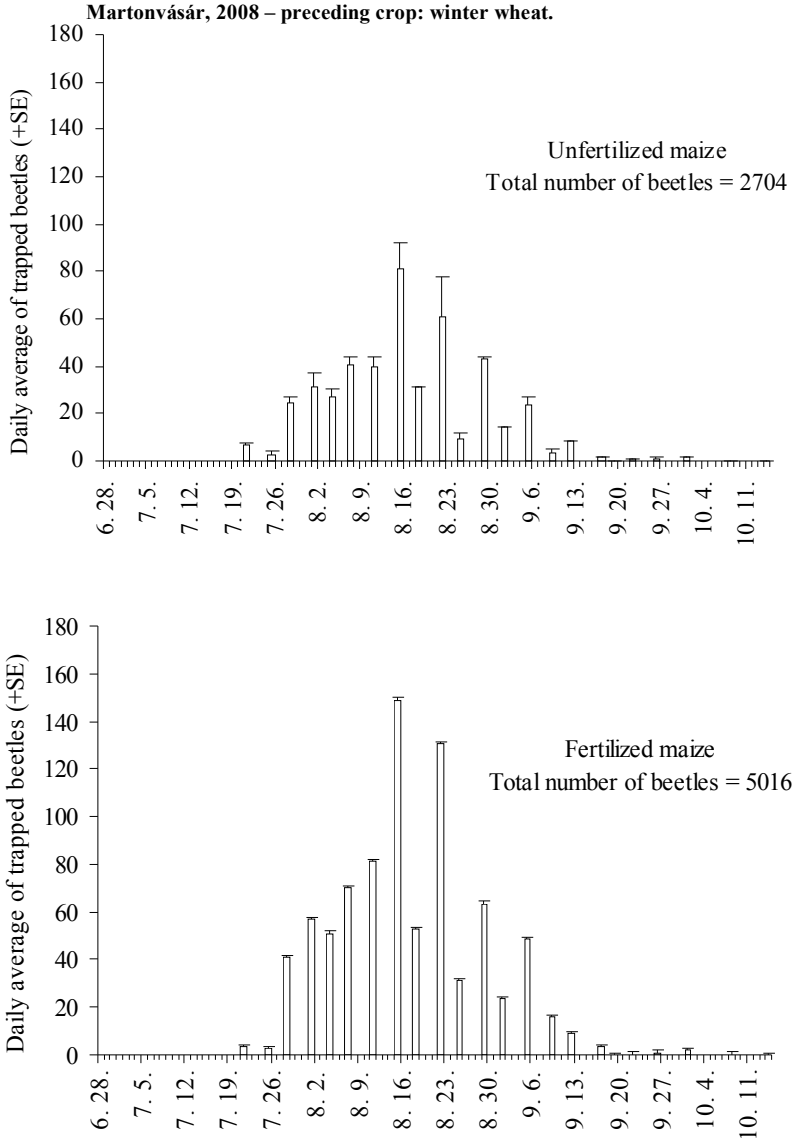
Table 1

Climatic features during maize vegetation period. Martonvásár, 2008 and 2009.

Month	Precipitation (mm)			Average temperature (°C)			Hot days [a]		
	Aver. [b]	2008	2009	Aver. [b]	2008	2009	Aver. [c]	2008	2009
IV.	43	36.2	2.1	11.3	11.8	14.0	0	0	0
V.	56	62.6	12.6	16.4	17.0	16.3	4	3	1
VI.	73	174.0	69.8	19.7	21.2	18.5	9	8	3
VII.	53	76.8	22.8	21.5	21.3	21.8	14	13	18
VIII.	46	44.5	42.8	20.7	21.0	21.5	12	13	10
IX.	41	88.6	26.8	16.6	15.3	18.5	2	5	2
Σ, or aver. IV-IX.	312	482.7	176.9	17.7	18.0	18.4	41	42	34
Σ, VII-IX.	140	209.9	92.4	19.6	19.2	20.6	28	31	30

Index: a - tmax ≥ 30oC; b – 30-year average; c – 10-year average;

Figure 1: **Dynamics of corn rootworm swarming in long-term fertilization experiments. Martonvásár, 2008 – preceding crop: winter wheat.**



In the maize stand grown after winter wheat, the average number of trapped corn rootworms increased continuously from the middle of July (*Fig. 1*). In the maize experiment located approximately 250 meters away from maize plots infested with larvae, the swarming peak occurred in the middle of August. The curve of swarming was similar, independently of the nutrition supply levels of plants, however, the intensity of swarming – i.e. the number of beetles caught – differed significantly on plants with different levels of nutrition supply. Overall, 2704 adults were found in the plots with no fertilization, while 5016 adults were trapped on plants in the fertilized plots. The highest daily average was 81.50 beetles in maize with nutrition deficiencies, and 148.83 beetles in well-supplied plots (15th August). It was only on 4 occasions out of the 23 detection dates when less corn rootworms were found on plants with higher nutrient supply, and these occasions were observed before or after the intensive swarming period. The vegetation period in the rainy production year was longer than the average, therefore, the beetles were present until the second decade of October.

In accordance with the number of corn rootworms trapped by the KLPflor non-sticky traps with floral bait placed out on 22nd June 2009, the first swarming peak in the maize produced after maize was on 3rd July (Fig. 2). The intensity of this peak – occurred after the months of April and May with practically no precipitation – was significantly lower that that of the previous year. The maximum of daily average catches in the plots with high levels of fertilization was less than thirty (27.75). Initially, the difference between the nutrition supply of plants did not influence the infestation caused by adult beetles. However, after the first swarming peak more corn rootworms were detected on well-supplied plants at each observation. Consequently, the overall number of trapped beetles was 700 on the plants with no fertilization supply, and 937 on the intensively fertilized plants. The number of beetles caught decreased continuously during the week after the first swarming peak, and a significant positive correlation was found between fertilization and the number of adult beetles. On 10th July, an average number of 1.50 corn rootworms were found on unfertilized maize plants, and 8.88 on fertilized ones.

Afterwards, the density of populations increased again gradually and to a small extent. The second swarming peak was observed on 17th July with the daily average catches of 11 beetles on plants with no fertilization and 21.25 beetles in fertilized maize plots. The number of female and male beetles has not been analysed yet, but the two swarming peaks must be the consequence of the fact that females swarm 2-3 weeks later than males (Bayar et al., 2003, Árendás et al., 2009). From the second swarming peak onwards, the number of beetles reduced at a great pace in the second half of July on the maize plants drying down quickly as a result of the high number of hot days (10 out of 14) and excessive lack of precipitation. Corn rootworms were last caught on 17th August by traps on unfertilized plants, and 31st August in fertilized maize plots. In accordance with determinate relation between the number of beetles and climatic parameters in the course of the entire swarming period (Table 2), the increase in the extreme values of solar radiation and temperature had positive effect on the activity of corn rootworms in the rainy production year. At the same time, the number of trapped adults decreased with the increase in atmospheric humidity. In the droughty production year, the average of daily catches had significant medium strong correlation with precipitation and relative humidity. Considering the main sections of swarming, it was found that weather conditions had less influence on the beetles' activity before the populations reached their swarming peak, i.e. during the stage of intensive population growth than afterwards. On the basis of the number of trapped corn rootworms, the more reliable influence of several climatic parameters could be observed after the swarming peak in the rainy production year.

Table 2

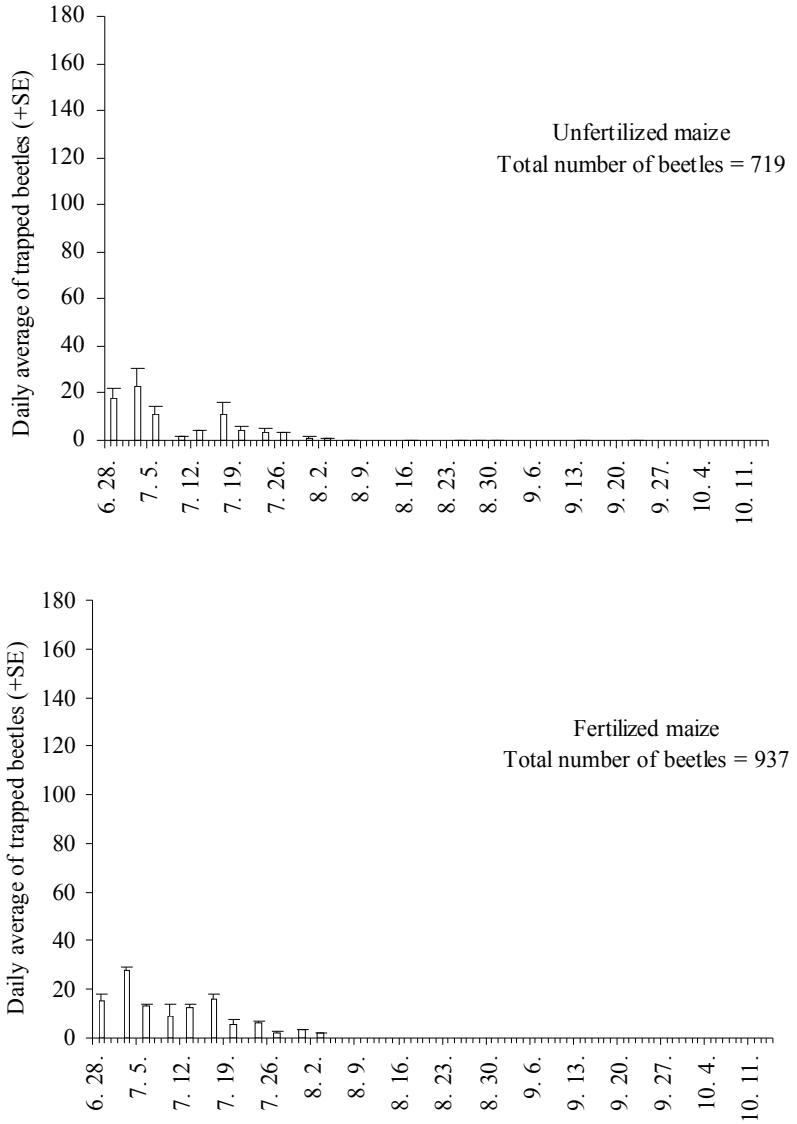
Relation between the swarming of corn rootworm and certain climatic parameters (r) in different production years. Martonvásár, 2008 and 2009.

Climatic parameter	2008			2009		
	21.07.-14.10. n=23	21.07.-15.08. n=8	16.08.- 14.10. n=15	29.06.-23.09. n=18	29.06-07.17. n=6	18.07.-23.09. n=12
Global radiation (MJ/m^2)	0.7575***	0.6176 ^{NS}	0.7823***	-0.0732 ^{NS}	-0.6673 ^{NS}	0.7416**
Max. temperature (°C)	0.6636***	0.5397 ^{NS}	0.6417**	-0.2135 ^{NS}	-0.1642 ^{NS}	0.4757 ^{NS}
Min. temperature (°C)	0.4848*	0.0969 ^{NS}	0.4452 $^{+}$	0.1653 ^{NS}	0.3632 ^{NS}	-0.0048 ^{NS}
Precipitation (mm)	-0.2011 ^{NS}	-0.2211 ^{NS}	-0.1154 ^{NS}	0.4793*	0.6909 ^{NS}	-0.2101 ^{NS}
Relative humidity (%)	-0.6183**	-0.8575**	-0.6515**	0.5743*	0.7330 ^{NS}	-0.2721 ^{NS}

2008 – number of traps: 4 , ∑ adult beetles = 7720. 2009 - number of traps: 4, ∑ adult beetles = 1656.

Confidence levels: *** - 0,1%, ** - 1%, * - 5%, + - 10%, NS: no significant correlation was found

Figure 2: **Dynamics of corn rootworm swarming in long-term fertilization experiments. Martonvásár, 2009 – preceding crop: maize.**



CONCLUSIONS

Beside the intensity of swarming, the dates of swarming peak, their maximum values and the duration of swarming also showed significant difference in the two examined years. One of the reasons is that the preceding crop of maize was winter wheat for two consecutive years before 2008. It is for certain that the adult beetles trapped during the experiment migrated from the nearby infested plots. In the rainy production year more favourable for the development of maize, the plants dried down later, which contributed to the higher stability of corn rootworm populations.

In accordance with the results of correlation analyses, the beetles' habits on searching for food were less influenced by changes in weather conditions in the droughty production year than in the rainy one. The food searching habit could not be reliably forecasted on the basis of any of the weather parameters under dry circumstances in the initial intensive stage of swarming.

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Abundance and species ratio of the multicoloured Asian ladybird beetle, *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) in some Hungarian habitats

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SUMMARY

The multicoloured Asian ladybird beetle (Harmonia axyridis (Pallas, 1773)) was used for a long time as successful biological control agent in the USA and Western Europe for reducing aphid, psyllid and scale populations in green houses, orchards and fields. However, it has been realized as an invasive alien species (IAS) threatening the diversity of native aphidophagous insects through competition and direct praying. In addition, H. axyridis became a horticultural pest consuming various fruits and adversely affecting the wine production. Regarding its direct influence to humans, it is now a nuisance when occurring at high densities in buildings and contacting people, furnishings and other articles. Unfortunately, little attention has been paid to the expansion and spread of feral populations of H. axyridis in many European countries, thus it has been found in 2008 also in Hungary, and regarding its establishment and spread in other European countries, it will occupy presumably quickly our territory. H. axyridis must be a hazard for our native ladybird beetle species as well as for other aphidophagous arthropods. In the New World and also in some European countries it became one of the dominant coccinellid species competing and preying on native ladybirds. Present study shows the abundance and species composition of coccinellid assemblages in some Hungarian habitats in order to assess the pressure of H. axyridis on native coccinellids and to report on its dispersion in the north-eastern part of the country.

Keywords: *Harmonia axyridis*, multicoloured Asian ladybird beetle, invasive species, Coccinellidae, Hungary, alfalfa, sunflower, peach tree, milkweed, sweeping, light trap, visual sampling, tritrophic interactions

INTRODUCTION

The multicoloured Asian ladybird beetle (*Harmonia axyridis* (Pallas, 1773)) is a well-known species overseas and in Europe which became of a well estimated natural enemy an invasive alien species (IAS), a pest. Its native distribution area includes southern Siberia, China and Japan (Chapin, 1965 in Koch, 2003). This coccinellid is often associated with trees and bushes in natural and agricultural settings in presence of available prey (Adriaens, Branquart, Maes, 2003). In addition, this semi-arboreal predator also occurs in various herbaceous habitats, including agricultural (Koch, 2003) and natural (Sebolt and Landis, 2004) systems. *H. axyridis* is an efficient predator preying mainly on homopterous insects (aphids, psyllids, scales; Hodek, 1996; Iablokoff-Khnzorian, 1982 in Adriaens et al., 2003) but feeding also on other insects (Lepidoptera, Coleoptera; Kalaskar and Evans, 2001) and nectar and pollen (LaMana and Miller, 1996). *H. axyridis* has had a great importance because of the voracity of its larvae capable for controlling aphid populations, and its cheap and simple rearing. It was released numerously as biological control agent in North America and Western Europe, and was used for controlling aphids in green houses, orchards and gardens (McClure, 1987 in Koch, 2003; Brown and Miller, 1998; Michaud, 1999). Biotop SAS (France), BioBest (Belgium) and Koppert (the Netherlands) as major companies commercialised it in Europe (Ferran et al., 1996).

Unfortunately, little attention has been paid to the expansion of feral populations of *H. axyridis* in most European countries. This is astonishing regarded the rising concerns over the negative influence of biological control agent introductions, and quick colonization of different American habitats by *H. axyridis*. In addition, the Asian ladybird beetle can be a household and fruit pest (Mannix, 2001; Foglia, 2002; Pickering et al., 2004; Huelsman et al., 2001). According to the most recent experiences, it is attacking natural or semi-natural ecosystems in numerous European countries (Adriaens, Branquart, Maes, 2003; Bathon, 2003, Majerus, 2005; Adriaens et al., 2003; Adriaens et al., 2008) and in Hungary, too. This exotic ladybird has been found first in 2008 in Hungary (Merkl, 2008), and regarding its spread in other European countries, it will likely be established within a year in our territory. *H. axyridis* must be a hazard for our native ladybird beetle species as well as for other aphidophagous arthropods by competition and intraguild predation (Cottrel and Yeargan, 1998; Snyder, Clevenger and Eigenbrode, 2004). In the New World and also in some European countries it became one of the dominant coccinellid species decreasing the native ladybird populations (Adriaens, Branquart, Maes, 2003; Koch et al., 2006; Adriaens et al., 2008; Brown et al., 2008).

The primary objective of present study was to target habitat types regarded as important for the more restricted or stenotopic Hungarian coccinellids which might be impacted by the Asian ladybird colonisation. A secondary objective was to assess the abundance and species composition of coccinellid assemblages in these habitats in order to estimate the influence of *H. axyridis* on native ladybird species and also to collect data on its dispersion in the north-eastern part of our country. A tertiary objective was to provide an overall coverage of ladybird beetles in the landscape of Gödöllő and Debrecen than that obtained formerly if any. Of necessity the scope of the survey was restricted to what could be achieved by one surveyor in one season.

MATERIALS AND METHODS

Coccinellid individuals (adults, larvae and pupae) were collected in 2009 from early April until late September in Gödöllő (abandoned orchard (3 ha), alfalfa (3 ha) and sunflower (2,5 ha) field, stinging nettle (4 x 40 m^2) and common milkweed patches (4 x 25 m^2), a peach tree) and Debrecen (botanical garden (1,8 ha), alfalfa field (1600 m^2), experimental area (1,5 ha), stinging nettle stand (4 x 20 m^2). When selecting the collection sites it was important to sample wooden and herbaceous, semi-natural and agricultural habitats, each of them suitable for the Asian ladybird. Captures and observations were obtained by sweeping net (4 x 25 sweeps), visual sampling and light trap. The individuals captured by sweeping were taken into a freezer, then dried for a while and identified immediately. Light trap collected insects were identified after emptying the trap. Observed individuals were identified at the moment of observing and noted at once.

The colour forms of specimens collected were also determined. The geographical coordinates of sites in which *H. axyridis* was recorded were measured using Google Earth (©2009 Google™). *Table 1* contains the basic data of sampling.

Table 1

Basic data of collection in Gödöllő and Debrecen (2009)

Site	Geographical position	Habitat	Catching method	Frequency	Number of coccinellid individuals caught
Gödöllő	47°35'39″ N 19°22'56″E 209 m	abandoned orchard	sweep net	weekly	2
Gödöllő	47°35'45″ N 19°22'45″E 209 m	alfalfa field	sweep net	weekly	284
Gödöllő	47°35'45″ N 19°22'45″E 209 m	stinging nettle stand	sweep net	weekly	0
Gödöllő	47°35'48″ N 19°22'36″E 205 m	sunflower field	visual observation	periodically	125
Gödöllő	47°35'51″ N 19°22'23″E 210 m	peach tree	visual observation	periodically	113
Gödöllő	47°35'04″ N 19°23'02″E 207 m	common milkweed stand	visual observation	periodically	42
Total in Gödöllő					566
Debrecen	47°33'01″ N 21°36'20″E 116 m	botanical garden	sweep net	weekly	7
Debrecen	47°33'07″ N 21°36'19″E 114 m	alfalfa field	sweep net	weekly	294
Debrecen	47°33'10″ N 21°36'05″E 114 m	stinging nettle stand	sweep net	weekly	0
Debrecen	47°33'10″ N 21°36'05″E 114 m	experimental orchard	light trap	daily	195
Total in Debrecen					496
Total					1062

RESULTS AND DISCUSSION

1062 individuals of 12 ladybird species have been collected during the sampling. 564 individuals of 7 species were captured in Gödöllő and 449 individuals of 12 species in Debrecen. No specimen were caught or observed in the stinging nettle patches and only very few in the abandoned orchard or the botanical garden. In case of both sites the alfalfa fields proved to be the most diverse and abundant. The data of the sunflower field, the peach tree and the milkweed stands cannot be compared with the other records because of the irregularity of their sampling: the sunflower field was tilled by the owner after the second sampling; the ladybird population disappeared rapidly from the peach tree because of the collapse of aphid population; coccinellids started to visit the milkweed only very late (in August), after the colonization of *Aphis nerii* Boyer de Fonscolombe, 1841. As to the data of the light trap, these are founded on daily basis. However, these records are suitable to conclude the plant-prey-coccinellid (*H. axyridis*) association, the species presence and dispersion, the intensity of spreading and somewhat the species ratio.

In Gödöllő *H. axyridis* became the third most dominant species after *Coccinella septempunctata* (Linné, 1758) and *Adonia variegata* (Goeze, 1777) (Table 2). The frequency and abundance of other species like *Adalia bipunctata* (Linné, 1758), *Propylea qutuordecimpunctata* (Linné, 1758), *Coccinula quatuordecimpustulata* (Linné, 1758) and *Subcoccinella vigintiquatuorpunctata* (Linné, 1758) were low. These coccinellids are aphidiphageous except *S. vigintiquatuorpunctata* which is a polyphageous plant eater. All the Gödöllő species are common species to be found in various habitats and on host plants but preferring mainly, except *A. bipunctata* and *P. qutuordecimpunctata*, the low growing plants. As to *A. bipunctata* and *P. qutuordecimpunctata,* they seem to be opportunists, preferring the plant stands supported high aphid densities (Honěk, 1985).

Regarding the parameters of Debrecen samples, abundance was lower, species richness was higher than those of Gödöllő (Table 3). Accounting the species apart those of mentioned above, *Vibidia duodecimguttata* (Poda, 1761), *Harmonia quadripunctata* (Pontopiddian, 1763), *Adalia decempunctata* (Linné, 1758), *Scymnus frontalis* (Fabricius, 1787) and *Exochomus quadripustulatus* (Linné, 1758) had to be added. When summarizing first the individuals, *H. axyridis* predominated, which was followed by *A. variegata, C. septempunctata, S. frontalis*, P. *quatuordecimpunctata*, *V. duodecimguttata*, A. *bipunctata*, *H. quadripunctata*, *Coccinula quatuordecimpustulata*, *E. quadripustulatus*, *S. vigintiquatuorpunctata* and A. *decempunctata,* respectively.

When omitting the light trap data, the order of dominance changed considerably: *A. variegata*, *C. septempunctata*, *S. frontalis*, *H. axyridis* etc. That means that the ratio of *H. axyridis* in Gödöllő could be approximately 16% and that of in Debrecen about 6%. Both data show the strong establishment and competition of Asian ladybird in the sampled sites and habitats. As to the light trap records (Asian ladybird amounted 87% of the total capture) these demonstrate the extraordinarily spreading ability of *H. axyridis*. *S. frontalis* and *V. duodecimguttata* prefer low herbaceous vegetation but *A. decempunctata* favours decideous trees and shrubs. As to *H. quadripunctata* and *E. quadripustulatus,* they have a preference for woody vegetation. *H. quadripunctata* can be almost invariably found on pine (*Pinus* sp.) trees *but E. quadripustulatus* is *c*ommon often on *Pinus* and *Picea* spp. but also on decideous trees. This is logical because there were various coniferous trees in both, the abandoned orchard and the botanical garden. All coccinellids studied are aphidophagous except the mycophagous *V. duodecimguttata* and the phyllophagous *S. vigintiquatuorpunctata.* These species evidently cannot be competitors *of H. axyridis* but they can serve as a prey for it. Regarding the different sites, the coccinellids generally visited habitats suitable for their preference. It was remarkable the very low coccinellid abundance and species richness of the orchard in Gödöllő and those of the botanical garden in Debrecen. The main reason for this can be the unusually scarcity of aphids. In contrast, the aphid population (*Acyrtosiphon pisum* (Harris, 1776), *Aphis craccivora* Koch 1854) was continually high in the alfalfa stands at both sites. Abundance and species composition of ladybirds in the alfalfa fields and the habitats with decideous and coniferous trees in both localities was similar. *H. axyrides* was caught and observed on *Anoecia corni* (Fabricius, 1775) (host plant (= hp) : *Cornus sanguinea* Linné 1753), *Aphis spiraephaga* Müller, 1961 (hp: *Spirea* x *vanhouttei* (Briot) Zabel 1884) in Debrecen, and on *Aphis fabae* Scopoli 1763 (hp: *Euonymus europeus* (Linné, 1753)) in Gödöllő. Aphid species used by *H. axyridis* and other coccinellids in the other sites are *A. fabae* Scopoli 1763 (hp: *Chenopodium album* Linné, 1753, *Ambrosia artemisiifolia* Linné, 1753) in the sunflower field; *Hyalopterus amygdali* on the peach tree; *A. nerii* on the common milkwood. The density of aphids was very important in the observed intervalum, that is the leaves were heavily infested by aphids. It was remarkable to observe that there were no *H. axyridis* individuals, either adults or larvae around the *Brevicoryne brassicae* colonies on the *Sinapis arvensis* Linné, 1753 plants, however, the *C. septempunctata* density (adults and larvae) was considerable. Leaves and shoots were completely covered by *B. brassicae* specimens. It seems that the polphageous *H. axyridis* did not prefer this aphid. This aversion of *H. axyridis* has not been documented yet (Koch et al., 2003; Koch et al., 2006; Brown et al., 2008). Most publications underlined the drastic competitive influence of *H. axyridis* on other coccinellids, however, they reported this rarely in terms of abundance or % of dominance (Adriaens, Branquart, Maes, 2003; Koch et al., 2006; Adriaens et al., 2008; Brown et al., 2008). *H. axyridis* was first found in the wild in Belgium in 2001. Five years later it was the most numerous lady beetle in Flanders (northern part of Belgium). Regarding its occurrence, it was the second highest occurring coccinellid after *C. septempunctata* in Flanders, and in Belgium as a whole its occurrence was the fifth highest after *C.*

septempunctata, P. quqtuordecimpunctata, A. bipunctata and *Thea vigintiduopunctata* (Brown et al. 2008). In Switzerland three years after the first detection of Asian ladybird it became the seventh most abundant species on trees and shrubs (Brown et al. 2008). *H. axyridis* comprised 1,5% (N=1110) of lady beetles collected in Manitoba (Canada) after a year of its first founding (Wise, Tarnack and Roughley 2001). In relation to these data, our records (*Table 2* and *3*) are realistic and show a quick and firm extension of *H. axyridis* in the sampled localities.

Table 2

Species composition of coccinellids collected in Gödöllő (2009)

Site	HA	C7	AV	A2	P14	V12	C14	H4	A10	S24	SF	E4	LC	Total
Abandoned orchard	2													2
Alfalfa field	6	92	165	2	7		6			4			2	284
Sunflower field	12	113												125
Peach tree	65	41	2	5										113
Milkweed stand	6	13	23											42
Total	91	259	190	7	7		6			4			2	566
%	16.1	45.8	33.6	1.2	1.2		1.1			0.7			0.4	

Abbreviations: HA: Harmonia axyridis, C7: Coccinella septempunctata, AV: Adonia variegata, A2: Adalia bipunctata, P14: Propylea quatuordecimpunctata, V12: Vibidia duodecimpunctata, C14: Coccinula quatuordecimpustulata, H4: Harmonia quadripunctata, A10: Adalia decempunctata, S24: Subcoccinella vigintiquatuorpunctata, SF: Scymnus frontalis, EQ: Exochomus quadripustulatus, LC: Larvae of Coccinellidae

Table 3

Species composition of coccinellids collected in Debrecen (2009)

Site	HA	C7	AV	A2	P14	V12	C14	H4	A10	S24	SF	E4	LC	Total
Botanical garden	7											2		9
Alfalfa field	13	44	132	2	17		2			2	35		47	294
Experimental orchard	168	1		6	1	9	2	5	1					193
Total	188	45	132	8	18	9	4	5	1	2	35	2	47	496
%	37.9	9.1	26.6	1.6	3.6	1.8	0.8	1.0	0.2	0.4	7.0	0.4	9.5	
Total[a]	20	44	132	2	17		2			2	35		47	301
%	6.6	14.6	43.9	0.7	5.6		0.7			0.7	11.6		15.6	

[a] without the light trap capture

Table 4

Percentage of different developmental and adult colour forms

Site	succinea	spectabilis	conspicua	L	P	Total
Abandoned orchard G	2					2
Alfalfa field G	4			2		6
Sunflower field G	12					12
Peach tree G	21	1	3	30	10	65
Milkweed stand G	3	3				6
Botanical garden D	4			3		7
Alfalfa field D	9	1		3		13
Experimental orchard D	161	3	4			168
Total	216	8	7	38	10	279
%	77.4	2.9	2.5	13.6	3.6	

L: larvae, P: pupae

The proportion of colour forms of the collected *H. axyridis* individuals have been counted (*Table 4* and *5*). All the in Europe reported colour forms have been observed at both localities. The colour form composition of Asian lady beetle was most similar to the Italian data (Burgio et al., 2008), though the form conspicua was not found there (*Table 6*).

Table 5

Percentage of adult colour forms

Site	succinea	spectabilis	conspicua	Total
Abandoned ochard G	2			2
Alfalfa field G	4			4
Sunflower field G	12			12
Peach tree G	21	1	3	25
Milkweed stand G	3	3		6
Botanical garden D	4			4
Alfalfa field D	9	1		10
Experimental orchard D	161	3	4	168
Total	216	8	7	231
%	93.5	3.5	3.0	

Table 6

Percentages of *H. axyridis* colour forms in some countries

Country	time	succinea	spectabilis	conspicua	melanic (unspecified forms)	N	Reference
Belgium	2004-2006	71	19	6	4	5164	Adriens et al. 2008
England	2005	79	14	7		6180	M. Majerus unpublished in Brown et al. 2008
Italy (northern part)	2008	98	2			1049	Burgio et al. 2008
Czech Republic	2006-2007	88			12	51	O. Nedved unpublished in Brown et al. 2008
Luxembourg	2004	85	11	4		28	Schneider and Loomans, 2006
Denmark	2006-2007	100				16	J. Pedersen unpublished in Brown et al. 2008

CONCLUSIONS

It was expected at the commencement of the survey that broadleaf trees, and particularly those near a woodland, would be productive. This was not the case. Of the two habitats predominated with broadleaf trees visited, both produced any ladybirds at all and these comprised small numbers of a common native species except of *H. axyridis*. The main cause of this could be the low aphid density and also the irregular temporary aphid distribution. E.g. there were practically no aphids (*A. fabae*) after 5 May in the abandoned orchard, and any of the trees and shrubs has been infested during the vegetation period. Similarly, only few aphids (*A. corni*)

were observed during a relatively short period on *C. sanguinea* and also the population of *A. spiraephaga* collapsed early in the botanical garden. *H. axyridis* was the 4th and 5th species in terms of abundance in the alfalfa fields which compared to the Belgian data (Adrians et al., 2008) shows a quick establisment and colonisation. Peach, milkweed and sunflower were more productive with 58, 14 and 10% of dominance of *H. axyridis*, respectively, making so it the first, second and third most abundant species. As to the stinging nettle stands, no coccinellids was found at both localities in spite of the relatively high aphid density in May. Regarding the variability of *H. axyridis*, all the in Europe reported colour forms have been observed at both localities.

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Faunistik und Phänologie der Schwebfliegen (Diptera: Syrphidae) im Tharandter Wald und Vergleich der Sammlungsmethoden

Fauna and phenology of hoverflies (Syrphidae) in tharandter wald and comparison of collection methods

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ZUSAMMENFASSUNG

Die Familie der Schwebfliegen (Syrphidae) enthalten mehr als 6000 beschriebenen Arten. In der paläarktischen Region leben fast 1600 Arten. Die Imagines ernähren sich von Nektar und Pollen. Viele Larven sind aphidophag. Die Sammlungen haben mit Malaise-Fallen, mit Netz und mit Schalen stattgefunden. Der Tharandter Wald liegt mit seinem 6000 ha im Mittelpunkt von Sachsen, südwestlich von Dresden und bei dem nördlichen Rand von Osterzgebirge. Insgesamt wurden 980 Individuen von 64 Arten Innerhalb der Sammlungsperiode gefangen. Die Fauna auf die zwei Gebiete war nicht sehr unterschiedlich. Die häufigsten Arten waren Episyrphus balteatus (De Geer, 1776), Sphaerophoria scripta (Linnaeus, 1758), Platycheirus albimanus (Fabricius, 1781), Melanostoma scalare (Fabricius, 1794), Melanostoma mellinum (Linnaeus, 1758) und Platycheirus peltatus (Meigen, 1822). Der Artikel bietet Information über Vergleich der Sammlungsmethoden und über die Phänologie der gefangenen Schwebfliegen auf die zwei Gebiete.

SUMMARY

Hoverflies (Syrphidae) are one of the most diverse families of the Diptera, comprising about 6000 species on the world. In the palearctic region is known about 1600 indentified hoverflies species. Adults feed on nectar and on pollen from flowers, many larvae of palearctic Syrphids are predator and the most of them feed aphids. The sampling was carried out on two areas of Tharandter Wald by Malaise traps, modified sweeping nets and yellow, blue and white traps. The Tharandter Wald (area 6,000 hectares) lies southwest of Dresden on the northern edge of the Eastern Erzgebirge. The collection yielded 980 specimens of 64 species. The fauna of the two areas was not so different. The species found in greatest numbers are Episyrphus balteatus (De Geer, 1776), Sphaerophoria scripta (Linnaeus, 1758), Platycheirus albimanus (Fabricius, 1781), Melanostoma scalare (Fabricius, 1794), Melanostoma mellinum (Linnaeus, 1758) and Platycheirus peltatus (Meigen, 1822). The available information on collection methods are reviewed and phenology of collected Syrphidae on the two areas is discussed.

Schlagworte: Malaise-Falle, Schalen, Netz, Sammlungsmethode, Syrphidae, Kahlschlag
Keywords: Malaise-trap, water traps, sweep net, sampling methods, Syrphidae, clear cutting area

EINFÜHRUNG

Die Schwebfliegen stellen eine gut abgrenzte Familie dar. Mit über 6000 beschriebenen Arten weltweit sind sie eine der größten Dipterenfamilien. In Europa leben knapp 800, in Deutschland etwa 400 Arten sowie in Ungarn. Von der paläarktischen Region sind bisher fast 1600 Syrphidenarten beschrieben (Röder, 1990).

Die Körpergröße der mitteleuropäischen Syrphida scwankt zwischen 4 mm und max. 20 mm. Sie haben sehr oft den Habitus von Bienen, Hummeln oder Wespen. Es gibt univoltine Arten, bivoltine und polyvoltine Arten. Die Schwebfliegen sind einer der wichtigsten tagaktiven Blütenbesucher und Blütenbestäubergruppen. Dabei wird entweder nur Pollen, nur Nektar oder aber beides gesammelt.

Über 30 % von 1600 paläarktischen Syrphidenarten leben als Larve von Blattläusen oder anderer tierischer Kost, etwa 30% sind phytophag, die übrigen meist sapro- oder micophag (*1. Tabelle*). Die Gefräßigkeit der aphidophagen Larven ist sehr groß: eine ausgewachsene Larve soll über 200 Läuse pro Nacht verzehren können. Die Larvalzeit ist oft kurz (8-14 Tage), aber die häufige Arten haben mehrere Generationen im Jahr. Die dezimierende Wirkung auf Blattlauskolonien ist meist beträchtlich und besser als die von Insetiziden, gegen die die Larven sehr empfindlich sind (Somaggio, 1999). Daher eignen sich aphidovore Schwebfliegen gut zum biologischen Pflanzenschutz. Die Aktivität der zoophagen Larven beschränkt sich zumindenst an warmen, sonnigen Tagen hauptsächtlich auf die Dämmerung und die Nacht. Tagsüber ruhen sie auf der Blattunterseite.

1. Tabelle

Ernährungstyp der Larven

Ernährungstyp der Larven	Beispiele
Phytophage	Cheilosia spp.
Saprophage	Eristalis spp., Helophilus spp., Xylota spp.
Zoophage (Aphidophag)	Syrphus spp., Episyrphus spp., Melanostoma spp., Epistrophe spp., Chrysotoxum spp., Spaerophoria spp., Platycheirus spp.

Damit ein Biotop für Schwebfliegen geeignet ist, muß er ausreichende Ernährungs-möglichkeiten für die Imagines und für die Larven bieten. Die bevorzugten Blütentypen sind im allgemeinen nicht sehr unterschiedlich. So kommen die Schwebfliegen überall dort vor, wo geeignete Blüten entfalten. Hinzu kommt, dass sie als gute Flieger sehr beweglich sind. Dennoch bei vielen Arten ist mehr oder weniger starke Biotopbindung erkennbar. Das liegt meist in erster Linie an den ökologischen Ansprüchen der Larven, und die sind innerhalb der Familie sehr unterschiedlich. Dementsprechend hängt das Vorkommen der Schwebfliegen von Biotopeigenschaften ab, z. B.: Waldcharakter, Strukturierung von Waldrändern, Vielfalt der Vegetation, Blütenangebot, Sonneneinstrahlung, Windschutz, Zivilisationeinflüsse. Bei der Stadt oder wo Luftverschmutzung ist, leben gleichfalls weniger Schwebfliegen als in einem natürlichen Biotop. Ihre Lebensräume sind die mehr oder weniger lichten Standorte im Waldbereich, z. B.: Waldränder, Hecken, Lichtungen, Waldwege, Kahlschläge. Überdies leben die Schwebfliegen in der Feuchtbiotope auch, z. B. lichte, feuchte Wälder, feuchte Wiesen, Moore. Zuletzt die blütenreiche Wiesen, z. B. Trockenrasen, Bergwiesen.

Die Bedeutung der Schwebfliegen für die Natur ist vielfältig. Die Imagines sind Pollenüberträger. Die phytophagen Larven bauen einen Teil der pflanzlichen Primärproduktion ab. Die Saprophagen nehmen an dem Abbau der toten organischen Substanz teil. Dank ihrer Gefräßigkeit, häufigkeit und langen Aktivitätsperiode treten die Aphidophagen Syrphidenlarven als machtvoller Regulator der Blattlaus-Vermehrung. Die aphidophage Gruppe ist artenreich und enthält viele häufige Arten. Durch mehrere Eigenschaften ihrer Lebensweise haben die Schwebfliegen besondere Bedeutung gegen der Blattläusen. Die begatteten Weibchen überwintern und erscheinen im Frühjahr. Die Fliegen haben große Beweglichkeit. Die Weibchen legen 500-1000 Eier in unmittelbarer Nähe der Blattlauskolonien ab. Die Larven haben große Gefräßigkeit, sie suchen ständig die Läuse und vernichten die Blattlauskolonien.

Ziele der Arbeit waren die verschiedenen Fangmethoden vergleichen, und die Wirksamkeit der Fangmethoden untersuchen. Dabei wurden die Faunistik und Phänologie der Schwebfliegen auf den verschiedenen Gebieten im Tharandter Wald festgestellt.

MATERIAL UND METHODEN

Die Untersuchungsflächen liegen im Tharandter Wald. Der Tharandter Wald (Lange: 13,43°-13,58°, Breite: 50,92°-51°) ist eine Landschaft mit 6000 ha im Mittelpunkt von Sachsen und liegt südwestlich der Forststadt Tharandt und von Dresden, südlich der Stadt Wilsdruff und bei dem nördlichen Rand von Osterzgebirge. Die charackteristische Vegetation sind Mischwald mit Fichten, mit Eiche Arten und Birken.

Ich habe in zwei Gebiete mit den Abteilungsnummern 321 und 434 gesammelt. Das erste war der Kahlschlag (L/B:13,48°,50,96°), wo die Bäume im Jahr 2007 durch einen Sturm zerstört wurden. Die Fläche wird von dichter Vegetation bedeckt, z. B. mit verschiedenen Gräser (*Holcus lanatus, Molinia coerulea, Juncus effusus, Deschampsia flexuosa, Calamagrostis villosa, Agrostis capillaris*), Blüten (*Digitalis purpurea, Matricaria recutita, Cirsium vulgare, C. arvense, Geranium robertsianum, Impatiens parviflora*), junge Bäume (*Picea abies, Quercus robur, Betula pendula*). Das Gebiet ist ziemlich offen, sehr windig und die Höhe ist 385 m.

Zweite Untersuchungsfläche (L/B:13,45°,50,94°) war eine Lichtung am Westrand von Tharandter Wald mit Obstbäume und Sträuche. Es wird durch eine Hecke von Nutzfläche getrennt. Aus westliche Richtung Acker, von Osten und Süden ist der Wald umgerandet. Die Flache ist blütenreich (*Ranunculus repens, Vicia cracca, V. Sepium, Urtica dioica, Rumex spp., Veronica chamaedrys, Trifolium spp., Galium mollugo, Lapsana communis, Prunella vulgaris*). Höhe ist 420 m.

Die Sammlungen haben zwischen dem 12. Mai und dem 25. August pro Woche stattgefunden. Zum Fang von Schwebfliegen sind die geeignetsten Mittel das modifizierte Schmetterlingsnetz, die gelben, blauen und weißen Schalen und die Malaise-Falle (Tóth 2001).

Das Netz ist eine für Schwebfliegen durchaus angewendete Methode. Einzelfänge liegen als standardisierte Protokolle mit Fangzeit von jeweiligen 30 Minuten (Ssymank, 1999). Die gesammelten Insekten wurden in einem Glas mit Essigether betäubt. Nach der Preparation wurden sie unter dem Stereomikroskop mit Anwendung einem Bestimmungswerk (van Veen, 2004) bestimmt.

Die Malaise-Falle ähnelt einem Zelt. Die Fangflüssigkeit in den Malaise-Fallen und den Schalen war Benzoesäure, um die Insekten zu töten und haltbar zu machen. Die Fallen wurden pro Woche geleert. Die Insekten waren in 70%igem Alkohol konserviert und später prepariert.

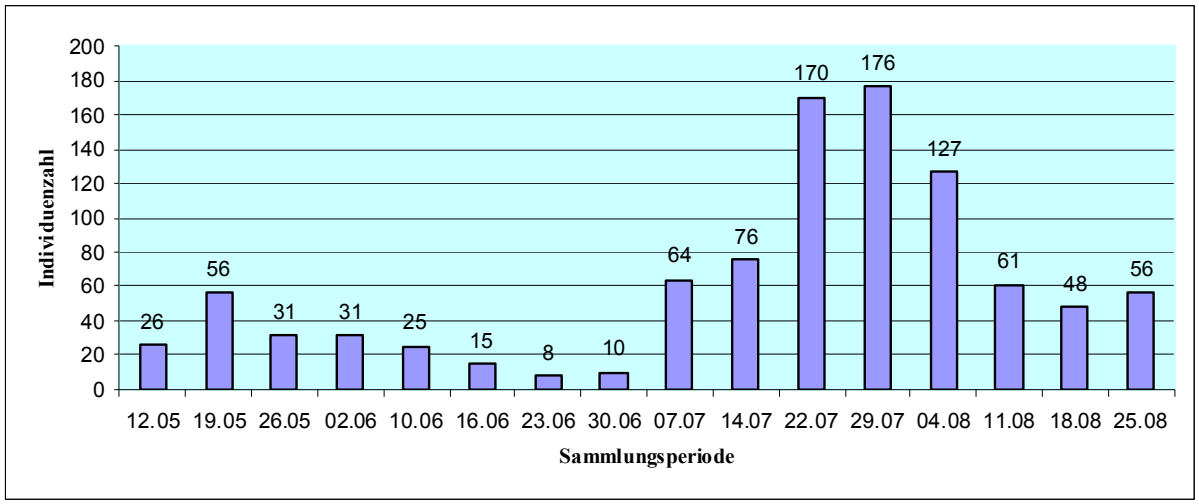
Die erste Malaise-Falle ist auf dem Kahlschlag aufgestellt wurden. Die zweite war am Westrand des Tharandter Waldes. Die dritte Falle war zuerst auf dem Kahlschlag, aber ich konnte damit keine Schwebfliegen fangen. Deswegen wurde sie später abgebaut und in dem dritten Gebiet (L/B:13,58°,50,98°) aufgestellt, die oberhalb des Cotta-Baus neben einer Weizenfläche auf eine Wiese mit Obstbäume liegt. Die Fangperiode war allerdings kürzer und demnach nicht standard denn diese Falle ist nur im Juli aufgestellt worden. Das gesammelte Material war weniger deshalb habe ich sie nicht bei der Auswertung berücksichtigt.

DISKUSSION DER ERGEBNISSE

Insgesamt wurden 980 Individuen von 64 Arten Innerhalb der Sammlungsperiode gefangen. Davon ernähren sich 43 Arten mit 731 Exemplaren als Larven von Blattläusen. Hinsichtlich der Individuenzahl ist die Häufigkeit von Aphidophagen Tieren 74,5%.

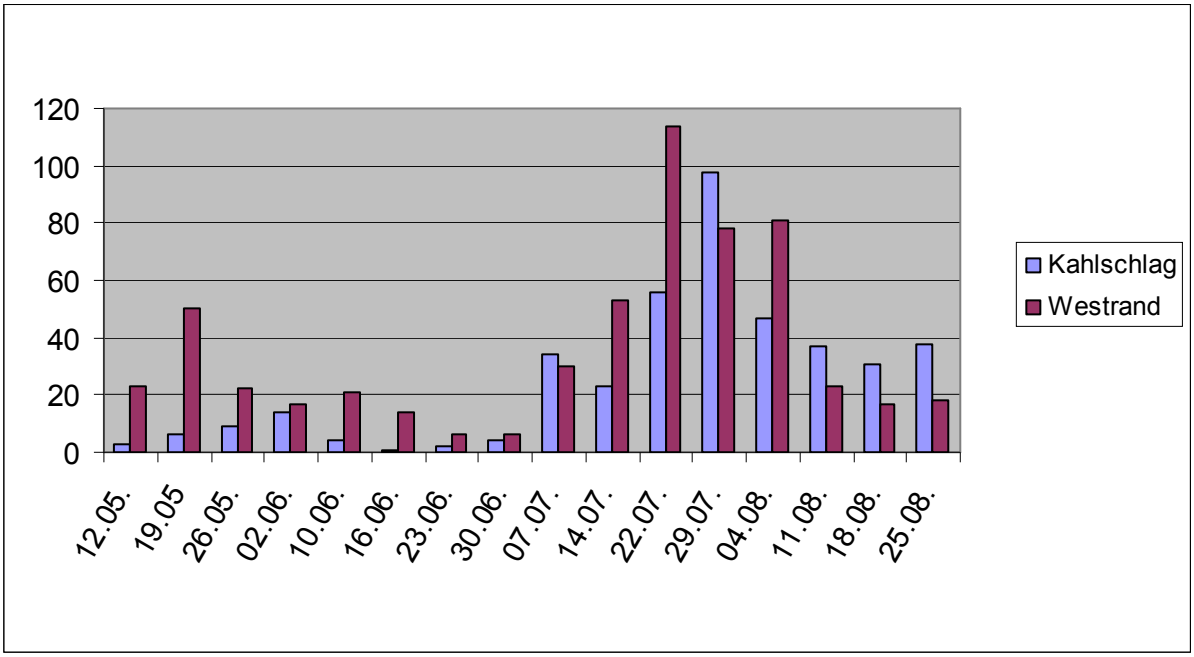
Im ersten Diagramm (*1. Diagramm*) kann man sehen, dass die Individuenzahl Anfang Mai ansteigend war, danach ist sie plötzlich wahrscheinlich wegen des Wetters abgestiegen. Der Juni war ziemlich kalt und regnerisch und es war ungünstige Bedingungen für die Schwebfliegen, besonders für die Larven. Allerdings mag es vorkommen, dass nach einer langen Schlechtwetterperiode manche Arten nicht mehr auftauchen, weil sie wahrscheinlich einfach diese Zeit nicht überlebt haben. Die andere Folge, dass die Larven sich nicht weiterentwickelt haben, sondern in Diapause geblieben sind. Die idealen Flugbedingungen für die Imagines liegen zwischen 21-27 Cº und die Luftfeuchtigkeit bei 75-97% (Röder, 1990), und natürlich ohne Regen. Aber das ist veränderlich bei den verschiedenen Arten. Anfang Juli ist die Individuenzahl wieder ansteigend und Mitte und Ende Juli erreicht sie ihr Maximum. Danach sinkt sie nach bis Ende August ab. Man kann vermuten, dass sich ein bis zwei Generationen entwickelt haben.

1. Diagramm: **Veränderung der Individuenzahl der Schwebfliegen während der Fangperiode im Tharandter Wald**



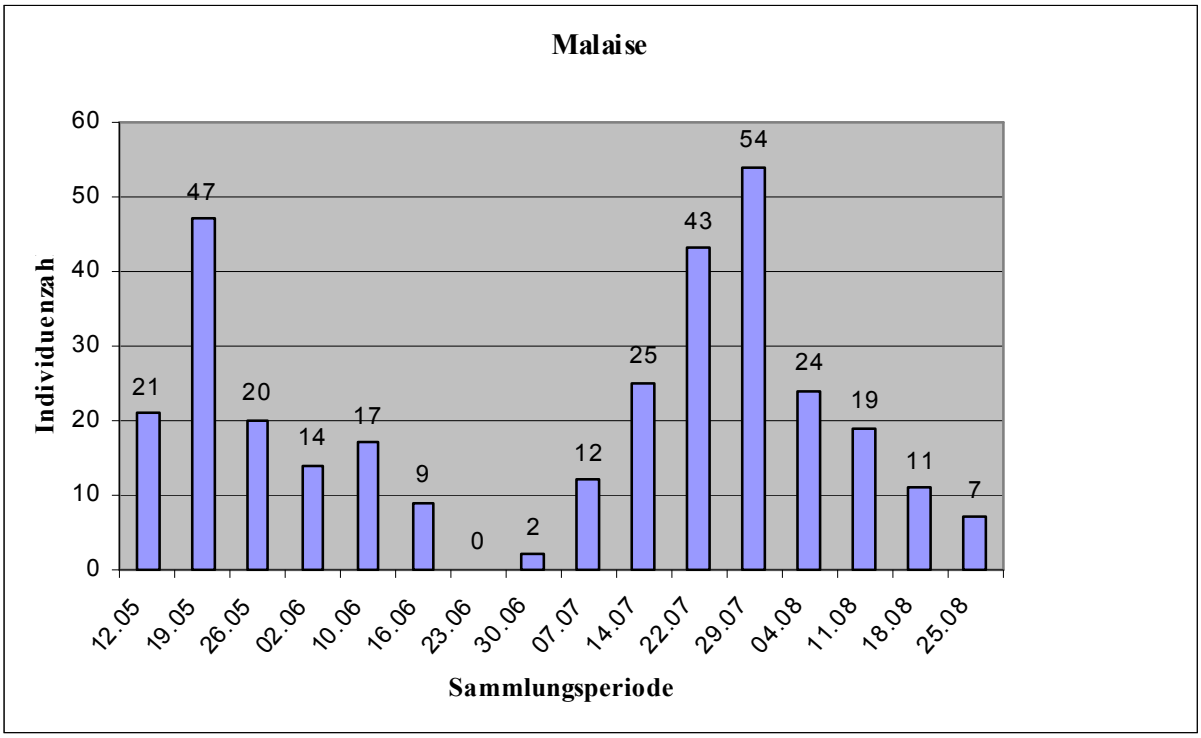
Im zweiten Diagramm (*2. Diagramm*) können wir sehen, dass am Westrand mehr Individuen waren, obwohl die Verhältnisse der Individuenzahlen sich umgekehrt haben. Am Ende der Sammlungsperiode sind mehr Exemplare auf dem Kahlschlag gefangen worden. Der Grund ist wahrscheinlich, dass mehr Blüten auf dem Kahlschlag Ende August entfaltet haben.

2. Diagramm: **Die Veränderung der Individuenzahl der Schwebfliegen auf dem Kahlschlag und am Westrand**

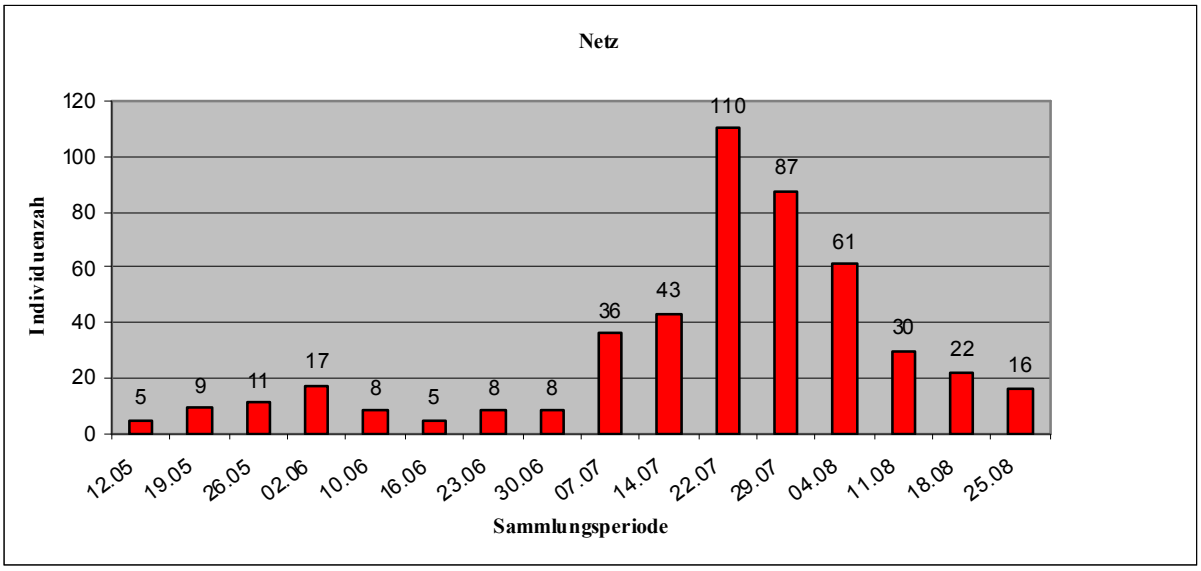


Man kann im dritten Diagramm (*3. Diagramm*) beobachten, dass die Malaise-Fallen während des Erfassungszeitraums wirksam funktioniert haben, obwohl die Individuenzahl relativ niedrig der Mitte des Sommers war als im Vergleich zu der mit dem Netz gesammelten Anzahl. Die Sammlungen mit dem Netz (*4. Diagramm*) während der Flugzeit ergab eine größere Individuenzahl als bei der Malaise-Falle. Die Malaise-Fallen haben insgesamt 325 Exemplaren gefangen. Diese Individuenzahl bedeutet 33% der Gesamtsmenge. Mit dem Netz konnte ich 476 Exemplaren sammeln, die 49% der Gesamtindividuenzahl sind. Die Schalen wurden im Juni gelegt, deshalb fanden sich in ihnen weinger Arten. In denen waren nur 17 Arten mit 179 Individuen, die 18% bedeuten.

3. Diagramm: **Mit den Malaise-Fallen gesammelten Schwebfliegenindividuenzahl**

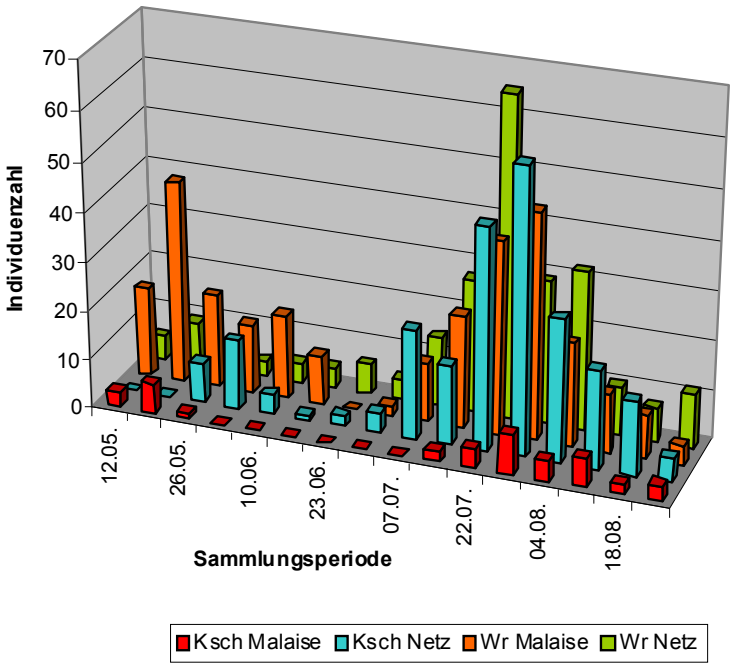


4. Diagramm: **Mit dem Netz gesammelten Schwebfliegenindividuenzahl**



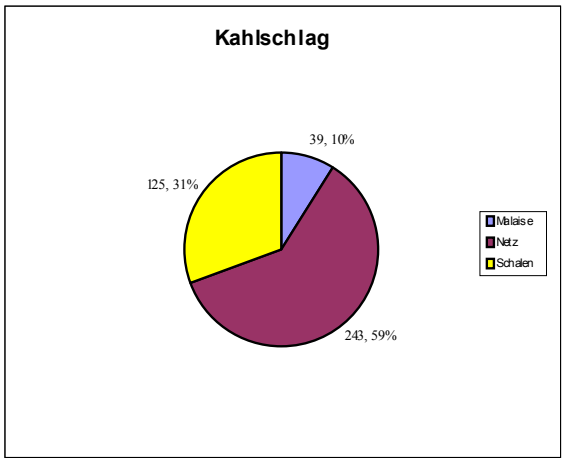
Beim Vergleich der Sammlungsmethoden in Bezug auf die Gebiete (*5. Diagramm*) kann man sehen, dass mehr Individuen durch die Malaise-Falle in beiden Gebiete am Anfang der Fangperiode gefangen wurden als mit Netz. Aber am Ende der Fangperiode hat sich dieses Verhältnis hauptsächlich auf dem Kahlschlag umgekehrt, aber am Westrand auch.

5. Diagramm: **Vergleich der Sammlungsmethoden der Schwebfliegen im Tharandter Wald in Bezug auf die Gebiete**

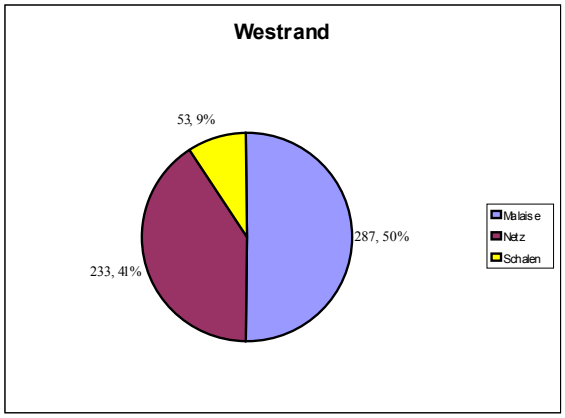


Auf dem Kahlschlag wurden 407 Individuen von 37 Arten und am Westrand 573 Individuen von 51 Arten gefangen. Man kann sehen, dass die Sammlung mit dem Netz auf dem Kahlschlag wirksamer gewesen war gegenüber am Westrand, wo die Malasie-Fallen 50% der Individuen gefangen hat (*6. und 7. Diagramm*). Die Individuenzahl war höher in den Schalen auf dem Kahlschlag, als am Westrand. Am Westrand waren mehr Arten und Individuen als auf dem Kahlschlag, aber die Diversität war niedriger. Die Diversität am Westrand auf Grund des Shannon-Indexes war H=1,97, auf dem Kahlschlag H=2,44. Vor Jahren 2007 war ein Nadelwald in dieser Fläche mit wenigen Bodenvegetation. Nach dem Sturm im Jahr 2007 ist die Bodenvegetation auf dem Kahlschlag gewachsen. Es könnte vorkommen, dass die gute Wanderarten der Schwebfliegen auf dem Kahlschlag erscheinen werden. Manche Arten, die die offene Gebiete bevorzugen, könnten auch auf das Gebiet zuwandern.

6. Diagramm: **Mit der verschiedenen Fangmethoden gesammelten Individuenzahl der Schwebfliegen auf dem Kahlschlag**



7. Diagramm: **Mit der verschiedenen Fangmethoden gesammelten Individuenzahl der Schwebfliegen am Westrand**



Auf Grund der Individuenzahl waren die häufigsten Arten in den zwei Gebieten nicht sehr unterschiedlich. Zwei Arten (*Episyrphus balteatus* (De Geer, 1776)*, Melanostoma scalare* (Fabricius, 1794)) konnten besser mit dem Netz gesammelt werden und eine (*Platycheirus albimanus* (Meigen, 1822)) war in höherer Zahl in den Malaise-Fallen. Mit größten Individuenzahl gesammelten Arten (*2. Tabelle*) sind im allgemeinen sehr häufig, meist polyvoltine Arten und ihre Larven sind aphidophag. Außer einer Art (*Xylota segnis* (Linnaeus, 1758)), ihre Larven leben in nassem Holzmulm, morschem Holz und in anderem verrottendem Pflanzenmaterial.

2. Tabelle

Die mit der größten Individuenzahl gesammelten Schwebfliegen Arten

Episyrphus balteatus (De Geer, 1776)	**241**
Melanostoma mellinum (Linnaeus, 1758)	**52**
Melanostoma scalare (Fabricius, 1794)	**96**
Platycheirus albimanus (Fabricius, 1781)	**102**
Platycheirus peltatus (Meigen, 1822)	**48**
Sphaerophoria scripta (Linnaeus, 1758)	**147**
Xylota segnis (Linnaeus, 1758)	**91**

Hinsichtlich der Ergebnisse kann man sagen, dass die Gebiete artenreich sind. Insgesamt wurden 980 Individuen von 64 Arten innerhalb der Sammlungsperiode gefangen. Davon ernähren sich 43 Arten mit 731 Exemplaren als Larven von Blattläusen.

Die Malaise-Fallen, die Schalen und das Netz waren zur Sammlung geeignet. Die Malaise-Fallen haben insgesamt 325 Individuen von 42 Arten gesammelt. Das Netz hat 49% der Gesamtindividuenzahl, 476 Individuen von 40 Arten, gefangen. Die Schalen wurden nur im Juni gelegt, deshalb fanden sich in ihnen weinger Arten und Individuen. Am Westrand waren mehr Arten und Individuen als auf dem Kahlschlag, aber die Diversität war niedriger. Die einzelnen Arten waren am Westrand mit höheren Anzahl vorgekommen, deswegen war die Diversität geringer als auf dem Kahlschlag, wo manche Arten sich nur mit ganz wenigen Anzhal

gefunden haben. Es könnte vorkommen, dass die gute Wanderarten der Schwebfliegen auf dem Kahlschlag erscheinen werden. Manche Arten, die die offene Gebiete bevorzugen, könnten auch auf das Gebiet zuwandern. Für faunistische und jahreszeitliche Messungen ist es besser mit der Malaise-Falle und dem Netz zu sammeln oder mit einem der beiden ergänzt durch Schalen.

DANK

Ich möchte mich den Mitarbeiter und den Mitarbeiterinnen des Professurs für Fortschutz der Technische Universität Dresden für die viele Hilfe bedanken.

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Natural tolerance of maize hybrids in Martonvásár against western corn rootworm (*Diabrotica virgifera virgifera* LeConte)

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SUMMARY

The corn rootworm, which has been introduced from North America, is becoming an increasingly serious problem for maize producers in Hungary. In several regions the damage it causes has reached the threshold of economic loss, making it the most problematic of all the biotic and abiotic stress factors faced by maize growers. The aim of the research was to determine the level of corn rootworm tolerance in various maize genotypes and to use selection methods to breed maize hybrids whose tolerance level provided satisfactory protection against this biotic stress factor. The present paper describes studies on the tolerance level of 41 Martonvásár hybrids at three locations in two years. Significant differences were found in the tolerance levels of the hybrids, and a close positive correlation was revealed in 2007 between root-pull resistance and yield. Root regeneration is also an important factor, good values of which were found for three hybrids.

Keywords: western corn rootworm, resistance breeding, tolerance level

INTRODUCTION

Maize production is one of the most important sectors of agriculture in Hungary. On the basis of profitability it has been one of the top-ranking field crops in recent years, and is grown on an area of around 1.1–1.2 million hectares. Until recently, apart from a few pathogens it has suffered little damage from pests, but this situation changed after the appearance of the corn rootworm in 1995. It is estimated that around 100 000 ha were affected on a third of which lodging has been recorded. No accurate data are available on the yield losses suffered in Hungary, but they probably amount to around 5% on a national scale. The yield losses caused by the pest may range from only a few per cent to as much as 70–80% (Sivcev and Tomasev, 2002, Széll et al., 2005). American data indicate that yield losses combined with the cost of control lead to a loss of income amounting to around a billion dollars a year (Krysan and Miller, 1986). In addition to agronomic, chemical and biotechnological control measures (Riedell et al., 1992, Keszthelyi et al., 2007, Árendás et al., 2009) work has been underway for several decades to breed maize varieties resistant to the pest (Owens et al., 1974, Hibbard et al., 1999, Ivezic et al., 2006, Šimić et al., 2007, Tollefson, 2007). Among the three basic mechanisms of host-plant resistance (non-preference, antibiosis, tolerance) defined by Painter (1951), conventional plant breeding can only be based on tolerance, where differences arise mainly as the result of diverse growth habits (stronger stalks, more robust root mass, better root regeneration). The present paper aimed to determine the corn rootworm tolerance levels of 41 maize hybrids.

MATERIALS AND METHODS

In order to determine the level of tolerance against corn rootworm, 41 Martonvásár maize hybrids were sown in experiments at three locations with three replications in 2007 and 2008. The extents of natural rootworm infection in the previous year and the type of soil were taken into consideration when choosing the locations, which included heavily infested chernozem soils only. The sites chosen for the experiments were Kőszárhegy, Lászlópuszta and Martonvásár, where the rates of root lodging in the past years were 55%, 40% and 30%. The two-rowed plots were 6 m in length with row and plant spacings of 0.7 m and 0.2 m, respectively. At each location the root-pull resistance was recorded on two occasions (end of June, middle of September) on 5 plants per plot for each genotype, after which the visible root damage was scored using the Iowa scale (1: no damage, 6: loss of three or more root levels) and the root diameter was measured. The latter values were used to determine the extent of root regeneration (by subtracting the June values from the September values). Root pull resistance was defined by measuring the force required to pull out the root, using a two-armed lever equipped with a dynamometer. After counting the number of lodged plants the yield was harvested from a 2 m section of each plot. The data were evaluated using analysis of variance and regression analysis.

RESULTS AND DISCUSSION

Different levels of root damage were recorded for the tested hybrids at the three locations and in the two years. The infestation was most severe in Kőszárhegy in 2007, followed by Lászlópuszta, with the least damage in Martonvásár. A higher level of infestation was expected based on the corn rootworm damage in the previous year. The infestation was most severe in Martonvásár in 2008, followed by Lászlópuszta, with the least damage in Kőszárhegy. Differences in the degree of infestation were also observed between the two scoring dates for root damage in 2007. At the second scoring date there was a substantial reduction in damage compared with the

first scoring date in Kõszárhegy, mainly due to root regeneration (*Figure 1*), which could be attributed partly to timely rainfall and partly to genetic differences in regeneration between the hybrids (*Figure 2*). It is clear from the figure that three hybrids exhibited a high level of root regeneration. There was no significant differences between the two sampling date in 2008.

Figure 1: **Root injury in different locations, averages of hybrids (2007-2008)**

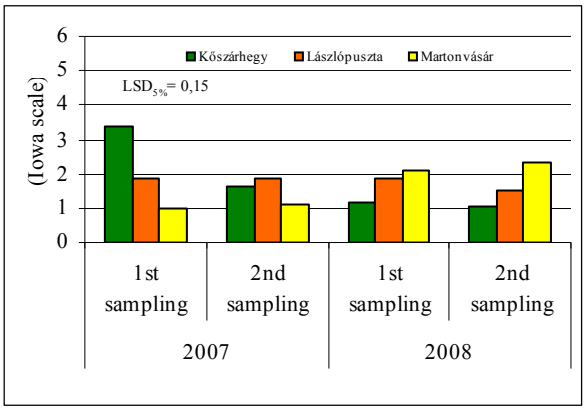
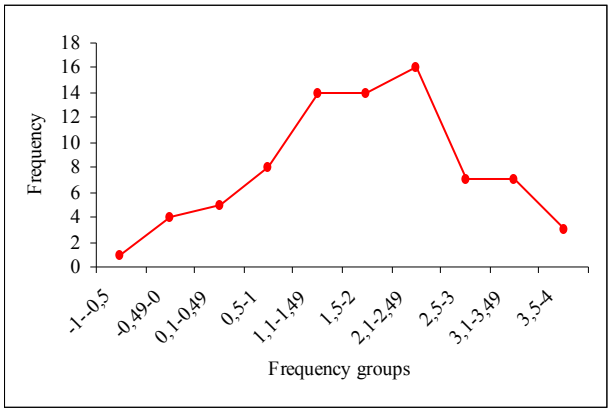


Figure 2: **Frequency distribution of root regeneration (Kőszárhegy, 2007)**



The 41 genotypes tested had different levels of tolerance of the pest, as shown by the considerable differences in root-pull resistance in different years and locations (*Figure 3*).

Figure 3: **Root-pull resistance in different locations, averages of hybrids (2007-2008)**

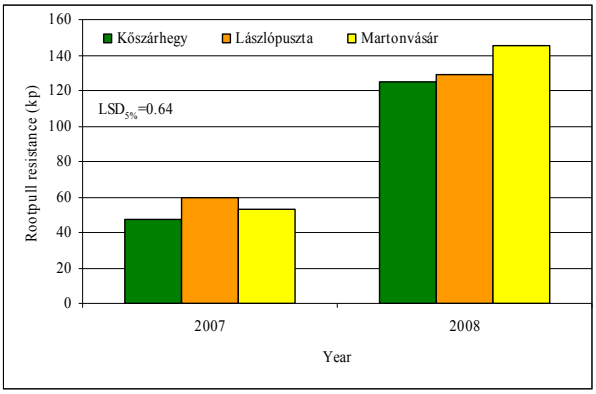
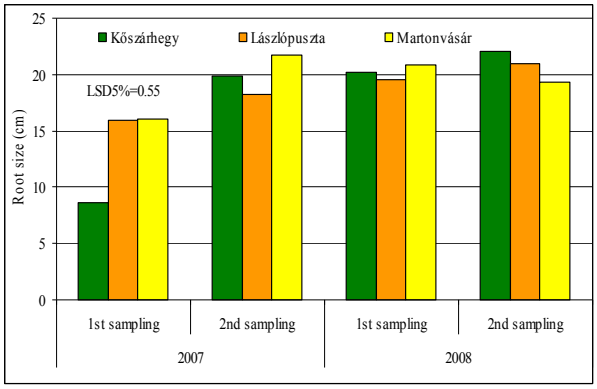


Figure 4: **Root size in different locations, averages of hybrids (2007-2008)**



The substantial difference between the results of the two years is apparent, in spite of that the extent of infestation demonstrated by the Iowa scale for the two years did not vary significantly. The reason for this difference is deemed to be the dissimilarity between rainfalls: the drought in 2007 inhibited plant growth, while the amount of precipitation in 2008 was more favourable for maize. This is also confirmed by the values of root diameter (*Figure 4*). The diameter of roots that survived the damage was considerably larger in 2008 than in 2007.

The yields also reflect the difference between the weather conditions in the two years (*Figure 5*). Although the infestation in 2008 was similar to that of the previous year, the average yields of hybrids - due to more favourable soil moisture levels - was nearly 50% higher than in 2007.

Even though the weather conditions were determinant in yield, infestation (Iowa scale) and yield showed a positive correlation with r=0,703*** in 2007, and with r= 0,409*** in 2008. The more severe the infestation was, the lower yield was produced. The correlation between root pull resistance and yield was significant only in 2007 (r=0,673***). These values demonstrate that western corn rootworm causes a reduction in yield, and the level of damage can be higher under dry conditions.

Figure 5: **Yield in different locations, averages of hybrids (2007-2008)**

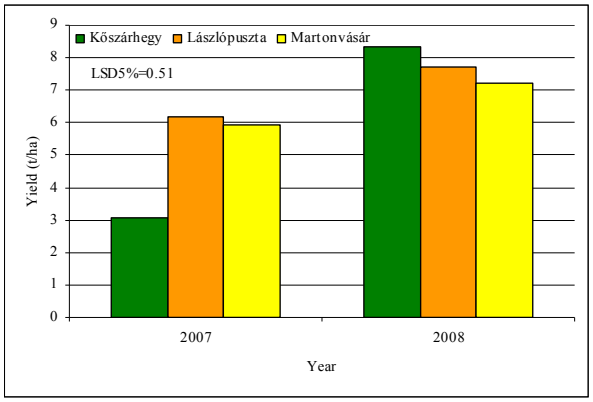
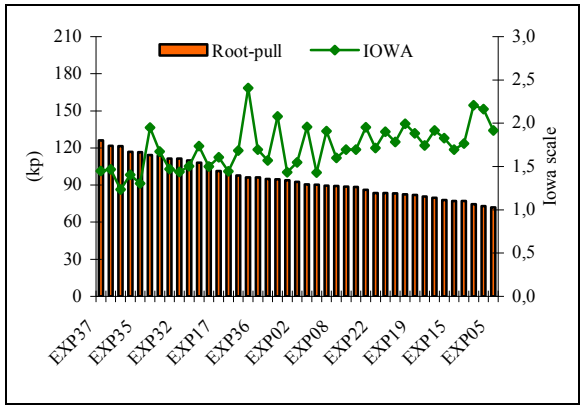


Figure 6: **Root-pull and root injury of the hybrids (average of 3 locations, 2 years and 2 samples)**



Root pull resistance was found to be a reliable indicator for the characterisation of root damage. The averages of the years and locations varied in a wide interval (72–126 kp) and had medium strong correlation (r=0,615***) with the values of the Iowa scale (1.2–2.4) (Fig. 6). Hybrids with greater root-pull resistance values exhibited significantly less root damage than those with weaker resistance. In the year with drier weather conditions (2007) this correlation was stronger (r=0,768***), while in the year with more precipitation (2008) it was weaker (r=0,378*).

CONCLUSIONS

The 41 maize hybrids tested were found to have different levels of tolerance against the corn rootworm. Tolerance is based mainly on external traits such as a stronger root system with better regeneration ability. Some hybrids had outstanding root regeneration ability. The selection method used in the experiments, based on root-pull resistance measurements combined with the counting of lodged plants and the scoring of root damage on the Iowa scale, proved to be suitable for the relatively rapid testing of the tolerance of large numbers of maize genotypes.

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Protected Orthoptera species of agro-ecosystems in Hungary

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SUMMARY

The locusts (Orthoptera: Acrididae) are one of the most feared pests in the world. Now in Hungary locust plagues are not a real danger. Orthopterans live in the Carpathian Basin are mostly 'friendly' moreover endangered members of natural, semi-natural and agro-ecosystems. Here we collect distribution and habitat preference data of protected orthopterans living in agricultural landscapes in Hungary and provide information about them in order to call attention of farmers and help its protection.

Generally we can assume that intensive land use and increasing use of chemicals in plant protection endanger species that live inboard and neighbouring of cultivated lands. The positive effects of traditional land use can be proved in case of more species. The conservation of these rare and vulnerable species needs activity of both nature conservation and agriculture.

Key words: species conservation, crickets, locusts, pests, land use

INTRODUCTION

The most known Orthopterans are locusts (Acrididae) which are one of the most feared pests in the world. In spite of pest control programs the locusts can consume vast swathes of crops and cause large damage in many regions of the world. In these days in the Carpathian Basin and especially in Hungary locust plagues are not a real danger. However up to the 19th century swarms of Migratory locust (*Locusta migratoria)* had swept through this region and than in the 20th century Moroccan locust (*Dociostaurus maroccanus*) caused large locust plagues.

Although some orthopterans are dangerous pests, most of them are 'peaceful' moreover endangered species of not only the natural and semi-natural habitats, but also of the agro-ecosystems. Beyond that considering their abundance and biomass orthopterans are often the main invertebrate consumers in grasslands and play significant role in the food-webs (Curry et al., 1994; Andersen, 2001).

In Hungary 121/124 Orthoptera species occur (Nagy, 2003; Nagy and Rácz, 2007) and 31 of them are protected (KÖM, 2001). Two of them – Eastern green bush-cricket (*Tettigonia caudata*) and Heath bush-cricket (*Gampsocleis glabra*) – especially prefer different kind of agricultural landscapes, while others live in hedges and abandoned patches in agricultural lands. Thus the protection of these species needs contribution of both conservation managers and farmers.

We collect distribution and habitat preference data of protected Orthoptera species living in agricultural lands in Hungary and provide information about them. We aimed calling farmers and agronomist attention to protection of these vulnerable species.

MATERIAL AND METHODS

We collected and summarized the distribution and habitat preference data of protected Hungarian Orthoptera species live in agro-ecosystems. In this paper eight Orthoptera species (6 Ensifera and 2 Caelifera) are characterised considering their relation to agriculture. During the study we survey more than 30 publications and our unpublished data. The main data of species are presented in the *Table 1*.

Orthoptera species

Subordo: Ensifera

Isophya modesta (Frivaldsky, 1867) – Hungarian name: szerény tarsza

Grass-green coloured medium-stratured micropter bush-cricket with short green wings. The average of body length: 23-27 mm. The ovipositor is 16-18 mm in length (Harz, 1957, 1969). Because of its colour and sheltering way of life the specimens are hard to recognize.

This Balkanian (Moesian) species (RÁCZ, 1998) occur in Central Europe. The sporadic populations are mostly well separated from each-other. *I. modesta* is rare in Hungary, it occurs in the Villány Hills, the Mecsek, the Mátra, and the Bükk Mountains (Nagy, 1981, 2002; Nagy and Rácz, 1996; Nagy and Nagy, 2000).

The specimens usually stay on large leafs of *Dicotyledonous* plants. They prefer the sunny hillsides covered by steppe grasslands and dense rocky grasslands. The adults can be found from end of May to beginning of July.

This species has no economical importance populations live only in the neighbouring natural and semi-natural grasslands of vineyards. It never occurs in cultivated areas and in the lowlands. The populations are

endangered by vineyards. The habitat loss caused by extension of vineyards has an indirect, while the intensive chemical plant protection has a direct effect on the *I. modesta* populations live in the neighbouring natural habitats. The species can not escape from disturbance because of their limited moving ability. It happens especially in the Villány hills where the *I. modesta* can be fund even in the abandoned vineyards and in the hedges and shrubs between existing vineyards (unpublished data of Nagy, A.).

Isophya modestior Brunner von Wattenwyl, 1882 – Hungarian name: illír tarsza

This medium-statured micropter bush-cricket is green with little reddish-brown dots. The elytra are yellowish-white and cover the third part of abdomen. The average of body length is 18-24 mm. The ovipositor is 11-14 mm in length (Harz, 1957, 1969).

This Balcanian (Ilyrian) chortobiont species (RÁCZ, 1998) occur in Southeast Europe. *I. modestior* is protected rare species in Hungary. This species is occurring in the Villány Hills, the Mecsek and the Kőszeg Mountains. The adults can be found from end of May to beginning of July. The extensions of vineyards are endangering this species as in case of *I. modesta*.

Polysarcus denticauda (Charpentier, 1825) – Hungarian name: fogasfarkú szöcske

P. denticauda is a large-stratured (28-36 mm), stocky, micropter bush-cricket. It is normally grass-green, sometimes reddish-brown. The ovipositor of females is 18-27 mm in length. The upper wings are yellow with brown veins. The look of specimens shows spatial differences. In East Europe multicoloured populations also can be found (Harz, 1957, 1969).

This Ponto-Mediterranean species is distributed in Central- and Southeast Europe. In Hungary *P. denticauda* occurs both in lowlands and mountainous areas. Although it is a rare species in Hungary in the mountain grassland (the Mátra and the Bükk Mountains, the Budai and the Pilis Hills) it is easy to find (Szelényi et al., 1974; Nagy and Rácz, 1996; Nagy, 1983, 1987, 1997, 2002; Nagy and Szövényi, 1999a). In western part of the country it can be locally common moreover it can cause local swarms in wet grasslands (Harcz, 1957, 1969; Nagy, 2002). The adults can be found from end of May to August depends on microclimate of habitats.

This species live only in dense wet grasslands dominated by *Dicotyledonous* plants. It has special microclimate needs, considering especially humidity of soil and air. In arable land never occur. The local outbreaks cause damages only in hayfields.

The lowland populations of *P. denticauda* are endangered by habitat loss caused by intensification of land use and mainly by the chemical plant protection, while the mountainous ones are unperturbed.

Tettigonia caudata (Charpentier, 1845) – Hungarian name: farkos lombszöcske

It is a large-statured (25-40 mm) bush-cricket with green body. Its legs are yellowish. It can be easy separated from other *Tettigonia* species occur in Hungary (*T. viridissima* and *T. cantans*) on the basis of morphological characteristics and its song. The females have long straight ovipositor (Harz, 1957, 1969).

The Ponto-Caspian *T. caudata* is distributed from Central Europe to West Siberia (Harz, 1957, 1969). In Hungary this species had been common up to the beginning of the 20th century (Pongur, 1918). During the 20th century it became rare or even it became extinct in some areas (e.g. in the Kőszeg Mountains (Szövényi and Nagy, 1999)).

This species has no special habitat needs. It can be found in mainly ago-ecosystems, in weed- and loess-grasslands and in meso- and hygrophilous mountain-grasslands (Kisbenedek, 1997; Nagy and Rácz, 1996). Gausz and Gallé (1968) found it in the *Cypero-Juncetum* community. The adults can be found from June to October.

In consequence of the intensification of land use, fragmentation and loss of habitats frequency of the species dramatically decreased during the 20th century (Kenyeres and Bauer, 2001). The extensive land use is favourable to *T. caudata*. The hedges and weed patches can provide both food source and suitable habitats for the species.

The fragmented and isolated populations are endangered by intensive land use and changes of landscape structure (disappearing of hedges and decreasing of habitat diversity). The increasing use of insecticides affects directly while the use of herbicides affects indirectly to the survival of the local populations.

Gampsocleis glabra (Herbst, 1786) – Hungarian name: tőrös szöcske

G. glabra is a usually green sometimes yellow coloured, medium-statured (20-26 mm) bush-cricket. Its pronotum is light brown with yellow margin on the lateral lobe. The first wings are green, longer than abdomen, with brown dots. The females have relatively large ovipositor 15-21 mm in length (Harz, 1957, 1969).

This Ponto-Caspian thamnobiont species (Rácz, 1998) occur in West-, Central- and East Europe. In Hungary it can be found only in the lowlands, where its distribution is sporadic (Varga and Rácz, 1986; Rácz, 1986; Nagy, 1983, 1987; Nagy and Szövényi, 1998, 1999a; Nagy et al., 1999). This bush-cricket is rare in the Hungarian Orthoptera fauna.

G. glabra prefers the hygrophilous grasslands, and meadows where the surface seasonally covered by water. This species lives in natural and semi-natural grasslands neighbouring to agricultural fields and in hedges. Sometimes it occurs inboard of arable lands but it has no economical importance. The adults can be found from June to August.

The intensification of land use, the undue mowing and the chemical plant protection also endanger the populations live in agricultural landscapes. Because of these reasons it already became rare in Austria and Germany.

Pholidoptera litoralis (Fieber, 1853) – Hungarian name: bújkáló avarszöcske

P. litoralis is a medium-statured (19-27 mm) micropter bush-cricket. In contrast with other *Pholidoptera* species live in Hungary it is mostly green, brownish-green or greenish-yellow. It has a black coloured pronotum with light margin. The wings are reddish-yellow. The females have long (23-25 mm) straight ovipositor (Harz, 1957, 1969).

P. litoralis is distributed in the north Mediterranean region. In the Pannonian biogeographical region it occurs mostly in mountainous areas. In Hungary it can be found only in the surroundings of the Körös River (southeast Humgary). This occurrence is unusual because of low height above the see level (approx. 80-90 m). These populations are connected with the nearest Transylvanian ones (Nagy and Szövéni, 1999a, 1999b; Nagy et al., 2000; Nagy, 2002.)

In Hungary this species prefer the high dense wet-grasslands, meadows and edges of park-forests (Nagy, 2002). The adults can be found from June to August.

In agricultural landscape it can be found only in the hedges, pastures and hayfields. It has no economic importance. The populations are endangered by intensive mechanical-mowing and chemical plant protection.

Table 1

Protected Orthoptera species live in agro-ecosystems in Hungary with its scientific name (Nagy, 2003) faunal- and life form type, geographical range (Rácz, 1998), frequency category in Hungary (on the basis of 10 x 10 km UTM distribution data; Nagy and Rácz, 2007) and protection status (KÖM, 2001; Council of Europe, 1992; Korm. Rendelet, 2004).

Species	Geog. range	Faunal type	Life forms	Freq. cat.	Protection
Subordo: Ensifera					
Isophya modestior Brunner von Wattenwyll, 1882	North-East-Carpathian	Balcanic (Ilyrian)*	Ch	I	P
Isophya modesta (Frivaldszky, 1867)	Central-Southeast-European	Balcanic (Moesian)	Ch	I	P
Polysarcus denticauda (Charpentier, 1825)	Central-Southeast-European	Ponto-Mediterranean	Ch	I	P
Tettigonia caudata (Charpentier, 1845)	Central-East-European	Ponto-Caspian	Ch-Th	I	P
Gampsocleis glabra (Herbst, 1786)	European-Asian	Ponto-Caspian	Th	II	P
Pholidoptera litoralis (Fieber, 1853)*	Southeast-European	North-Mediterranean	Ch-Th	I	P
Subordo: Caelifera					
Odontopodisma rubripes (Ramme, 1931)	North-East-Carpathian	Dacian	Th	I	P, N-2000, AII, AIV
Locusta migratoria Linnaeus, 1758	Cosmopolitan	Policenric	Ch*	I	P

*: faunal- and life form types and geographical range on the basis of unpublished data of I. A. Rácz.

Th: thamnobiont (species live in only high and dense grasses, shrubs and forests); Ch: chortobiont (grass-dwelling species, live in different kinds of grasslands); P: protected species in Hungary; N-2000: listed in the NATURA 2000 species list; AII and AIV: listed in the Annex II and Annex IV of the Habitats Directive (Animal and plant species of community interest whose conservation requires the designation of special areas of conservation (Annex II) or need a strict protection (Annex IV)); Frequency categories: I: rare (relative frequency: <0.0625); II: scattered (relative frequency: 0.0626-0.125)

Subordo: Caelifera

Odontopodisma rubripes (Ramme, 1931) – Hungarian name: erdélyi hegyisáska

O. rubripes is a medium-statured (15-23 mm) grass-green micropter locust. This species has very small and narrow wings. Its legs are singularly scarlet (Harz, 1957, 1975; Nagy, 2002).

This Carpathian subendemic (Dacian) species occur only in the eastern part of the Carpathian Basin. The central part of its area located in Transylvania (Romania). In Hungary it can be found only in the Bereg and in the Szamos-hát. Near Bátorliget village there is an isolated population in the Bátorligeti-ősláp (Bátorliget Marsh)

(Nagy, 1990a, 2002). Considering phenology *O. rubripes* is an early species, adults can be found from end of May to July (unpublished data of A. Nagy).

This species prefer especially dense and wet vegetation types as meadows, marshes, shrubs in the bank of ditches and hedges. Secondarily it occurs in forest clearings and roadside shrubs (Nagy, 2002).

The *O. rubripes* has no economical importance and never occurs in arable lands, but prefer hedges and bank of ditches. The populations are endangered by loss and fragmentation of their habitats. The intensive mechanical-mowing and the drainage have especially negative effect. The intensive insecticide use in the orchards, which is a common land use in the Bereg region, also influences the survival of local populations live in neighbouring habitats.

Locusta migratoria Linnaeus, 1758 – Hungarian name: keleti vándorsáska

It is a large-statured (30-60 mm) robust, generally green, rarely brown macropter locust species. The third legs are light red. Specimens show large differences in colouring (Harz, 1957, 1975). The species has two different forms the 'peaceful' soliter and the migrant gregaria. The two type show differences considering both their morphology and behaviour. The gregaria type is larger, more agile and dark coloured than soliter, however the soliter also can good fly (Uvarov, 1921).

The species have wide distribution, it occurs in Central-, East- and South Europe and in the greatest part of Asia. In central part of Africa and in Madagascar different subspecies occur (Harz, 1957, 1975).

L. migratoria is hygrophilous it prefer wet habitats as meadows, river banks and marshes (Harz, 1957). In these days the gregaria type still makes large swarms in different part of their geographical range. During locust plagues it can be found in all kinds of natural, semi-natural and agricultural habitats otherwise it prefers fallow lands and natural habitats.

In the Carpathian Basin in the beginning of the 20th century was the latest locust plagues caused by *L. migratoria*. The large swarms swept through the region and reached even south Germany. After the regulation of the Tisza River and its tributaries habitats of the species desiccated and finally mainly disappeared. Because of human activity the species nearly have became extinct, while the other xerophilous pest the Moroccan locust (*Dociostaurus maroccanus*) appeared in this area (Nagy 1964, 1988, 1990b, 1995).

Now there are only some soliter populations in northeast part of the Hungarian Great Plain (Alföld) e.g. near Hajdúbagos village and city of Debrecen. Consequently for today remain populations are protected by law. For the last isolated populations are endangered by drying of their restricted habitats and intensive pasturing.

DISCUSSION

In this study we characterized protected orthopterans living in agro-ecosystems in Hungary. We collect distribution and habitat preference data of species and studied the role of agriculture in the protection of them. There are 121/124 Orthoptera species in Hungary (Nagy, 2003; Nagy and Rácz, 2007) 31 of them are protected (KÖM, 2001). Seven of these species are directly or indirectly affected by the agriculture. In these days neither of them has economical importance. Only Migratory locust (*Locusta migratoria*) can bee seen as a pest but in Hungary only some remain soliter type populations live in this time. The near totally extinction of the species caused on the one hand by regulation of the Tisza River and its tributaries and on the other hand by drainage of marshes, bogs and meadows (Nagy, 1995, 2002).

However these species has large natural protection value and we have large responsibility for protect them especially in case of endemic, rare and threatened species. In order to protect these species there is a need for cooperation of conservation biologists, managers, farmers and agronomists.

Tettigonia caudata and *Gampsocleis glabra* lives mainly in agro-ecosystems and prefer hedges and fallow lands. The occurrence of these species can be expected mostly in the lowlands. The populations are endangered by habitat fragmentation and habitat loss caused by intensification of land use and intensive use of insecticides (Kenyeres and Bauer, 2001; Nagy, 2002). The larger habitat diversity and the extensive land use generally favours to survival of local populations.

Polysarcus denticauda generally lives in hayfields and pastures. Sometimes there are local outbreaks but it has no significant economic importance moreover the intensive land use (pasturing and mowing) endangers these local populations (Harz, 1957; Nagy, 2002).

The other four species hardly ever occur inboard of cultivated lands. They live in hedges and neighbouring natural and semi natural habitats. The intensification of land use generally causes fragmentation and loss of these habitats and the increasing use of pesticides and other chemicals directly endangers the local populations (Nagy, 2002).

Both *Isophya modestior* and *I. modesta* occur in the Villány Hills (Nagy and Nagy, 2000). They live in natural and semi natural grasslands fragmented and isolated by vineyards. The fragmentation and the use of insecticides also endanger these species (Nagy, 2002). Maintaining existing and establish new connections between subpopulations and cautious use of chemicals can help survival of local populations.

Pholidoptera litoralis occur only in the surroundings of the Körös River therefore the local organisations (both conservational and agricultural) have large responsibility for protect these populations (Nagy et al., 2000).

Finally *Odontopodisma rubripes* has to been emphasized. This subendemic species listed in international lists of protected species as Annex II and IV of Habitat Directive and NATURA 2000. The Hungarian conservation biology has a large responsibility for this species. It occurs only in the Bereg region and the Szamos-hát and lives in hedges and neighbouring of cultivated land thus the responsibility of farmers and agronomist are also decided.

Generally we can assume that intensive land use and increasing use of chemicals endanger species that live inboard and neighbouring of cultivated lands. The positive effects of traditional (extensive) land use were proved in case of many species (e.g. *Tettigonia caudata*, *Gampsocleis glabra*).

Conservation of these species needs further investigations in order to collect more detailed data on distribution and habitat preferences. In order to detect changes caused by human activity the populations must be monitored. Finally the participation of agricultural organisations, agronomist and farmers is essential both in conservation planning and activities.

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Possibilities of biological control with insects against ragweed (*Ambrosia artemisiifolia* L.)

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SUMMARY

It is an overview about possibilities of biological control of ragweed (Ambrosia artemisiifolia L.)

Keywords: ragweed, *Ambrosia artemisiifolia*, biological control

INTRODUCTION

Ragweed, which can be considered as a pioneer species of plant successive processes, was gotten into Europe from North America through grain shipments (Béres and Hunyadi 1980). The plant has been progressively spread since then and on the basis of national weed surveys it became the most widely spread weed species in Hungary since 1997 (Tóth 2003). Ragweed has an increased importance because it is the most allergenic weed species, which it is indispensable to root out. Unfortunately, protection against it was not taken seriously, it has been studied only just for a couple of years (Kiss et al. 2003).

Beside traditional protection methods, biological ones come to the front increasingly within the fight against ragweed. Biological methods are used with success in certain countries, in spite of the fact that chances of biological protection against weeds are slight. Biological methods are mainly spread against pest insects but there are more and more possibilities of biological protection against plant pathogens too. The main problem of biological protection against weeds is, that an introduced plant for which the abiotic environmental factors of the given country are favourable, does not have specialized natural enemies, which damage solely the given weed species.

Polyphagous pests (e.g. cockchafer grubs, wireworms, caterpillars of dart-moths) cannot be used because they damage the wide range of cultivated plants as well. Import of an insect (ragweed beetle *Zygogramma suturalis*), which is used in foreign countries with success, can also raise a problem, because we do not know the degree of damage it can cause in Hungarian agricultural fields. Notwithstanding, there were made some successful attempts with different insects against ragweed in the past years. In the case of these attempts, insects (*Ophraella communa, Zygogramma suturalis*) from the original home of the plant were tested under the new circumstances (Schwarczinger and Polgár 1999, Evans et al. 2001). These insects were used in practice only then they were not harmful to other cultivated plants (Klingmann and Coulson 1982, Cook et al. 1996). The greatest number of scientific studies on ragweed pests was made in North America (Harris and Piper, 1970). There were found about 200 insect species feeding on ragweed. Unfortunately, the bulk of these insects were oligophagous of polyphagous so there were only a few monophagous species, feeding exclusively on ragweed. There were conducted different studies in other countries too, 10 species were found in the former Soviet Union (Reznik et al. 1994) and 28 species in the former Yugoslavia (Igrc and Ilovai 1996), which fed on ragweed. There were performed some experiments in Japan with the species *Ophraella communa*, but the results were inconsistent. The greatest number of experiments was conducted with ragweed beetle (*Zygogramma suturalis*) in North America, Yugoslavia and China (Julien and Griffits 1998, Teshler et al. 2002). It was conducted to the given countries without any risk because it is a specialized monophagous ragweed pest. The attempts for import were successful everywhere, there was not observed any damage of these insects on other plants. Unfortunately, they were not able to fulfil the hopes set on them because their damages were not serious on the stocks of ragweed. Similar results were derived from the experiments made with other insect species too. *Conotrachelus albocinereus* weevil in Australia (McFadyen 2000), *Zygogramma bicolorata* leaf-beetle, *Stobaera concina* leafhopper and *Epiblema stenuana* moth in Australia, *Tarachidia candefacta* dart-moth *Trigonorhinus tomentosus* beetle and *Euaresta bella* fly in the former Soviet Union were used unsuccessfully (Julien and Grifits 1998, Teshler et al. 2002).

In addition to field experiments, laboratoy essays were also performed to study the effectiveness of different phytophagous insects on ragweed. These experiments were ended without any success because the test insects were not able to damage the ragweed plants or selected cultivated plants as food plant. Such species was Liothrips spp. originated from Argentina, but it was quite harmful to sunflower plants, which is not characteristic in its original home (McFadyen 2000). The same problem was revealed in Australia with *Ophraella communa,* which also caused severe damages on the leaves of sunflower plants (Palmer and Goeden 1991). However, this insect was not harmful to sunflower in Japan (Palmer and Goeden 1991, Yamazaki et al. 2000).

On the basis of these results we can establish, that the applied entomological experiments till now were not able to work out all possibilities of biologicl control against ragweed.

Entomological studies in the same topic started in 1998 in Hungary, with leafhoppoer species (Benkő 1998). Based on Benkő's (1998) experiments Kiss et al. (2007) performed their studies with the leafhopper *Eupteryx atropunctata*. These experiments revealed that leafhoppers fed with the mesophyllum cells of ragweed leaves causing tiny bright spots on their surface. Unfortunately, this damage on the surface of leaves did not affect the flowering of plants. It was also revealed that the leafhopper has more generations per year and is able to develop feeding extraordinarily on ragweed. Besides *Eupteryx atropunctata,* other leafhopper species (*Philaneus spumarius*, *Empoasca pteridis* and *Emelyanoviana mollicula*) can also feed and develop on ragweed.

Kiss (2006) performed further studies on insects damaging ragweed. According to expectations, a low number of phytophagous insects were collected and determined. These insects were mostly leafhoppers, ahids, bugs and leaf-beetles. The most abundant and most harmful group wa aphids. The number of insects was maximal in the summer, then it decreased rapidly from September. Among aphids *Myzus persicae, Brachycaudus helichrysi* and Aphis spp. were the most frequent developing more generations on ragweed. Among leafhoppers *Eupterix artopunctata* was observed in large numbers, developing more generations on ragweed during the summer. As a consequence of their damage the growing of ragweed plants decreased. The leafhopper species *Aphrodes bicinctus* and *Philaenus spumarius* was also found in the netted material, but they did not damage the ragweed plants. Among bugs, larvae and imagines of *Adelphocoris lineolatus* was also observed without any damage. Among beetles *Longitarsus pellucidus* was the most abundant feeding mostly on leaves of ragweed seedlings. They were able to survive four month on the plants. The nettings also caught some other insects (Frankliniella occidentalis, Trialeurodes vaporarium, Oecanthus pellucens and Agapanthia spp.) without any considerable damage.

There were some biological control experiments with aphids carried out in Hungary, which are linked with the names of Magyar and Basky (2007). They studied the feeding, life cycle and damage of *Aphis fabae*, *Brachycaudus helichrysi* and *Myzus persicae* on young (4 leaf stage) ragweed plants. As a result of aphids' damage, the height of plants, the number of polliniferous flowers, the amount and the germinative ability of pollen was decreased within the whole period. Owing to the decreased atmospheric pollen concentration, allergenic load can also be lower.

Based on these encouraging results we can establish that aphids fulfil the requirements of biological control against ragweed.

MATERIAL AND METHODS

Our studies, carried out in the autumn of 2008 included two different fields:

1. Using a sweep net, we collected phytophagous insects on ragweed plants of the experimental field. The time of sweep netting was 26th August 2008 and 10th September 2008. Location of our investigation was an experimental field near Balatonszentgörgy Somogy County, Hungary. On both occasions four times ten net strokes were made, collected material were preserved and later separated and determined. Determinations of collected insect species were made by Balázs Keresztes.
2. Cockchafer grubs were tested under greenhouse circumstances in small plastic pots to investigate whether the larvae feed on the roots of ragweed. Our experiments were carried out in the greenhouse of the Institute of Plant Protection (University of Pannonia Georgikon Faculty Keszthely, Hungary) on 18th September 2008. Two different plastic pots with size were used. 500 and 2000 grams of soil (brown forest soil) derived from the experimental field were placed into the pots. Ragweed plants grubbed up in the experimental filed in 6-10 leaves developmental stages were planted into each pot. One specimen of cockchafer grub (*Melolontha melolontha*) in L_2 larval stage, derived from a sunflower field in Székesfehérvár (Fejér County, Hungary) was placed into each pot. The experiment was evaluated on 28th November of 2008.

RESULTS

1. Results of sweep nettings:

Following insect species were found during the nettings:

Collection on 26th August 2008

Plant bugs (Heteroptera)

Lygaeidae

Nysius thymi 2 specimens

Nysius senecionis 1 specimen

Miridae

Adelphocoris lineolatus larvae, 3 specimens

Adelphocoris lineolatus imagines, 4 specimens

Lygus spp. larvae 3 specimens

Leafhoppers (Hemiptera)

Cicadellidae
Eupteryx spp. 4 specimens
Empoasca spp. 1 specimen
Delphacidae
Laodelphax striatellus 3 specimens
Butterflies (Lepidoptera)
Geometridae larvae, 7 specimens
Noctuidae
Helicoverpa armigera gyapottok-bagolylepke lárva 1 darab

Collection on 9th September 2008
Plant bugs (Heteroptera)
Miridae
Adelphocoris lineolatus imagines. 7 specimens
Lygus rugulipennis 2 specimens
Lygus spp. 3 specimens
Leafhoppers (Hemiptera)
Cercopoidea - Aphrophoridae
Philaenus spumarius 18 specimens
Cicadellidae
Eupteryx spp. 8 specimens
Empoasca spp. 5 specimens
Butterflies (Lepidoptera)
Noctuidae
Helicoverpa armigera larvae 52 specimens

1. Our results are the same as the results of the former Hungarian studies, namely leafhoppers and plant bugs were mostly collected on ragweed. On the other hand, it was surprising that we did not find any aphids. We can establish as a new result that we found the larvae of *Helicoverpa armigera* in quite large number feeding on ragweed. There is not any data in the native and international scientific literature about *Helicoverpa armigera* as a ragweed pest. Unfortunately, it is a polyphagous pest with more than 150 host plants (e.g. cotton, maize, sunflower, pea, alfalfa, pepper, tomato vine) so it cannot be used in the biological control against ragweed.
2. Our experiment was evaluated on 28th November of 2008. There was not any larval damage on the roots of ragweed plants. The cockchafer grubs were all alive so it is inferred that they did not feed on the roots.

Investigations in 2009

Our plane was to find out the phytophagous fauna of the ragweed stocks of the experimental field at Balatonszentgyörgy (Somogy County, Hungary). Unfortunately, we were not able to carry out any investigations, because the field was broken up before.

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Research on food / floral attractants, pheromones and their interactions: a review of recent results of our team

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SUMMARY

Recently the research on food-derived attractants (i.e. compounds playing a role in locating the host plant or other food sources) gained emphasis worldwide. One practical argument explaining this is that the vast majority of known pheromones applied in agriculture fall within the class of sex pheromones, and they attract only one sex, usually the males. Food-derived attractants can be expected to attract also females, so female-targeted lures can be developed. In the present paper three examples of interactions between pheromones and food-derived attractants is given from recent results of our team.

First, in the case of Lepidoptera, both in noctuids and phycitids the iso-amyl alcohol based female-targeted attractants did not show positive or negative interactions with the sex pheromones of the respective species. In this case the practical application of both types of attractants has no advantage.

Second, in A. ustulatus click beetle, the presence of the pheromone increased catches of females attracted to the floral bait, while on its own the pheromone was highly active in attracting males also. In this case the joint application of the pheromone and floral bait can be recommended, although both can be used separate as well.

Third, the pheromone of flea beetles showed very little activity on its own, but readily synergized the activity of the plant-derived isothiocyanates (which showed some activity on their own. In this case the joint use of the pheromone and plant-derived attractants is clearly superior, but for limited use the plant compounds on their own can also be applied. The pheromone on its own is useless.

This suggests that possible interactions and best application use of pheromones and food-derived attractants should be studied separately in each pest or pest group and specific scenario to determine best application opportunities.

Keywords: pheromones, food attractants, floral attractants, female-targeted lures, interactions, Lepidoptera, Noctuidae, Phycitidae, Coleoptera, Elateridae, Chrysomelidae

INTRODUCTION

Recently the research on food-derived attractants (i.e. compounds playing a role in locating the host plant or other food sources) gained emphasis worldwide. One practical argument explaining this is that the vast majority of known pheromones applied in agriculture fall within the class of sex pheromones, and they attract only one sex, usually the males. Food-derived attractants can be expected to attract also females, so female-targeted lures can be developed. Once a food attractant is characterized, it is necessary to study its possible interactions with the pheromone of the given taxon, and in a lucky case the action of the two types of attractants may complement each other.

In the present paper I review results some recent example studies from our team in this field.

MATERIAL AND METHODS

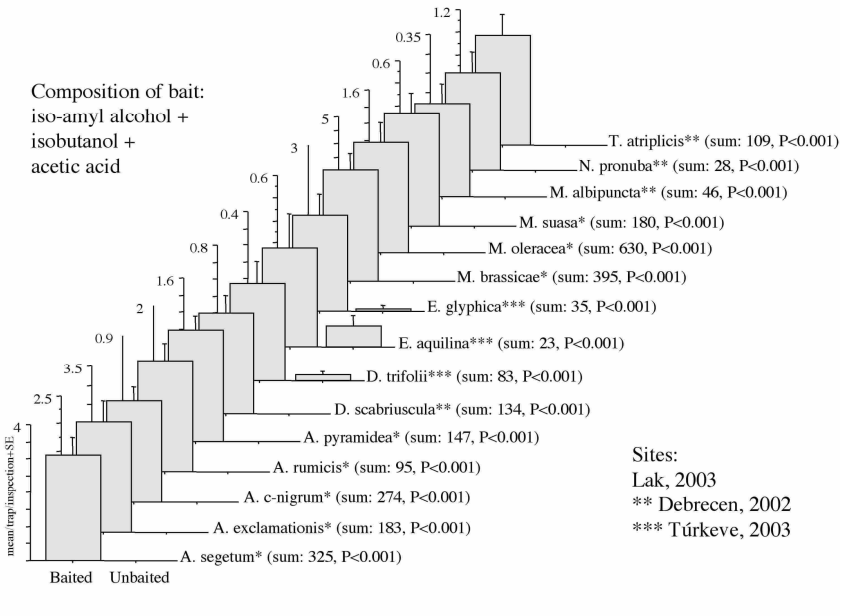
Experiments were conducted by internationally accepted and well established methods.

RESULTS AND DISCUSSION

Female-targeted attractants for Lepidoptera

Following the pioneering discoveries of Landolt (Landolt, 2000; Landolt and Alfaro, 2001; Landolt and Hammond, 2001) on the attraction of short-chain alcohols plus acetic acid for female noctuids in North America, we tested blends of iso-amyl alcohol, isobutanol and acetic acid for field activity on the noctuid fauna in Europe, in the hope of finding female-targeted attractants for agricultural pests.

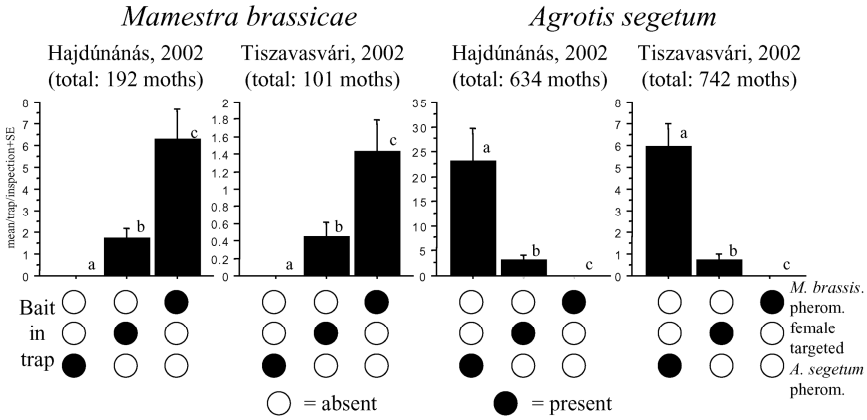
Figure 1: **Catches of noctuids in traps baited with a blend of iso-amyl alcohol, isobutanol and acetic acid vs. unbaited traps in Hungary. P values result from Student t test.**



In field tests in Hungary significant attraction for several noctuids was demonstrated (*Figure 1*). The best blend attracting most species contained all three compounds. Several of the noctuids attracted were pests of agriculture, as for example the turnip moth *Agrotis segetum*, or *Mamestra spp.*, etc.

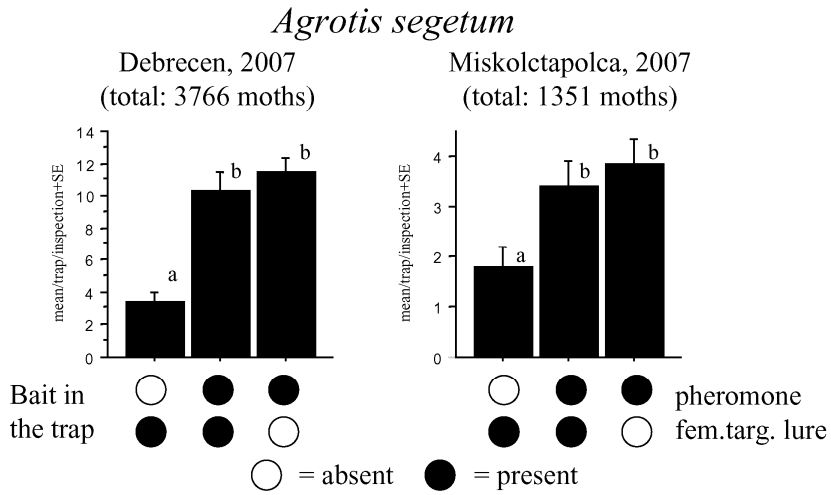
A large portion of the catch in baited traps was females: female ratio ranged from 20 to 70% depending on species and experimental site.

Figure 2: **Catches of two noctuids in traps baited with their respective pheromone or with the female-targeted lure (consisting of iso-amyl alcohol + isobutanol + acetic acid) in Hungary. Columns with same letter within one diagram not significantly different at P=5% (ANOVA, followed by Games-Howell test).**



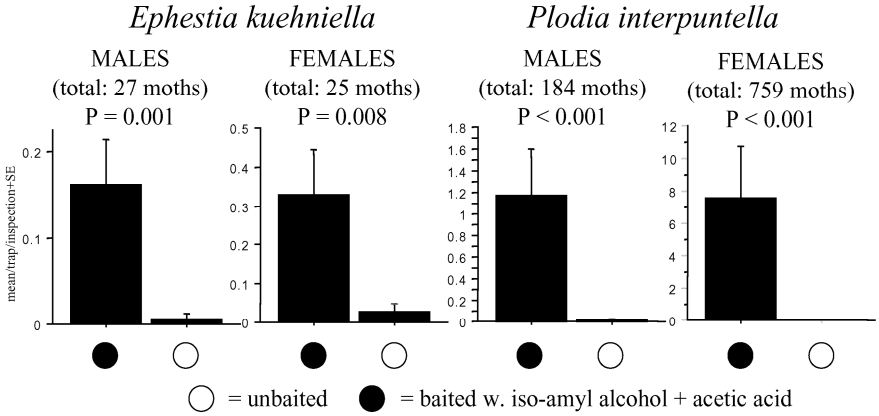
However, when on *M. brassicae* and *A. segetum*, the most important selected pest species, the performance of the female targeted lure was directly compared with that of the respective pheromones, the traps with the female-targeted lure caught only ca. 5-20% of the amount caught in pheromone traps. This may not be strong enough for general application in agricultural practice.

Figure 3: **Catches of the turnip moth in traps baited with its pheromone, with the female-targeted lure (consisting of iso-amyl alcohol + isobutanol + acetic acid) or with the two baits together in Hungary. Significance: see *Fig 2*.**



In case of the turnip moth, when both the pheromone and the female-targeted lure were placed into the same trap, no synergistic increase in overall catches was recorded, despite the fact that the female-targeted lure on its own caught sizeable numbers. Further studies are needed to clarify this controversy. Tests on other important pest noctuids are underway (*Figure 3*).

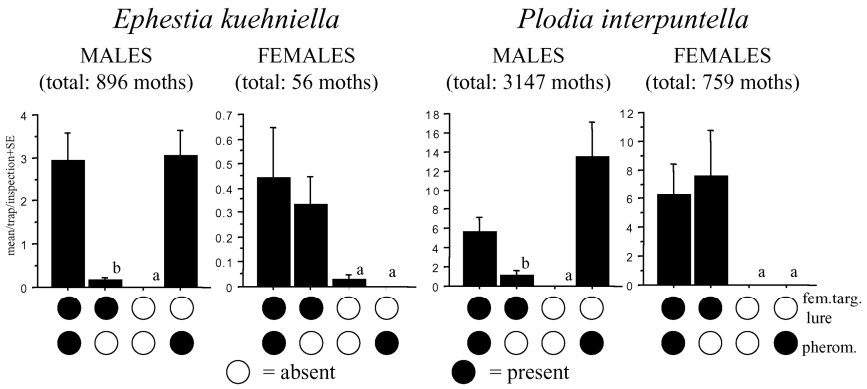
Figure 4: **Catches of two phycitids in traps baited with the female-targeted lure (iso-amyl alcohol + acetic acid) or in unbaited traps in Hungary (Kompolt, 2003). P values derive from Student *t* test. (after Tóth et al., 2002)**



In the course of the noctuid studies, we accidentally discovered that the blend of iso-amyl alcohol plus acetic acid attracts both sexes of *Plodia interpunctella* and *Ephestia kuehniella* (Tóth et al., 2002), both highly important stored products pests (*Figure 4*).

When both the pheromone and the female-targeted lure were placed into the same trap, no significant interference of the two types of baits was recorded in neither sexes (*Figure 5*). However, the activity of the female-targeted bait was negligible as compared to that of the pheromone. Further research efforts are directed towards increasing the activity of the female-targeted bait.

Fig. 5: **Catches of two phycitids in traps baited with the female-targeted lure (iso-amyl alcohol + acetic acid), their respective pheromone or the two baits together in Hungary (Kompolt, 2003). Significance: see *Fig 2*.**

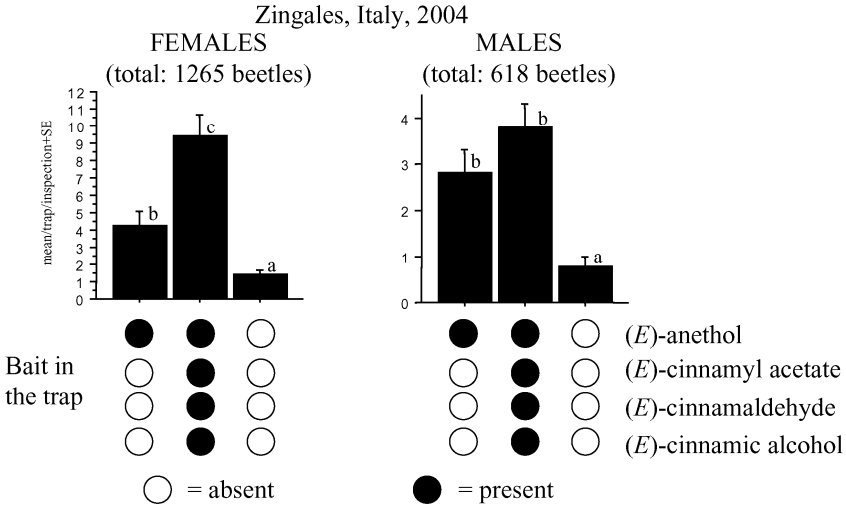


Click beetles (*Coleoptera*, *Elateridae*)

Click beetles are generally thought to use classical sex pheromones, which are emitted by the females, and responded to by the males. *Agriotes ustulatus* is among the most important pest click beetles in Europe. The female-emitted sex pheromone has been characterized as (E,E)-farnesyl acetate, and this compound attracts large numbers of males into traps (Kudryavtsev et al., 1993, Tóth et al., 2003).

Adult *A. ustulatus* can frequently be seen feeding on flowers, and as chance finding we observed some attraction to (E)-anethol, a common flower scent compound. In subsequent tests with floral compounds, the addition of cinnamic compounds clearly increased catches of females by (E)-anethol (*Figure 6*).

Figure 6: **Catches of *A. ustulatus* in traps baited with (*E*)-anethol and its mixture with cinnamic compounds vs. unbaited traps in Italy. Significance: see *Fig 2*.**



When the cinnamic compounds were added singly, there were no striking differences (*Figure 7*). As the binary blend with (E)-cinnamaldehyde caught numerically the most beetles, later this was used as a female-targeted lure for *A. ustulatus*.

Curiously, in the presence of the pheromone, female catches with the female-targeted lure increased dramatically (*Figure 8*). No interaction of the two types of lures was recorded in male catches.

This suggests that perhaps the pheromone of *Agriotes ustulatus* is not a classical sex pheromone, but shows characteristics of an aggregation pheromones also. Confirmation tests to prove this hypothesis are underway. From the practical point of view it is advantageous to use both the floral and the pheromonal baits in the same trap because then we shall capture the largest numbers from both sexes.

Figure 7: **Catches of *A. ustulatus* in traps baited with (*E*)-anethol and its mixtures with cinnamic compounds vs. unbaited traps in Hungary. Significance: see *Fig 2*.**

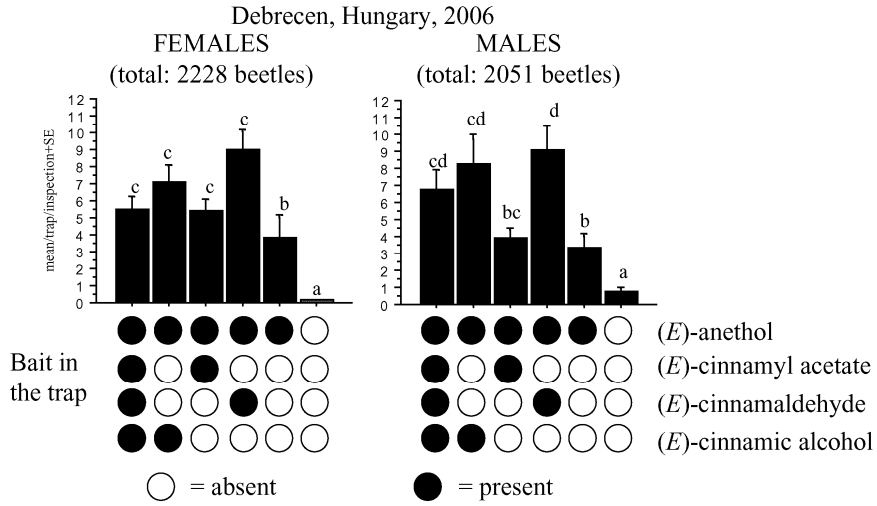
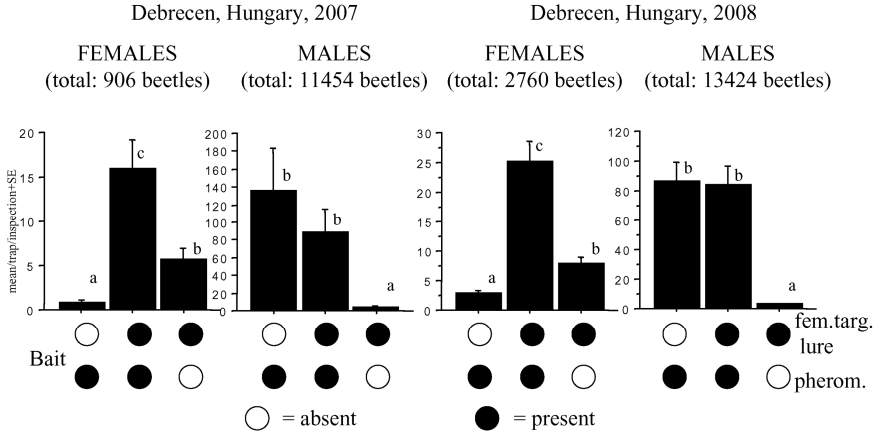


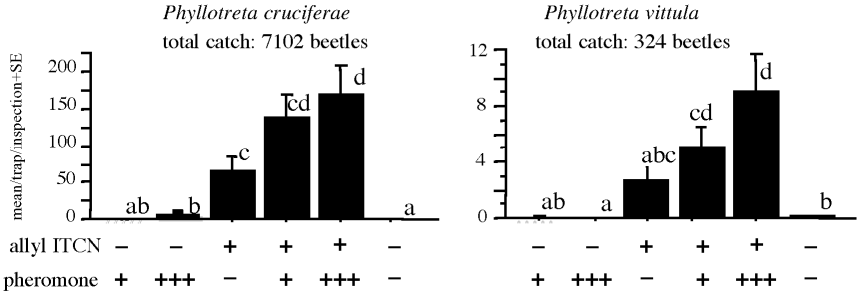
Figure 8: **Catches of *A. ustulatus* in traps baited with the female targeted bait [(*E*)-anethol + (*E*)-cinnamaldehyde], the pheromone or their mixture in Hungary. Significance: see *Fig 2*.**



Cabbage flea beetles (*Phyllotreta spp.*)

Cabbage flea beetles can cause significant damage to various cruciferous crops worldwide. Host plant derived volatile isothiocyanates assist flea beetles in locating their hosts. As for pheromonal communication, Bartelt et al. (2001) identified several male-specific compounds (mostly of himachalene structure) from *Phyllotreta cruciferae*, which were thought to have pheromonal function.

Figure 9: **Catches of *Phyllotreta* species in traps baited with allyl ITCN, pheromone, and both baits together. (Nadap, 2002). Signifcance: see *Fig 2*. (after Tóth et al., 2005)**



When we tested the Bartelt compounds A, C, D, E and H in Hungary, they showed good activity on *Ph. cruciferae* only, when presented together with allyl isothiocyanate (allyl ITCN) (*Fig. 9*, Tóth et al., 2005), confirming a parallel study in the US (Soroka et al., 2005). However, to our surprise, the same effect was recorded on the related *Ph. vittula* also, suggesting that the pheromone components of the two species may be similar.

As a result of later studies we found that compound A [(6R,7S)-2,2,6,10-tetramethylbicyclo[5.4.0.]undeca-9,11-diene] alone had the same level of activity as the full mixture, in both *Ph. cruciferae* and *Ph. vittula* (*Figure 10*, Tóth et al., 2005).

This again suggested that compound A may be a common pheromone component in both species.

When we collected volatiles from male *Ph. vittula*, the same range of compounds was identified in the extracts, with comp. A being the main one.

We subsequently identified the presence of these compounds in similar ratios also from male volatiles of *Ph. undulata*, *Ph. nemorum* and *Ph. nigripes*.

Fig. 10: **Catches of *Phyllotreta* species in traps baited with allyl ITCN or its blend with compound A of the pheromone. (Pusztazámor, 2005). P values result from Student *t* test. (after Tóth et al., 2005)**

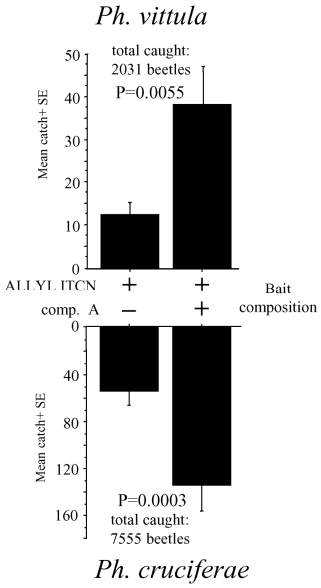
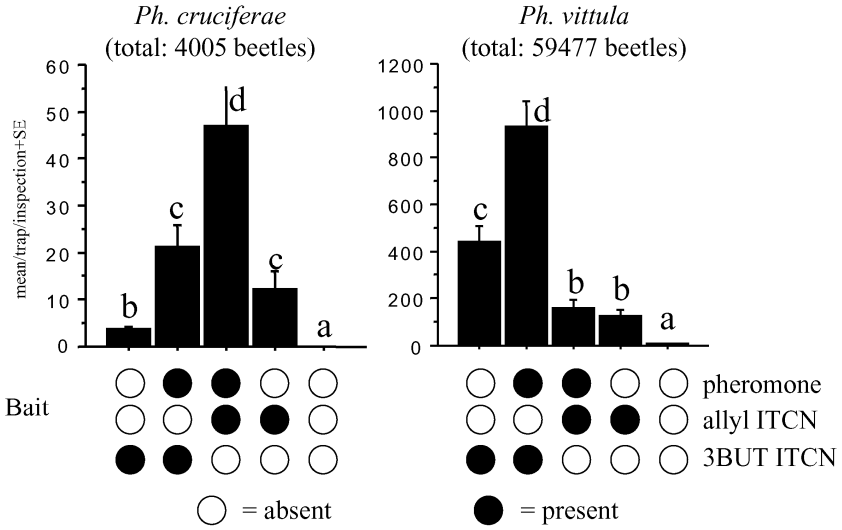


Figure 11: **Catches of *Phyllotreta* species in traps baited with allyl ITCN, 3BUT ITCN or their blend with compound A of the pheromone. (Kápolnásnyék, 2006). P values result from Student *t* test.**



In other studies on the preference of flea beetles to selected isothiocyanates we found that *Ph. vittula* responded best to 3-butenyl isothiocyanate (3BUT ITCN) (Csonka et al., 2007).

When testing combinations of the two isothiocyanates and comp. A, highest catches of *Ph. cruciferae* were recorded with comp. A plus allyl isothiocyanate, while of *Ph. vittula* with comp. A plus 3BUT ITCN (*Figure 11*).

Consequently these two flea beetle species show differing preferences in their host-plant related communication, but are very similar in their pheromonal communication.

In general, the pheromone and the food attractants show strong synergistic interaction in cabbage flea beetles, common to many other pheromones of the aggregation type. The pheromones of flea beetles are a special case

since on their own they show little activity, so there is little sense in using them without the addition of the plant-derived attractant.

CONCLUSIONS

Three examples of interactions between pheromones and food-derived attractants were given from recent results of our team.

First, in the case of Lepidoptera, both in noctuids and phycitids, the iso-amyl alcohol based female-targeted attractants did not show positive or negative interactions with the sex pheromones of the respective species. In this case the practical application of both types of attractants together has no advantage.

Second, in *A. ustulatus* click beetle, the presence of the pheromone increased catches of females attracted to the floral bait, while on its own the pheromone was highly active in attracting males also. In this case the joint application of the pheromone and floral bait can be recommended, although both can be used separate as well.

Third, the pheromone of flea beetles showed very little activity on its own, but readily synergized the activity of the plant-derived isothiocyanates (which showed some activity on their own). In this case the joint use of the pheromone and plant-derived attractants is clearly superior, but for limited use the plant compounds on their own can also be applied. The pheromone on its own is useless.

The above suggests that possible interactions and best application use of pheromones and food-derived attractants should be studied separately in each pest or pest group and specific scenario to determine best application opportunities.

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Chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu in China and in Hungary

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SUMMARY

The chestnut gall wasp (Dryocosmus kuriphilus Yasumatsu) is one of the major pests damaging chestnut. It is native in China. It was first reported in Japan in 1941. Dryocosmus kuriphilus belong to so-called "oak-gall wasp" (Cynipi tribus) of Cynipidae family Several species of Cynipi tribus are native in Hungary, which live on Quercus cerris. The chestnut gall wasp has been accidentally introduced to distant continents as North America and Europe. Its occurance in Europe was first recorded in Piemonte region, near Torino, Italy in 2002. In Hungary it was found in May 2009 on a young single chestnut tree in Budapest. The pest distributes over 16 provinces covering all the chestnut productive area in China with yield damage 15-30% every year. It was observed only 1 generation each year. Over wintering as young larvae within the host buds. The adult eclosion is at end of male flower blooming. There have been 28 parasitical wasps reported as the nature enemies of the pest up today. Among them, Torymus sinensis Y. is the most predominant one. Pollarding in winter, releasing natural enemy during April to June, using black light trap from June to September, and utilizing resistant cultivars are the main methods of IPM.

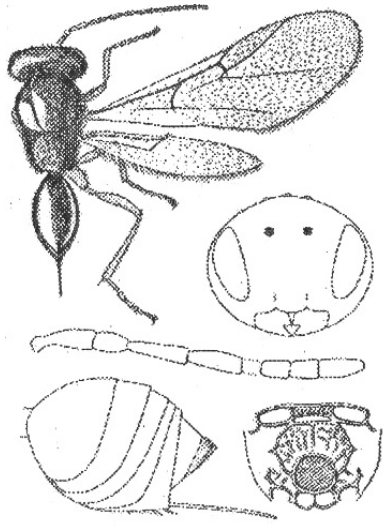
Keywords: *Dryocosmus kuriphilus*, China, gall wasp, *Castanea sativa, Quercus spp.*

INTRODUCTION

The chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu) is one of the major pests damaging chestnut. It is native in China. It was first reported in Japan in 1941 (Yasumatsu, 1951) (*Figure 1),* and it had got its official name in Japan what is current now (Fukuda and Okudai, 1950). *Dryocosmus kuriphilus* belong to so-called "oak-gall wasp" (*Cynipi* tribus) of *Cynipidae* family (Melika et al., 2003). Several species of *Cynipi* tribus are native in Hungary, which live on *Quercus cerris* (Csóka, 1997, Melika et al, 2000).

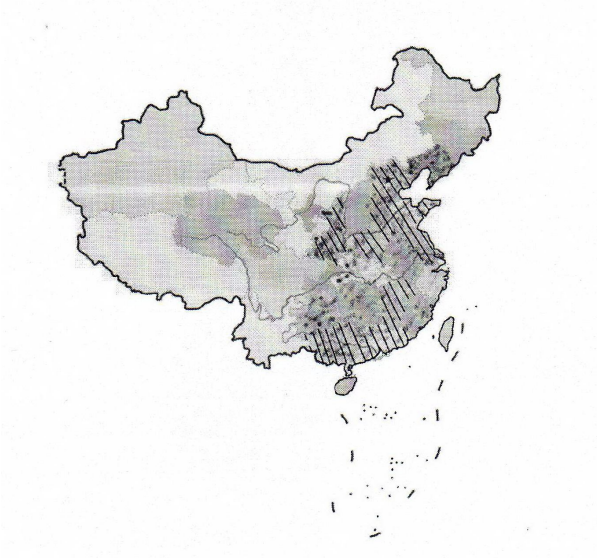
It is reported that the rate of the pest damaging trees and buds are up to 100%, respectively, which leads yield loss over 80%. In China, the pest is widely distributes in many provinces and municipalities where there are the main chestnut productive areas (*Figure 2*). The chestnut gall wasp a main pest which often outbreak. It is clear that the pest is such a specialized parasitic insect living in the chestnut buds. Adults lay eggs in the buds. The buds of the chestnut tree cannot grow shoots and get seeds, and will grow ball-like galls in the next spring. When the infested bud breaks, larvae induce the formation of the gall; make stop the growth of shoots. This will lead to a poor production of the chestnut. The damage may continue for many years.

Figure 1: **Chestnut gall wasp**



(Drawing by K. Yasumatsu, 1951)

Figure 2: **Dictribution of *D. kuriphilus* in mainland of China**



In the years around 1990, the damaged area was over 13 000 ha, which makes up 60% of total chestnut productive regions of the Jian'ou City of Fujian. The yield of infected plants in weight was about 20% of that of healthy plants; and the wasp even caused the death of some plants, resulting in heavy financial losses. In 1998, in Pinggu of Beijing, the ratio of damaged trees reached 96% and the ratio of damaged chestnut branches reached 70%. The yield loss was 30%. In 2004, the serious damaged area was over 333 hectares, which makes up 71% of total chestnut productive regions in Shennongjia in Hubei Province. The yield was about 20% of that in former years. And the economic losses reached 200 000 RMB yuan (Yan et al., 1995, Wu, 2005, Feng et al., 2006).

According to the research history of chestnut gall wasp in China, as early as in 1929, damage records on chestnut were reported by Gao Zhulin. In 1952, serious damages on the chestnut gall wasp were found by Zou Zhonglin in Wangting of Jiangsu province when a large number of insects galls appeared. The chestnut gall wasp was reared and the samples were identified as *Dryocosmus kuriphilus* Yasumatsu.

The oriental sweet chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu 1951) has been accidentally introduced to distant continents as North America and Europe. Its occurance in Europe was first recorded in Piemonte region, near Torino, Italy in 2002 (Brussino et al, 2002)(*Figure 3*). It was expected that gall wasp will appear in Hungary soon (Melika et al., 2003). It was first found in Hungary in May 2009 on a young single chestnut tree in Budapest. It is possible that the tree was carried from Northern Italy (Csóka et al, 2009).

BIOLOGY AND CHARACTERISTICS OF CHESTNUT GALL WASP

Life Cycle

Chestnut gall wasp has one generation annually in the mainland of China (Shandong, Henan, Chongqing and other areas) It overwinters as a small larva in the bud of the host plant. The larva feeds in the bud during chestnut shooting in the following spring, which leads to form a hard insect gall gradually instead of growing on the damaged shoots, leaves or buds. Each gall has 1-5 pest rooms and can hosts 1-16 larvae. 2 to 5 larvae were observed in each gall in most cases. The larva lives in the gall for 30-70 days, generally for 50 days (Ding et al., 2004).

The occurrence and damage activities of the chestnut gall wasp have close relations with chestnut phonologic phases (Ao et al., 1980). The overwintered larvae began to feed following with chestnut bud beginning to sprout in spring. The larvae in the bud grow and develop rapidly accompanying the shooting of their host chestnut buds. During the male flower initial bloom stage the larvae of the wasp get into pupation. At the and of the male flower blooming of host tree the pests in the galls (*Figure 4*) develop into adult stage. About 15 days later, the wasps get into the reproductive period. When getting out off host galls the adults are ready for ovideposition. Under natural conditions, the zooecium number was positively related to the quality and volume of the gall (Zang and Zang, 1966, Ding et al., 2004).

Chestnut gall wasp occurrence is observed cyclically and regionally throughout mainland of China. It was said that the regulation of its natural enemies is the main reason, mainly of its parasitic wasps. There was such phenomenon that if a heavy damage in practice with an outbreak of the pest lasted for 2-3 years, then the pest occurred just mildly in scale for about 10 years (Zu, 1993, Li et al., 2003).

In Shandong, Henan, overwintering larvae of the wasp began to be active and growing rapidly from early April. The pupation of the wasp is observed from mid-May to late June. Emergence of its adults occurs from late May to the end of June. The insect forms galls since late April. After its emergence the adults stay about 10 days in the gall and complete the development of ovaries during this period. They then bite a hole and get out off the host gall, from early June to early July. Female adults ovidepost their eggs in the buds parthenogeneticly. Since the August the larvae hatch, feed, and form a larger zooecium in the bud, which will develop into shoot, leaves, flower next year. Since the mid-September they gradually get into overwinter stage and spend the whole winter in the zooecium (Jin et al., 1995, Li et al., 2003).

Habitat

The adults stay about 10-15 days after the emergence stage in the galls before they get out. After getting out off the gall, the adults begin to lay parthenogenetic eggs; they are mostly found from 6 to 11 am. The habits of supplementary nutrition of the adult was not found but it shows phototaxis to black light lamps. The eggs are mostly laid in chestnut buds, generally the last 5-6 buds down from the top bud. Each bud has 1-10 eggs, usually two or three. Duration of eggs is about 15 days. Over 90 % of eggs are laid into the upper half side of a bud, and about 80% are located above the growing point, which would lead to the production of a gall instead of a branch. Some eggs are laid into edge side of growing point and would lead a weak stick with a gall. Eggs are laid on rudiment would lead insects gall in the vein of leaves (Sun and Fan, 1965, Luo, 1989, Li et al, 2003).

THE NATURAL ENEMIES OF CHESTNUT GALL WASP

Almost all of the natural enemies of chestnut gall wasp are the larvae stage parasitic wasps, in which 28 species have now been found (Guo et al, 1997). They are belonging to 12 families such as Torymidae, Eurytomidae, Ormyridae, Encyrtidae, Eupelmidae and so on. Among the parasitic wasps of chestnut gall wasp, the minority is kinds of monophagous parasitic wasps such as *Torymus sinensis* Kamijo, *Ormyrus* punctiger

Westwood, and the majority is kinds of oligophagous parasitic wasps such as *Torymus geranii* Walker and *Eupelmus urozonus* Dalman, which are mainly parasitoids of the gall-making insects on the plants of beech family Generally more than 10 kinds of initial parasitoids such as *Torymus sinensis* Kamijo and *Megastigmus nipponicus* Kamijo are hosted in phytophagous insects. About 5 to 8 kinds of them such as *Eurytoma variegate* Cuvtis and *Eurytoma decatoma* Concinna were epiparasitism parasitoids (*Table 1-2*).

Table 1

The recorded natural enemies of chestnut gall wasp

Scienfitic name	Known distribution
Torymus sinensis Y. et K.	China
Torymus geranii (Walker)	China, Japan, Korea, Europe
Torymus koreanus Kamijc	Korea
Torymus beneficus Y. et K.	Japan
Megastigmus nipponicus Y. et K	Japan, Korea
Megastigmus maculipennis Y. et K	China, Japan, Korea
Eurytoma brunniventris R.	China, Japan, Korea, America
Eurytoma setigera Mayr	China, Japan, Central-Asia,Europe
Eurytoma schaeferi Y. et K	Japan
Sycophila variegate (Curtis)	China, Japan, Korea, Europe
Ormyrus punctiger W.	China, Japan, Korea, Europe
Ormyrus flavitibialis Y. et K	Japan, Korea
Eupelmus urozonus Dalman	China, Japan, Korea, Central-Asia Europe, North-Africa
Peleumus ferrierer Y.	Japan
Eupelmus spongipartus F.	China, Europe, Russia
Cynipencyrtus flavus Ishii	Japan
Pteromalus apantelpohagus C.	Japan
Amblymerus amoenus japonicus Y.	Japan
Tetractishus sp.	Japan, China
Apsilota yasumatsui	Japan

Table 2

The parasitic natural enemies of chestnut gall wasp and their emergence period

Species	Emergence period
Torymus sinensis Kamijo	from February to March, July
Torymus geranii Walker	early May
Megastigmus nipponicus Kamijo	from March to April, June
Megastigminae toryminae	March
Torymidae	early July
Eupelmus urozonus Dalman	April, from June to August
Eupelmus sp. (undetermined sp.)	from July to August
Euryto manilensis	From March to April, June
Encyrtidae (undetermined sp.)	from March to April, from June to August

Sun (1965) reported a kind of adult parasitic wasps of the chestnut gall wasp in vitro. The larva of the pest was parasitized by Diptera insects. Yamamoto (1987) found that three kinds of hymenoptera adults were emerged from the Chestnut Gall, including species of Ceraphronidae (shield small-bee), Bethylidae (bethylids) and *Elaehertus* sp. (Scarcity Festival Wasps), whether they were the parasitic natural enemies of chestnut gall wasp or not, had not been confirmed. According to another report (Forestry Farming Bureau of Forestry Ministry, 1980), the natural enemies of chestnut gall wasp also included a kind of weevil, a spider and a pentatomid bug.

Among lots of parasitic natural enemies of chestnut gall wasp, *Torymus sinensis* Kamijo was the dominant specific parasitic natural enemy in many areas (Luo, 1985, Yan, 1995, Guo, 1997). The nearer *Torymus sinensis* Kamijo was to North, the more obvious the advantage was (Luo et al., 1986). There were certain differences in the dominant species in different region. According to the survey, in the damaged area by chestnut gall wasp, they were distributed both in Hebei Province and in Beijing, with a generation per year. The mature larvae overwinter in the parasitic dry galls of last year. The larvae overwintered would begin to pupate in mid-February, hatch in mid-April, and adult emergence in early May of next year (Dai and Lin, 2000).

After mating the adults of Torymidae Wasps find the new developed galls with the larvae of chestnut gall wasp, then insert their ovipositor into the chestnut gall, lay eggs in the inner wall of the zooecium of the pest, chestnut gall wasp. One or two days after hatching, its larva break the tegument of chestnut gall wasp larva with its large jaw, and suck their body fluids to live. Generally, one Torymidae Wasp larva is only hosted in one chestnut gall wasp larva. In Changping, Huairou, Miyun County, and other areas in Beijing, the ratio of chestnut

gall wasp hosting parasitic natural enemies was 0.9 to 1.5% in 1978, when the ratio of the destroyed new shoots was 60.5 to 90.0%. In 1979 the ratio of damaged shoots was 23.9 to 54.2%, but the ratio of the destroyed new shoots declined to 30.5% comparatively. It was also reported that the ratio of chestnut gall wasp hosted by parasitic natural enemies was 24.23% in 1978 and 81.15% in 1980 respectively; the ratio of damaged shoots was 56.83% in 1978 and 9.9% in 1980 respectively in chestnut-producing areas in Qianxi County of Hebei Province (Zu, 1993, Dai and Lin, 2000).

IPM OF THE CHESTNUT GALL WASP IN CHINA

A lot of job has been done on the IPM (Integrated Pest Management) of the chestnut gall wasp in China. Wei (1990), Guo et al. (1991, 1997) Jin et al. (1995) and Liu et al. (2000) reported chemical control technologies. Huang et al. (1988) and Zhang et al. (2002) reported their research results on the application of classification, biology of the natural enemies of the chestnut gall wasp. Tang et al. (1991), Huang et al. (1998), Wu et al. (2002) and Li et al. (2003) reported the physical and chemical resistance of the host trees against the chestnut gall wasp. At this moment it is considered that to control chestnut gall wasp the methods of agriculture should be used as the basement, keeping and utilizing the natural enemies should be considered before using chemicals. The main guidelines of the IPM are as follows:

Strengthening Quarantine

Transporting nursery stocks and scions from infected areas to the new chestnut development areas is forbidden, to prevent the proliferation of the chestnut gall wasp (Jin et al., 1995).

Strengthening the Management of Trees

To improve soil for nourishment, to culture root mass, to promote the growth of crown are good methods to strength the trees. Mean swell, breeding of insect-resistant chestnut is a basic method. There are different resistance sources to the chestnut gall wasp in different kinds of chestnut cultivars: Firstly, in the adult phase of chestnut gall wasp, chestnut gall wasps do not like to lay eggs on the shoot buds which grow so slowly that avoid the favorite period of the pest. This is named avoidance in resistances. Secondly it is difficult for a chestnut gall wasp to lay eggs on kinds of chestnut with slim buds with outer layer flakes hugged closely, which is seemed as insect-resistance. Thirdly, it is well known that the shoots of susceptible variety lure chestnut gall wasp adults to lay egg because they contain such chemicals that induce chestnut gall wasp adults into laying eggs. (Li, 2003, Ding et al., 2004, Wu, 2005).

Agriculture Control

Prevention and control measures widespread carried out in orchards are as follows: winter pruning to decreasing the number of the overwintered chestnut gall wasp. Artificial destroying gall in the begging of new gall in the spring, placing overwinter dry gall cut last year in chestnut orchard to keep the parasitoids to control chestnut gall wasp and so on. The way to control withered gall through continuous annual winter pruning has been proved to be the best one combining with various measures in building forestry. After the practical application of it, the rate of controlling can come to 97.55% (Wei, 1990).

Physical Control

Using black light lamp tolure and kill adults of the chestnut gall wasp from early June to September every year. Setting up a lamp per 667 m² chestnut orchard can lure and kill the adults partly (Guo and Liu, 1992).

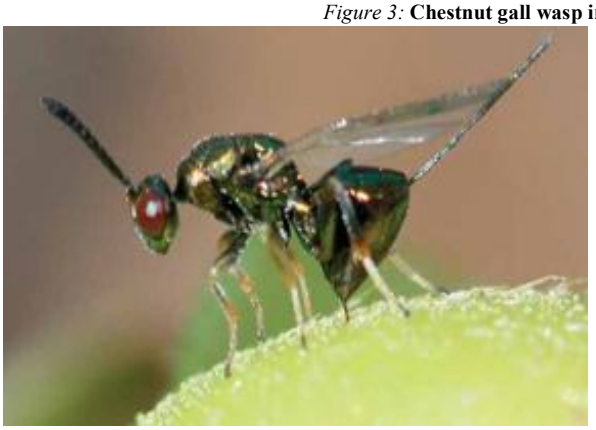
Biological Control

Using the parasitoids that can parasitize the chestnut gall wasp gall larvae is the best way for biological control. After winter pruning, preserving the dry gall and transferring them to a cage mask, which can keep lots o the natural enemies, then placing them into chestnut orchard in April to May next year. After all parasitoids are entirely emerged, the dry galls are burned (Huang et al., 1988, Guo and Liu, 1992, Tong et al., 2005).

Chemical Control

It is proved that spraying systemic insecticides such as methamidophos and omethoate is good at adult control of chestnut gall wasp. It can be used from the start to the top peak period with a 6-7 days interval. Totally two to three continuous times can significantly reduce the insect population density the next year. Meanwhile in the red stage of the buds to the stage of leafing of chestnut, injecting 1 ml the systemic insecticides into a hole, totally 3-6 holes depending on the diameter of stem, can prevent and treat the overwintering larvae in shoots. In some experiments the results showed also brushing branches using 50% methamidophos, 40% omethoate or phloem after the bark spread with 40% water solution had good effect to control gall wasp larvae in chestnut. These methods were basically non-injury to chestnut, and also effective to control other pests with piercing-sucking mouth parts on chestnut trees (Tang et al., 1991, Liu et al., 2000)

Figure 3: **Chestnut gall wasp in Italy, Europe**



(Photos: G. Brussino)

Figure 4: **Galls on chestnut branches in Italy and in China**

(Photos: G. Brussino and G. Tarcali, 2009)

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New data on the appearance of rape stem weevil (*Ceutorhynchus napi* Gillenhal 1837) in oilseed rape in Hungary

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SUMMARY

There is insufficient professional information on the rape stem weevil in Hungary. According to the general source of agricultural entomology in Hungary (Manual of the plant protection zoology) its damage is rare in Hungary. This paper summarizes the most important information of the technical literature and shows the results of recent investigations on the occurrence and damage of C. napi in the centre of Hungary. On the basis of these the rape stem weevil occurred commonly in the spring of 2009 in the fields of Kisbag, Domony, Bag, Hévízgyörk and Galgahévíz (Pest county), its frequency was high and low in the rape. Regarding this experience, the continually developing oilseed rape growing and former Hungarian observations, Ceutorhynchus napi occurred commonly in Transfanubia and in the north and centre of Hungary, consequently its damage can be frequent. Comparing the results of our former and present paper to the information of the Hungarian entomological references, it seems to be worth following with attention the presence and damage of C. napi in Hungarian rape fields because either the former information on the pest based on few data was not exact or the rape stem weevil must be spreading.

Key words: *Ceutorhynchus napi*, rape stem weevil, Curculionidae, oilseed rape, damage, Hungary

INTRODUCTION

Occurrence, importance: Its distribution area is large, it can be found in the north of Africa and in the whole territory of Europe. *C. napi* is a native species, it occurs often in Hungary (Marczali, 2006). Sáringer (1990) reported *C. napi* is an important pest of rape in Western and Northern Europe but its damage is rare in Hungary, thus the Hungarian agricultural literature did not deal with it. The judgement of importance of *C. napi* is not uniform either in Western Europe because it is thought as a considerable pest of cabbage in Germany (Jancke 1953 in Keilbach, 1966) but others reported that *C. napi* attack the well developed, vital oilseed rape plants and damages them strongly (Günthart 1949 in Marczali, 2006). In France experts esteem it besides pollen beetle as one of the key pest of oilseed rape (Lerin 1988 in Marczali, 2006). In spite of these, there is an interesting Germen observation: *C. napi* causes but a small damage unless the shoots break because of the strong wind or they are rotted by wet weather (Schmidt, 1962).

Morphology: Body length of adults is about 3.2-4.1 mm. Elytra are black leaden ornated with fine stripes separated by large intervals, covered in short greyish hairs. Length of rostrum amounts one third of the body length. When the weevil places the rostrum on the ventral surface of the body it reaches the coxa of the second leg. Elytra are vaulted, shoulder tubercles are explicit. Pronotum is laterally rounded. There is a tooth on each of the second and third femur. Larva is a yellow-whitish curculionid larva. Head capsule width of developed (L3) larva is about 0.83 mm, body length 6-8 mm. Head of the first and second larval stage is blackish, that of the third yellow. Pupal type is a yellowish *pupa libera* with 3.4 mm length. Eggs are whitish, length 0.65 mm (Anonim, 2007a, Keilbach, 1966, Schmidt, 1962).

Host plants: Adults feed on various Brassicaceae but they lay eggs only on oilseed rape, cabbage, turnip and *Sysimbrium officinale* Linnaeus (Schmidt, 1962, Keilbach, 1966, Marczali, 2006). According to German references, females prefer for oviposition mainly oilseed rape and turnip and eggs are laid on cabbage only in lack of the preferred plants (Keilbach, 1966).

Development: *C. napi* has one generation a year, adults overwinter in the soil of the oilseed rape field of previous year. Adults start leaving their winter refuge prepared in the upper soil layer when soil temperature reaches 7 °C. Mass swarming occur if *soil temperatures* are above 9 °C. Adult colonization into the rape stand needs al least 9 °C air temperature, which becomes intensive above 12 °C. This occurs generally in the middle of March. Colonisation of the weevils can be followed well with yellow pans. Warm weather with much sunshine is extraordinarily favourable for the beetles. For controlling *C. napi* in oilseed rape the 12-14 day interval after the appearance of the first weevils is optimal (Schmidt, 1962).10-20 days feeding and copulation after the appearance of the first beetles at 18 °C females lays eggs one by one into the rape stem. Eggs are laid exactly into the pith of the main shoot immediately into the part below the apical bud, but just into the lower offshoots. The number of eggs is 12-60 per female. The duration of embryonic development is 6-20 days. Larvae live and feed in the shoot until finishing their development (32-47 days). At that time (in May or June) at the level of the lower leaf the larva bores a hole in the shoot, drops to the soil surface and burrows *into the soil* to a maximum depth of 6 cm to form an overwintering chamber and develops to praepupa, pupa finally adult. Weevils diapause till the end of autumn, leave the soil chamber and remained in the soil until next spring (Jancke, 1953 in Keilbach,,1966, Günthart, 1949 in Marczali, 2006, Schmidt ,1962).

Damage: There are various ideas about the cause of the damage. First a stab trace, later a short fissure on the rape shoot appears as a consequence of oviposition. Both, main and offshoots thickened and become deformed. Kelbach (1966) thought substance produced by the female at oviposition may cause this deformation and thickening. Kazda (1958 in Sáringer, 1990) reported bacteria from the ovipositor of female get into the shoot and they are responsible for the deformation. Le Pape et Bronner (1987 in Marczali, 2006) disprove this, because on the basis of their data, the structure of deformity caused by *C. napi* differed from the cells of neoplasm triggered by insects, because these contained much normally operating cells besides those with pathological activity. The same authors also stated that stabs without eggs caused similar symptoms. They proved as well that stem weevil females did not secrete any substance at oviposition. They concluded the deformity developed as a result of the defensive, the wound covering mechanism of the plant. The shoot split long after one or two weeks and lost the stability. A shoot like that cannot keep the weight of plant and curves with deformity. The curved shoot rot and the yield will be lost. The early withering is the consequence of *Phoma* infection of split stem (Anonym, 2007a). The yield loss can reach 70% in dry years (Anonym, 2007b).

Control: Cultural control: The most important preventive method is crop-rotation (Anonym, 2007b).

Natural enemies: *Phaonia trimaculatus* Bouché (Tachinidae), *Tersilochus moderatus, Tersilochus fulvipes* Gavenhorst (Ichneumonidae) are important endoparasitoids of *C. napi* larvae. 0.99% of parasitosis was found in 13000 larvae in Poland (Anasiewicz, 1978 in Marczali, 2006). The rate of parasitosis amounted 18,5-50,3% in Germany (Klingenberg és Ulber, 1994), but *T. fulvipes* parasitized 76% of the larvae investigated (Kraus és Kromp, 2002). Parasitoid infection of *C. napi* larvae with *Tersilochus* spp. can reach 95% in France and 81% in Austria (Alford, 2000). Except parasitoids also predators and nematodes can diminish larvae and pupas of *C. napi* I the soil. These can be the carabids and staphylinids as well as species of the genera *Heterorhabditis* and *Steinernema* (Alford, 2000).

Chemical control: Insecticide active ingredients proposed by EPPO are as follow here: cypermethrin, deltamethrin, esfenvalerate, fenvalerate, lambda-cyhalotrin and permethrin. It is worth choosing chemical control if the number of adults in four yellow pans during 3 days are above 25 (Anonim, 2007b).

MATERIALS AND METHODS

Information on the localities and the survey are showed in *Table 1*.

Table 1

Characteristics of the sites and the oilseed rape stands in Pest county (2009)

Sites	Geographical position	Field size (ha)	Time of survey	Average plant height (cm)	Developmental stage BBCH (Meier, 2001)
Kisbag	47°38′06,35″ N 19°27′17,88″ E	82	05.01.	123.7	67
Domony	47°38′57,40″ N 19°27′04,01″ E	10	05.01.	117.0	67
Bag	47°38′25,41″ N 19°28′36,76″ E	39	05.01.	134.2	69
Hévízgyörk	47°38′09,39″ N 19°31′57,85″ E	6.1	05.15.	125.1	68
Galgahévíz	47°37′45,69″ N 19°33′22,13″ E	6.3	05.15.	124.9	73

Survey method: Four times 20 plants were examined from the field border diagonally, along a transect. Plants were chosen randomly after making five steps. There were 50 steps between repetitions. The chosen plants were categorized as follows here:

1. intact plant
2. stabbed plant (a stab or short fissure on the shoot; damage value 1)
3. split plant (a fissure of 5-10 cm on the shoot, the pith can be seen; damage value 3)
4. curved plant (length of fissure is longer than 15-20 cm, the shoot is curved in form of U or S; damage value 5)

Except of the plants showing the symptomes of *C. napi* damage every fifth plant was slit in order to constat the presence of larvae.

Evaluation: *C. napi* infection of the fields was compared on the basis of the number of damaged plants and the sum of damage values per field. Data were examined by one way ANOVA (Sváb, 1980). The least significant difference (LSD) was calculated by Tukey test (Armitage, 1971).

RESULTS AND DISCUSSION

Results are presented in *Table 2*. According to the results the occurrence and damage of *C. napi* was important only in Galgahévíz. Frequency of damage and damage value were merely negligible in the other fields. The damage was found on 24% of the plants observed. This means that the symptomes were found on almost every fourth plant in the rape field of Galgahévíz. Larvae of rape stem weevil were found in each damaged plant shoot.

Table 2

Number of damaged plants and the measure of damage in Pest county (2009)

Sites	Number of damaged plants (%)	Sum of the damage value
Kisbag	0.00 (0.0)	0.00
Domony	0.00 (0.0)	0.00
Bag	0.25 (1.2)	0.25
Hévízgyörk	0.25 (1.2)	1.25
Galgahévíz	4.75 (23.7)	15.500
LSD	2.824***	8.297***

*** ANOVA computed significant difference between the values at $p < 0.05$. LSD calculated by Tukey test)

Dwarfism of plants did not occur. Dead, dried plants or perishing plants have not been found. Further survey would has been needed to estimate the final damage in terms of the yield but our aim was simply to assess the presence and frequency of damage of stem weevil this occasion. According to our survey *C. napi* occurred in one of the studied oilseed rape fields in Pest county, where its frequency and damage value were considerable. Consistent with our results in 2009, 2008 (Bozsik, 2008), 2007 (Bozsik et al., 2007) (*Table 3 and 4*), former observations of Szarukán in the north of the Great Hungarian Plain (I. Szarukán, 2007, unpublished data), the experiences of Farkas (I. Farkas, 2008, unpublished data) as well as the examinations of Marczali (2006) (*Table 5*), rape stem weevil must commonly occur in northern Hungary, in Pest county and in Transdanubia, and its harm can be extensive in the country. However, there was a remarkable difference between the severity of the symptomes of the surveys realized in different years showing that environmental factors and perhaps the intensivity of plant protection practice (e.g. crop rotation, chemical control) can infuence heavily the occurrence of the pest.

Table 3

Number of damaged plants by C. napi and the measure of damage (Újfehértó, Érpatak, 2007) (Bozsik et al., 2007)

Sites	Number of damaged plants (%)	Sum of the damage value
Újfehértó	10.00 (50)	41.00
Érpatak1	9.00 (45)	36.00
Érpatak2	6.00 (30)	23.50
$SD_{5\%}$	5.160	22.680

Table 4

Number of damaged plants by C. napi and the measure of damage in Pest (PM) and Hajdú-Bihar (HM) counties (2008) (Bozsik, 2008)

Sites	Number of damaged plants (%)	Sum of the damage value
Kisbag1 (PM)	8.00 (40)	31.5
Kisbag2 (PM)	2.00 (10)	8.00
Domony (PM)	0.00 (0,0)	0.00
Galgahévíz (PM)	2.25 (11,2)	8.25
Kismacs1 (HM)	1.25 (6,2)	3.00
Kismacs2 (HM)	0.50 (2,5)	0.75
LSD	2.875***	9.876***

*** ANOVA computed significant difference between the values at $p < 0.05$. LSD calculated by Tukey test)

Table 5

Dominance values (%) of *Ceutorhynchus* spp. in oilseed rape (Keszthely Újmajor) (Marczali, 2006)

Year of occurrence	*C. pallidactylus*	*C. obstrictus*	*C. napi*	*C. pleurostigma*
1999	47	43	9	1
2000	41	52	7	1
2001	43	46	10	1
2002	48	41	10	1

In addition, it seems to be worth following with attention the presence and damage of *C. napi* in Hungarian rape fields because either the former information on the pest based on a few data was incertain or populations of the rape stem weevil must be spreading. In case of the second case the main cause of spreading and damage of this pest can be either the increase of oilseed rape sowing area as well as the lack of crop rotation and proper plant protection practice.

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Recent questions on the plant invasions – an overview in international and Hungarian aspect

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SUMMARY

This article contains information on plant invasions as an overview in international and Hungarian aspects. International activity on invasive plants of the European Commission, the Council of Europe and the European and Mediterranean Organization are presented. The main results of the Hungarian national monitoring programmes on invasive plant species are outlined. Plants that cause the most significant nature conservation and economy risk effects in Hungary are presented. Finally the most important Hungarian books on invasive plants are mentioned.

Keywords: plant invasion, Hungary, Asclepias syriaca, Cyperus esculentus var. leptostachyus, Fallopia×bohemica, Senecio inaequidens.

PLANT INVASIONS

Nowadays biology invasion is considered as one of the factors, which mostly endanger the biodiversity. Prevention on biological invasion and control against invasive species are stressed for nearly all of habitats around the world.

Based on the widely used international definition according to Botta-Dukát et al. (2004) neophytes are adventive plant species, which appeared in Europe after the great geographical discoveries (1492). Consider as an invasive plant species, their distribution area and population size are rising monotonous on their suitable habitats, in given place- and timescale. Inside the invasive plant species the transformer plant species cause significant change in the structure (species content, physiognomy) as well as function of the conquest community during their invasion.

Long tradition provides the fight of prevention and control against invasive species in New-Zealand, Australia and the North-American countries. These countries apply regulation on invasive species, which based on pest risk assessment. In Europe the fight against invasive species has beginning later than in the mentioned countries. The base framework of control against invasive species is presented by *European strategy on invasive alien species* (Genovesi and Shine 2003), which was published in Hungarian by the Ministry of Environment and Water (Genovesi and Shine 2007).

The communication of the European Commission and the European detailed impact assessment, which were published on 3 December 2008, are considered as milestone in control on invasive species in the European Union (EU 2008a, 2008b). The Commission presented four policy options for an EU Strategy on Invasive Species in its communication. A) Business as Usual. B) Maximising the use of existing legal instruments together with voluntary measures. B+) Adapted existing legislation. C) Comprehensive, dedicated EU legal instrument. According to the impact assessment of the European Commission the damage caused by invasive species and the necessary control measures are estimated as costing at least EUR 12 000 million annually, according to the available documented information.

Inside the Sixth framework of the European Commission was carried out the *Delivering Alien Invasive Species Inventories for Europe* (DAISIE) project, which was aimed at gathering all of information on the invasive species in Europe. Based on the surveys of the DAISIE project, the 100 of the most dangerous species list contains eighteen terrestrial invasive plants, in which eight species cause problems in Hungary: *Ailanthus altissima, Ambrosia artemisiifolia, Echinocystis lobata, Fallopia* section *Reynoutria, Heracleum mantegazzianum, Impatiens glandulifera, Prunus serotina, Robinia pseudoacacia*. *Handbook of alien species in Europe* (DAISIE 2009) presented comprehensive overviews on the aspect of biological invasions in Europe. According to the DAISIE data 11.000 alien species were determined in Europe. About 15 % of these alien species can cause economic damages and endanger the biological diversity, environment, habitats as well as native plants, animals and micro-organisms.

European and Mediterranean Organization established an ad hoc panel on invasive plants (EPPO IAS Panel) in 2002 for prevention caused damages by invasive plants. During the panel meetings more than thousand plant species were discussed. Sixty-four of them are listed on the EPPO List of invasive alien plants and the EPPO Alert List.

EPPO List of invasive alien plants contains thirty-nine plant species and twenty-eight of them are presented(#) in Hungary. Pest risk assessments were carried out for seven species(*): *.Acacia dealbata, Acroptilon repens, Ailanthus altissima*[#]*, Ambrosia artemisiifolia*[*#]*, Amelanchier spicata, Amorpha fruticosa*[#]*, Azolla filiculoides*[#]*, Baccharis halimifolia, Bidens frondosa*[#]*, Buddleja davidii*[#]*, Cabomba caroliniana*[*#]*, Carpobrotus acinaciformis, C. edulis, Cenchrus incertus*[#]*, Cortaderia selloana, Cyperus esculentus*[#]*, Egeria densa*[#]*, Elodea nuttallii*[#]*, Fallopia japonica*[#]*, F. sachalinensis*[#]*, F. × bohemica*[#]*, Helianthus tuberosus*[#]*,*

Heracleum mantegazzianum#, H. persicum*, H. sosnowskyi*#, Impatiens glandulifera#, Lagarosiphon major#, Ludwigia peploides#, L. uruguayensis#, Lupinus polyphyllus#, Myriophyllum aquaticum, Oxalis pes-caprae, Paspalum distichum, Prunus serotina#, Rhododendron ponticum, Senecio inaequidens*#, Sicyos angulatus*#, Solidago canadensis#, S. gigantea#.* Nineteen plants are listed on the EPPO Alert List, five of them are presented in Hungary *Eriochloa villosa* (Partosfalvi et al. 2008), *Fallopia baldscuanica, Humulus japonicus, Pistia stratiotes,* and *Salvinia molesta* (Király eds. 2009).

Recently six plant species are listed in EPPO A2 List, which contain pests that are locally presented in the EPPO region and recommended for regulation as quarantine pests. One of them, *Hydrocotyle ranunculoides* is occurred in Hungary too (Vidéki et al. 2008, Király eds. 2009).

In the course of the EPPO analysis the *Actual list of neophytes in Hungary, and their classification according their invasiveness,* titled list (Balogh et al. 2004) was considered as a Hungarian reference list.

EPPO published the *Guidelines for the management of invasive alien plants or potentially invasive alien plants which are intended for import or have been intentionally imported* (EPPO Bulletin 2006) titled article as a first guideline, which presented the fundamental principles for the management of invasive and potentially alien plants. *National regulatory control systems* provide control possibilities against pests. The first control system against invasive alien plants was published in the case of *Ambrosia artemisiifolia* (EPPO Bulletin 2008). In 2007 EPPO published the *Council recommendation on plants for renewable energy and Invasive Alien Plants,* in which it called attention to the member states for the risks of cultivated plants for renewable energy (EPPO 2009a). EPPO and the Council of Europe organised a workshop on *Codes of conduct on horticulture and invasive alien plants*, which is the first voluntary code of conduct in the fight against invasive plants in Europe. Participants recommended for the governments and national plant protection organizations to adapt it as a national code of conduct in their countries (EPPO 2009b).

In Hungary significant distribution of the invasive plants are presented by the following national programmes: Mapping of the Hungarian Flora Programme (Balogh et al. 2008), MÉTA Programme (Ecological Vegetation Database & Map of Hungary) (Botta-Dukát 2009) and the Fifth National Weed Survey on Arable Lands in Hungary (Novák et al. 2009). Based on the results of the MÉTA Programme the (semi-)natural habitats are covered by invasive alien plants at the rate of 13.1% (Botta-Dukát 2009). According to the last national weed survey on the arable lands in Hungary (2007-2008), which carried out on control (untreated) sample quadrants, the average cover of *Ambrosia artemisiifolia* has raised, the volume was 5.3% (Novák et al. 2009). According to the *Actual list of neophytes in Hungary, and their classification according their invasiveness* (Balogh et al. 2004) titled article seventy-one plants are invasive and thirty-three of them are transformers. The listed plants cause significant damages on (semi-)natural and/or agricultural habitats.

Nowadays, nature conservation and economy risk effect and distribution of *Asclepias syriaca,* which is one of the invasive listed plants, is getting increasing based on the results of the Hungarian Flora Programme and the last national weed survey on the arable lands. *Fallopia ×bohemica* is spreading significantly in settlements and their surroundings as well as along riverbanks and channels. Occurrences of *Cyperus esculentus* var. *leptostachyus* present disperse pattern in Hungary, so this species can became a wide spread weed. *Eriochloa villosa* (listed on the EPPO Alert List) was discovered near Miskolc (NE-Hungary) in 2007; further occurrence is expected in Hungary. *Senecio inaequidens* is a South-African of origin invasive plant, which was discovered in Hungary in 1998, since than this species is getting spreading near infested railway stations in Northwest Hungary (Bauer and Schmidt 2005). Its further distribution is expected in Central and Eastern Hungary. Nowadays *Senecio inaequidens* endanger for the native vegetation, and as a poison plant infests pastures and meadows.

The following books present detailed information for the management of invasive plants in Hungary based on the professional experience. *Serious 48 weed* titled book (Benécsné Bárdi et al. eds. 2005) comprises monographs on the most important agricultural weeds, included invasive species, providing guidelines for management of invasive alien plants. Two volumes of the *Biological invasions in Hungary, Invasive Plants* titled books (Mihály and Botta-Dukát eds. 2004, Botta-Dukát and Mihály eds. 2006) and the English edition of the most important monographs presents detailed information on each species (Balogh and Botta-Dukát eds. 2008).

In the fight against invasive plants in Hungary the frameworks of the *European strategy on invasive alien species* and the EPPO recommendations can be provided solution.

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Changes of allelopathy affected by water supply and temperature

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SUMMARY

Allelopathy of three noxious weeds (cocklebur – Xanthium italicum Mor., velvetleaf – Abutilon theophrasti Medic., jimsonweed – Datura stramonium L.) was studied used three test plant: cress (Lepidium sativum), maize (Zea mays) and sunflower (Helianthus annuus).Weed species were grown under controlled conditions watered on 4 different levels. Dried and ground shoots and roots of weeds were used in bioassays. Differences of water supply and temperature modified the effects of the three weeds on test plant on differ way depending on species of donor plants and test plants. Effects of cocklebur shoot extracts ranged from 88% inhibition to 1% insignificant effect in case of cress, from 46% inhibition to 1% insignificant effect in case of maize, from 41% inhibition to 9% insignificant effect in case of sunflower, effects of root extracts ranged from 50% inhibition to 169% stimulation in case of cress, ranged from 75% inhibition to insignificant values in case of maize and from 84% to 34% inhibition in case of sunflower depending on water supply and germinating temperature. Effects of the other two weed species were observed wide bounds, as well. Sensitivity of maize increased mostly when temperature was lower. Surges of allelochemicals in samples were also influenced by water supply of donor plants, however in most part of samples there was ineffective amount of them in case of certain compound.

Keywords: allelopathy, cocklebur, velvetleaf, jimsonweed, maize, sunflower, cress

INTRODUCTION

Most of spreading, difficult to control weeds seem to be allelopathic and it supposed to play determinant role in their excellent competitiveness (Mikulás 1979; Béres and Kazinczi 2000; Béres et al. 2003; Dávid et al. 2005; Buzsáki et al. 2008).

However, real role of allelopathy is controversial in competition of plants and in non-herbicidal weed management methods. One of main reason of antinomic estimation of allelopathy is its changeability: allelopathy of plants or microorganisms expresses in different degree and manner in several experiments.

Rice (1964) called attention to several factors which can modify allelopathical relationship of plants and microorganisms. He found that age of plant parts influence their allelophatic effects depending on species. He observed differences in allelopathy of plants falling within the same species depending on phenological stages, and found differences when there was no inequality in maturity. In these cases he supposed that environmental factors can modify allelopathy.

According to Kazinczi et al. (1991) main factors which can influence effect of allelopathy are used plant parts (leaf, stem or root), maturity of plant parts, examined live process, plant species (perhaps cultivars).

Dias and Dias (2000) examined effect of drought stress on expressing of allelopathy in case of jimsonweed as donor and cucumber as test plant. Differences were found among effect of plants grown under different water supply levels, furthermore age of plant parts proved to be determinant in amounts of several allelochemicals.

Casini (2004) examined allelopathic effect of cocklebur extracts on maize hybrids, which react differ to the same treatments. Maize hybrids showed different sensitivity against water extracts of residues, however, the order of sensitivity was different in case of root exudates.

Szabó (2000) proposed to unify experimental methods to decreased methodical differences and make better reproducibleness of experiments.

A uniform methodology may make better reproducibleness of experiments, but it must be based on knowledge of main factors which can effect on allelopathy.

MATERIALS AND METHODS

Allelopathy of *Xanthium italicum* Mor., *Abutilon theophrasti* Medic. and *Datura stramonium* L. was examined. Donor plants were grown in greenhouse in pots on sandy soil in August and September in 2008. Soil of plant was kept on 70% (treatment 1, 5, 9, 13, 17, 21), 50% (treatment 2, 6, 10, 14, 18, 22), 30% (treatment 3, 7, 11, 15, 19, 23) of minimum water capacity or dried until unavailable water content of soil in case of treatment 4, 8, 12, 16, 20, 24 (*Table 1.*). Shoots and roots were collected separatedly at 4 or 5 leaves stage of plants. Collected plant samples were stored frozen until drying at 50^{o}C then samples were stored cooled until use. Extracts were made of 2,5g dried and ground shoot or root in 100 ml water. Extraction lasted 24 hours at 21^{o}C in darkness. Bioassays were conducted in Petri dishes (diameter 9cm) with cress (*Lepidium campestre*) (50 seeds in a dish) and maize (*Zea mays* L.), sunflower (*Helianthus annuus*) (30 seeds in a dish) under 12 hours light /12 hours dark light regime. In one case bioassays were conducted at temperature of 20^{o}C (18^{o}C under dark

period/22°C under light period) and in other case at temperature of 10°C (8°C under dark period/12°C under light period). Root and shoot growth of test plants were measured.

Besides germination trials, we followed-up the quantitative changes of some allelochemicals: coumarin, p-coumaric acid, trans-cinnamic acid, chlorogenic acid, ferulic acid, 2-phenilpropionic acid, 4-hydroxybenzoic acid.

For the quantitative determination of the 4 compounds we prepared an extract from dried crop samples with distilled water, using a sample of 2.5 g and distilled water of 100 cm^3, which was shaken for 2 hours. After filtering, the agents were identified with Merck-Hitachi HPLC equipment. The circumstances of separation were the following:

column: Lichrospher 100RP-18, 125x4mm;

12:15:1 mixture of eluent: water: methanol: acetic acid;

flow: 1ml/minute.

Detecting was performed with a L-4500 Diode Array Detector at a wave length of 275 nm. For quality identification we used a comparative solvent liquid containing coumarin, p-coumaric acid, trans-cinnamic acid, chlorogenic acid, ferulic acid, 2-phenilpropionic acid, 4-hydroxybenzoic acid (SIGMA-ALDRICH).

The quantitative identification of allelochemicals took place in the Institute of Food Processing, Quality Control and Microbiology, Faculty of Agronomy, University of Debrecen.

Table 1

Treatments in bioassays

Abbr. of treatments		Treatments
0	Control	Water
1	XS70	Shoot extract of cocklebur grown at 70% of minimum water capacity
2	XS50	Shoot extract of cocklebur grown at 50% of minimum water capacity
3	XS30	Shoot extract of cocklebur grown at 30% of minimum water capacity
4	XSuw	Shoot extract of cocklebur grown in soil dried near to unavailable water content
5	XR70	Root extract of cocklebur grown at 70% of minimum water capacity
6	XR50	Root extract of cocklebur grown at 50% of minimum water capacity
7	XR30	Root extract of cocklebur grown at 30% of minimum water capacity
8	XRuw	Root extract of cocklebur grown in soil dried near to unavailable water content
9	AS70	Shoot extract of velvetleaf grown at 70% of minimum water capacity
10	AS50	Shoot extract of velvetleaf grown at 50% of minimum water capacity
11	AS30	Shoot extract of velvetleaf grown at 30% of minimum water capacity
12	ASuw	Shoot extract of velvetleaf grown in soil dried near to unavailable water content
13	AR70	Root extract of velvetleaf grown at 70% of minimum water capacity
14	AR50	Root extract of velvetleaf grown at 50% of minimum water capacity
15	AR30	Root extract of velvetleaf grown at 30% of minimum water capacity
16	ARuw	Root extract of velvetleaf grown in soil dried near to unavailable water content
17	DS70	Shoot extract of jimsonweed grown at 70% of minimum water capacity
18	DS50	Shoot extract of jimsonweed grown at 50% of minimum water capacity
19	DS30	Shoot extract of jimsonweed grown at 30% of minimum water capacity
20	DSuw	Shoot extract of jimsonweed grown in soil dried near to unavailable water content
21	DR70	Root extract of jimsonweed grown at 70% of minimum water capacity
22	DR50	Root extract of jimsonweed grown at 50% of minimum water capacity
23	DR30	Root extract of jimsonweed grown at 30% of minimum water capacity
24	DRuw	Root extract of jimsonweed grown in soil dried near to unavailable water content

X: cocklebur extract, A: velvetleaf extract, D: jimsonweed extract, S: shoot extract, R: root extract, 70: plants grown at 70% of minimum water capacity of soil, 50: plants grown at 50% of minimum water capacity of soil, 30: plants grown at 30% of minimum water capacity of soil, uw: soil of plants were dried until unavailable water capacity.

RESULTS

Affects of extracts on cress

Inhibitory affects of shoot extracts of cocklebur on cress became stronger as donor plants were exposed to drought stress: inhibition was 50% in case of extracts of cockleburs grown at 70% of soil minimum water capacity, but extract of cockleburs of which soil were dried near to unavailable water content reduced growth of cress by 88% compared to control plants at germination temperature of 20°C. In case of 10°C germination temperature similar tendency was observed, however, the affect was slighter: Shoot extracts of plant watered abundantly did not inhibit growth of test plants, and plants dried to unavailable water content of soil decreased growth by 65% (*Figure 1*).

Cocklebur's root extracts inhibited growth of cress at 20°C in case of treatments XR70 and XRuw, but XR50 and XR30 extracts did not have significant effect on that. At the lower germination temperature extracts of donor plants watered well stimulated growth of test plants by 60-169%, but similar effect was not observed in case of plants exposed to drought stress.

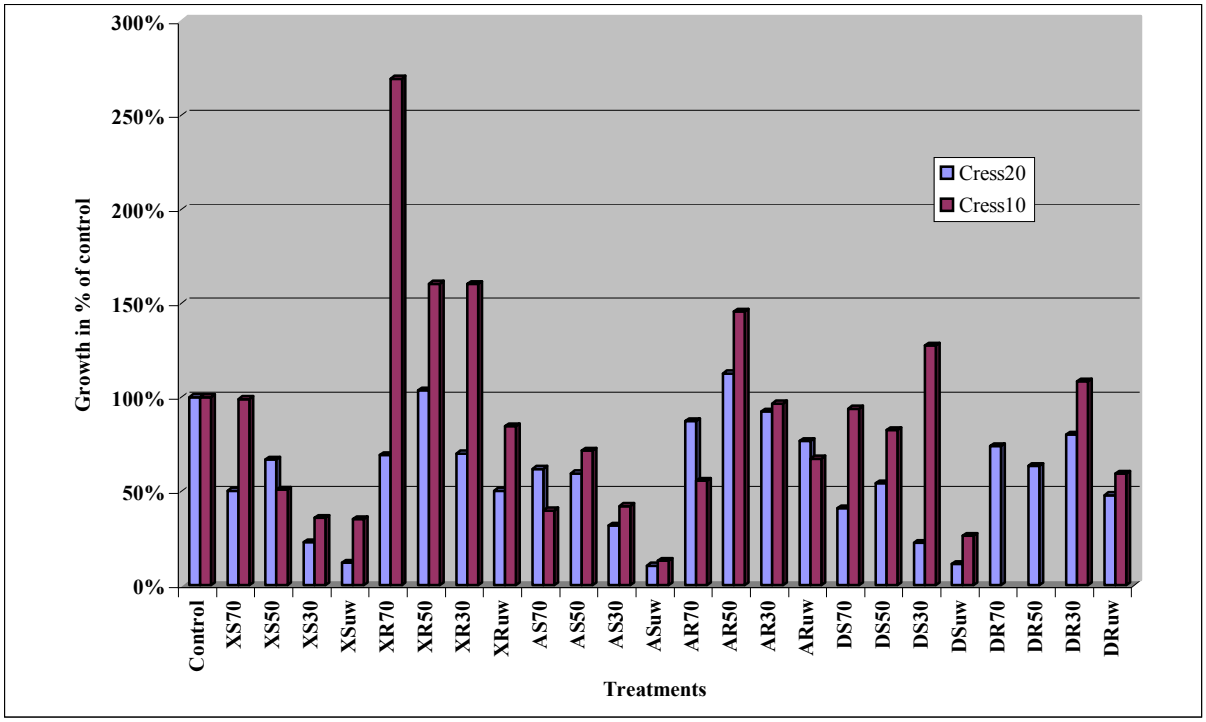
Velvetleaf's shoot extracts showed stronger and stronger inhibitory effects as lack of water of donor plants increased at 20°C. At the lower temperature similar effects were observed, except extracts of plants grown at 50% of minimum water capacity, which did not have significant inhibitory affect.

Root extracts of velvetleaf did not influence growth of cress.

Jimsonweed's shoot extracts, like the two other weed species, showed stronger and stronger inhibitory effects (59-89% inhibition) as lack of water of donor plants increased at germination temperature of 20°C. At 10°C the same extracts did not have significant inhibitory effects, except extracts of plants exposed to the strongest drought stress, which decreased growth of test plants by 74%.

In case of root extracts of jimsonweed DR50 and DRuw treatments inhibited growth of cress significantly (by 37 and 52%) germinated that at 20°C.

Figure 1: **Effects of extracts of weed species on cress**



Cress10: cress germinated at 10°C, Cress20: cress germinated at 20°C. DR70 and DR50 (Maize10) are missing values. LSD5% (Cress10): 47%, LSD5% (Cress20): 31%.

Affects of extracts on maize

Growth of maize was inhibited by extracts of shoots of cockleburs exposed to extreme drought stress by 26%, but other ones did not reduced it at 20°C, however, inhibitory effects were 32-46% at germination temperature of 10°C, except treatment XS70 (*Figure 2*).

In case of cocklebur root extracts the inhibition was about 40% - except extracts of plants grown on 50% of minimum water capacity of soil – at 20°C. At 10oC germinating temperature inhibitory effects were 47-75% depending on water supply of donor plants.

Velvetleaf shoot extracts did not have significant affects on maize germinated at 20°C, however, every extracts inhibited growth of maize at 10°C, AS70 and ASuw treatments had the strongest inhibitory effects (62-64%).

Similar effects were observed in case of root extracts of velvetleaf. The strongest inhibition (75%) belonged to velvetleaf plants grown on soil dried to unavailable water.

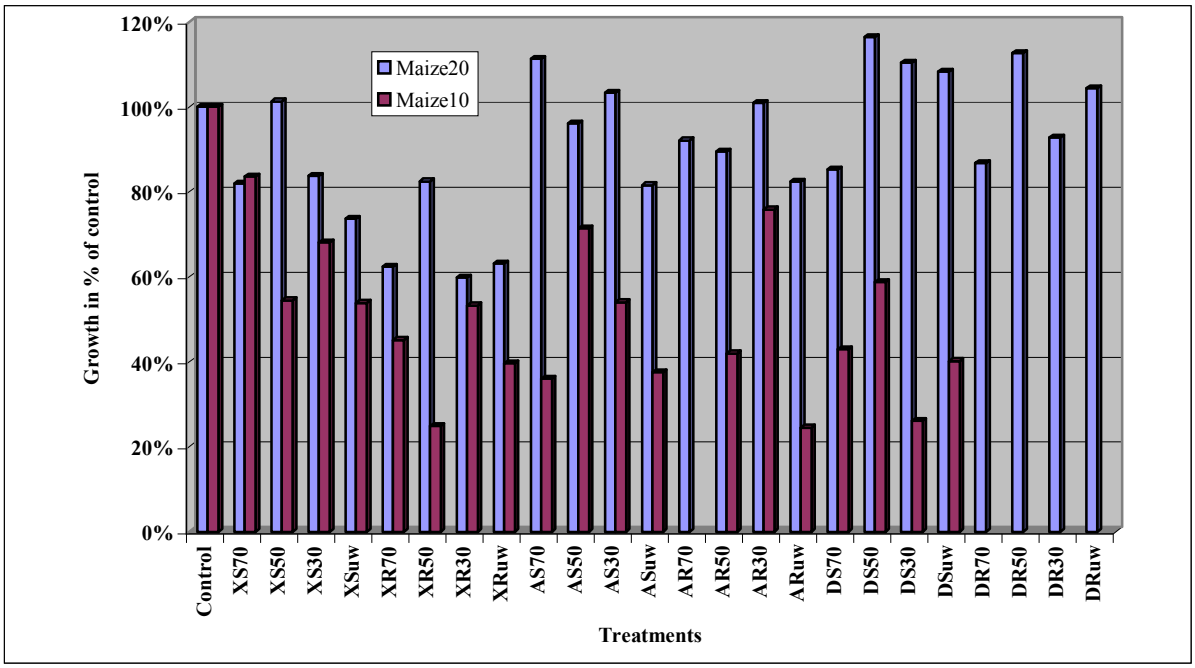
Jinmsonweed shoot extracts did not have significant affects on maize germinated at 20°C, however, every extracts inhibited growth of maize by 41-71% at 10°C, the strongest inhibition was observed in case of treatment DS30. Root extracts of jimsonweed did not influence significantly the growth of maize at 20°C.

Affects of extracts on sunflower

Inhibitory affects of extracts of cocklebur shoots ranged from 19% to 41% at 20°C, and slightly stronger inhibition was observed in case of donor plants which were grown drier conditions. Inhibition (34%) was

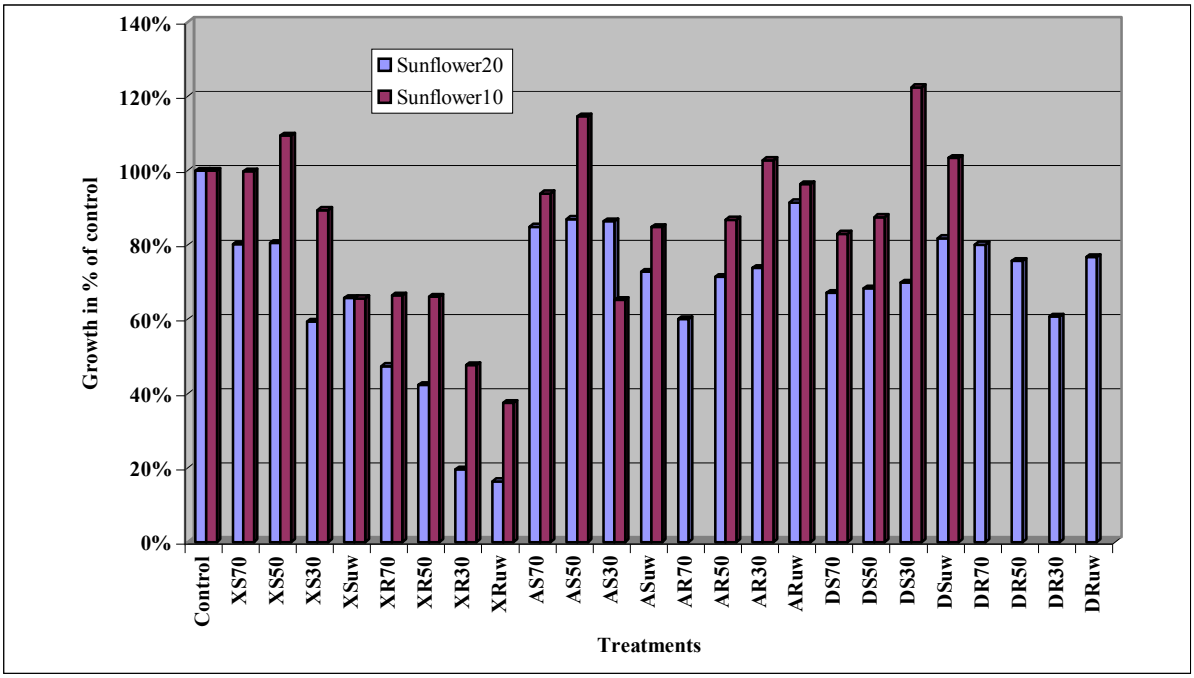
observed only in case of cockleburs which were exposed to extreme drought stress when sunflower germinated at 10°C (*Figure 3*).

Figure 2: **Effects of extracts of weed species on maize**



Maize10: maize germinated at 10°C, Maize20: maize germinated at 20°C. AR70, DR70, DR50, DR30, DRuw (Maize10) are missing values. LSD5% (Maize10): 19%, LSD5% (Maize20): 22%.

Figure 3: **Effects of extracts of weed species on sunflower**



Sunflower10: sunflower germinated at 10°C, Sunflower20: sunflower germinated at 20°C. AR70, DR70, DR50, DR30, DRuw (Maize10) are missing values. LSD5% (Sunflower10): 16%, LSD5% (Sunflower20): 16%.

Root extracts of cockleburs caused stronger and stronger inhibitory effects on sunflower when drought stress of donor plants increased at both of examined germinating temperature. The effects were stronger at the higher one (53-85%).

Velvetleaf shoot extracts inhibited growth of sunflower only in case of extreme lack of water of donor plants at 20°C, and at 10°C only AS30 treatment had inhibitory effect.

Root extracts of velvetleaf plants – except ones which were exposed to the strongest drought stress – reduced growth of test plants by 30-33% at 20°C, but none of extracts had significant inhibitory effects when sunflower were germinated at 10°C.

Jimsonweed shoot extracts had similar inhibitory effects on sunflower (18-33%) at 20°C, but those extracts did not decreased significantly growth of test plant at 10°C, except one which was made from jimsonweed plants watered abundantly (treatment DS70).

Root extracts of jimsonweed inhibited sunflower growth by 20-39% at 20°C.

Surges of allelochemicals influenced by water supply

Amounts of allelochemicals ranged between wide bounds in samples of weed species affected by different level of drought stress. P-coumaric acid was detected in every sample in concentration of 0.036-4.146, 0.487-0.616, 3.301-3.470, 0.123-0.685, 0.159-0.288, 0.159-0.288 in shoot/root samples of cocklebur, shoot/root samples of velvetleaf, shoot/root samples of jimsonweed, respectively. 2-phenylpropionic acid was found in all three weeds but not in every samples. Ineffective quantity of allelochemicals were measured in many samples, but significant surges were observed in another ones (*Table 2*).

Table 2

Minimum and maximum amounts of several allelochemicals in weed samples affected by water supply (mg/100g dry matter).

	Cocklebur		Velvetleaf		Jimsonweed	
	shoot	root	shoot	root	shoot	root
Coumarin	0	0	0.008-0.054	0.208-0.240	0	0
P-coumaric acid	0.036-4.146	0.487-0.616	3.301-3.470	0.123-0.685	0.159-0.288	0.159-0.288
Trans-cinnamic acid	0	0-0.047	0.252-0.562	0	0.003-0.028	0
Chlorogenic acid	0-1.196	0-2.452	0	2.219-2.967	0-0.262	0-0.912
Ferulic acid	0-1.954	0	0	0	0-0.018	0
4-hydroxybenzoic acid	0.037-0.736	0.013-0.697	0	0	0	0-0.015
2-phenylpropionic acid	0-5.578	0.065-11.656	0-1.213	2.329-8.516	0-0.912	0-2.930

CONCLUSIONS

Effects of the three weed species were different in many cases and sensitivity of test plants was also different against certain treatments.

Responses of cress on treatments with shoot extracts showed increasing inhibitory effects as donor plants were exposed to stronger and stronger drought stress, however, such a clear tendency could not be observed in case of the two other test plants.

Generally, root extracts did not inhibit growth of test plants as strong as shoot extracts of the same donor plants, but in some cases (e.g. in interactions of cocklebur and sunflower) affects of them were stronger.

Maize was the most sensitive test plant against low temperature (it could be observed in slow growth of seedlings, as well), and the same extracts had stronger inhibitory effects on that test plants germinating at 10°C than at 20°C.

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Efficacy of herbicides influenced by spray carrier water pH and hardness

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SUMMARY

Field experiments were conducted to study affects of pH and hardness of spray water on efficacy of some herbicides influenced by several pH adjusters and adjuvants in Debrecen, Hungary in 2008 and 2009.

Favourable or unfavourable effects of pH and hardness of spray water could be observed under field conditions. Evaluation of weed control efficacy is suitable for examination of affects of spray water pH and hardness on herbicides.

Sensitivity of three herbicides was significantly different against the pH and hardness of spray carrier: mezotrione did not respond to hardness significantly and was only slightly affected by pH, the efficacy of nicosulfuron was influenced moderately by pH and hardness, and terbutilazine responded to either pH or hardness spectacularly.

Certain pH adjusters (e.g. ammonium nitrate) can lessen harmful affects of water hardness effectively.

Significant loss of efficacy of sensitive herbicide was found in hard water (by about 50-60%), and surfactants was not able to eliminate that harmful affect. However, biological activity was the same as in soft water with ammonium nitrate which can overcome antagonism of salts. That pH adjuster had a more significant affect on the efficacy of the herbicide than surfactant had in that experiment.

Keywords: herbicide, pH, hardness, pH adjuster, adjuvant, nicosulfuron, mezotrione, terbutilazine

INTRODUCTION

Herbicides come in contact with several salts and another compounds in spray carrier water, which may influence biological activity of them. Regarding of those factors contribute to make better efficacy of herbicides, however, disregarding of them lead to less success of herbicidal treatments in many cases. Interactions of herbicides, dissolved salts and pH of spray carrier depend on chemical properties of herbicides, amount and kind of salts which may determine pH of spray carrier.

A lot of kinds of herbicides are weak acids, and pH can determine which form of them exists in spray carrier: protonated, neutral form of herbicide is predominant when the pH is below the pK_a, however, lots of herbicides dissociate at an increased pH, and exist in ionic form (Green and Hale 200;, Gronwald et al. 1993; McMullan 1996). It depends on dissociation constant (pK_a) above which pH herbicides are ionic. pK_a values of many herbicides range from 3 to 5 (e.g. glyphosate, bentazon, picloram, dichlorprop, acifluorfen, imazethapyr, clorimuron, phenoxy acetic acids, cyclohexanediones), so dissociation happens at a mild acidic pH of spray carrier (Gronwald et al 1993). Dissociation constant of sulfonylurea herbicides also is in the acidic range, pK_a values of them range from 3.3 to 5.2. Those compounds are in ionic form at a neutral pH or slightly below that, as well. Some herbicide do not dissociate with an increasing pH, e.g. aryloxyphenoxypropionates, which are ester compounds and are unable to ionize unless the ester linkage is hydrolyzed. Changes of pH do not influence them (McMullan 1996).

Neutral form of herbicides is more favourable to diffuse into cuticle, penetrate cell wall and plasma-membrane: penetration is faster and higher proportion of herbicides is taken up (Green and Hale 2005; Gronwald et al. 1993; Liebl et al. 1992). Water solubility of protonated herbicides is low, but that becomes better spectacularly as pH increases: the water solubility limit of nicosulfuron is 360 ppm at pH5, 12200 ppm at pH 6.9 and 29200 ppm at pH 8.8. Lots of herbicides become more soluble, and crystallization and forming residual deposits are not as likely as in neutral form. However, the negative charge if ionized herbicides slows penetration through the lipophil cuticle, and the negatively charged cell wall (Green and Hale, 2005). In that case adjuvants are necessary to fast penetration.

According to Green and Cahill (2003) nicosulfuron dissolves rapidly under alkaline conditions, then its biological activity increased with crop oil concentrate and hydrophilic nonionic surfactants. Similar phenomenon were observed by Woznica et al. (2003) in case of quinclorac: it dissolved slightly in water without a pH adjuster retaining a cloudy suspension, but the mixture was clear with triethanolamine, indicating that the herbicide had completely dissolved. In case of several herbicides a slightly acid spray carrier proved to be optimum to penetrate (Green and Hale 2005; Gronwald et al. 1993; McMullan 1996). Green and Hale (2005) examined solubility and biological activity of water-dispersible granule formulation of nicosulfuron influenced by pH. Adding to spray carrier K_3PO_4 or K_2HPO_4 they got an alkaline buffer, in which the herbicide dissolved completely. That treatment enhanced activity of nicosulfuron, which could be increased adding adjuvants with 13-17 HLB (hydrophilic-lipophilic balance) values. When pH was reduced below the pK_a value by H_3PO_4, the herbicide converted into its neutral form, but was not precipitated at either rate. In the latter case HLB optimum values of adjuvants ranged from 10 to 15.

Spray carrier pH also influence stability of sulfonylureas. Half-life times of protonated forms indicated that hydrolysis occurred more rapidly under acidic than neutral and alkaline spray carrier pH, but efficacy was not adversely affected by chemical degradation using the mixture in normal time (Green and Hale 2005; Matocha and Senseman 2007).

Lots of compounds are suitable to make a buffer and achieve a favourable spray carrier pH (e.g. K_3PO_4, K_2HPO_4, KH_2PO_4, Na_2CO_3, H_3PO_4, $(NH_4)_2SO_4$, NH_4NO_3, triethanolamine) (Green and Cahill 2003; Green and Hale, 2005; Gronwald et al. 1993; Matocha et al. 2006; Woznica et al. 2003).

Dissolved salts in spray carrier (e.g. $(NH_4)_2NO_3$, NH_4HCO_3, $(NH_4)_2CO_3$, NH_4NO_3, NH_4Cl, $NaHSO_4$, Na_2SO_4, $NaHCO_3$, Na_2CO_3, $NaNO_3$, NaCl, $CaSO_4$, $CaCO_3$, $Ca(NO_3)_2$, $CaCl_2$, $MgSO_4$, $MgCO_3$, $Mg(NO_3)_2$, $MgCl_2$, $ZnSO_4$, $ZnCO_3$, $ZnCl_2$, $MnCl_2$, $FeSO_4$, $FeCl_2$, $Fe_2(SO_4)_3$, $Fe(NO_3)_3$, $FeCl_3$) and other compounds used as pH adjusters not only modify spray carrier pH, but also interact with herbicides. Herbicides in tank mixture can also effect on each other, moreover that interaction may be modified by dissolved salts (Matysiak and Nalewaja 1999; Nalewaja and Matysiak 1991, 1993; Nalewaja et al. 1989).

Several dissolved cations in spray carrier are antagonists of sulfonylureas and other types of herbicides forming less efficacious sodium, calcium or magnesium salt complexes, so modifying of pH is not advantageous with compounds containing them. Dissolved cations have different effects on herbicides, and several herbicides respond to the same cation different ways. Generally, calcium and magnesium ions are more harmful than sodium and potassium ions. Furthermore certain ammonium fertilizers overcome the salt antagonism of the weak acid herbicides (Woznica et al 2003). Sodium and potassium salts due to them pH modifying affect increased biological activity of nicosulfuron (Green and Cahill 2003; Green and Hale 2005). In case of glyphosate, which also a weak acid herbicide, ammonium nitrate overcame sodium, but not calcium antagonism (Nalewaja and Matysiak 1991). Moreover, nicosulfuron efficacy was enhanced by sodium bicarbonate, which increased its solubility, and was further increased by ammonium nitrate, which probably overcame sodium antagonism while maintained a high pH (Woznica et al. 2003).

MATERIALS AND METHODS

Water samples were collected from several spray carrier sources in Hajdú-Bihar county in 2008. Conductivity and pH of samples were measured by a pH and conductivity tester. Two of those samples (a slightly hard – conductivity: 496µS, pH 8,13 and a very hard one - conductivity: 1823µS, pH 7.31) were chosen for further examining. Several artificial fertilizers and other pH adjusters (ammonium nitrate - AN, ammonium sulfate – AS, monopotassium-phosphate – MPP, dipotassium-phosphate – DPP, Control DMP (a. phosphoric acid based adjuvant) – CDMP and triethanolamine – TEA were added to them, and conductivity and pH were measured in buffers, as well.

Field experiments were conducted in 3 replications, in small plots by a plot sprayer to study influence of spray carrier pH and hardness on efficacy of three herbicides in Hajdúböszörmény and Debrecen, Hajdú-Bihar county, Hungary in 2008 and 2009. Examined herbicides were nicosulfuron, 40g a.i. ha^{-1} (in Milagro® 40 SC – Syngenta Ltd.), and mezotrione, 119g a.i. ha^{-1} and terbutilazine, 561g a.i. ha^{-1} (in Calaris® – Syngenta Ltd.). Etoxylated octylphenol – EO, 0.1% (in Extravon® concentrate – Syngenta Ltd.) was added to some mixture. 250l ha^{-1} spray water were used in each treatment, doses of pH adjusters were 4000g MPP, 313 ml CDMP, 4000g AN (active ingredient is 33%), 500g DPP, 250ml TEA (*Table 1, 2*).

Table 1

Nicosulfuron treatments

Abbr.	Treatments in 2008	Abbr.	Treatments in 2009
M801	Weedy control	M901	Weedy control
M802	nicosulfuron + EO + MPP in slightly hard water	M902	nicosulfuron + EO + MPP in slightly hard water
M803	nicosulfuron + EO + MPP in very hard water	M903	nicosulfuron + EO + MPP in very hard water
M804	nicosulfuron + EO + CDMP in slightly hard water	M904	nicosulfuron + EO + CDMP in slightly hard water
M805	nicosulfuron + EO + CDMP in very hard water	M905	nicosulfuron + EO + CDMP in very hard water
M806	nicosulfuron + EO + AN in slightly hard water	M906	nicosulfuron + EO + AN in slightly hard water
M807	nicosulfuron + EO + AN in very hard water	M907	nicosulfuron + EO + AN in very hard water
M808	nicosulfuron + EO + DPP in slightly hard water	M908	nicosulfuron + EO + DPP in slightly hard water
M809	nicosulfuron + EO + DPP in very hard water	M909	nicosulfuron + EO + DPP in very hard water
M810	nicosulfuron + EO + TEA in slightly hard water	M910	nicosulfuron + EO + TEA in slightly hard water
M811	nicosulfuron + EO + TEA in very hard water	M911	nicosulfuron + EO + TEA in very hard water
M812	nicosulfuron + EO + in slightly hard water	M912	nicosulfuron + EO + in slightly hard water
M813	nicosulfuron + EO + in very hard water	M913	nicosulfuron + EO + in very hard water

Table 2

Terbutilazine + mezotrione treatments

Abbr.	Treatments in 2008	Abbr.	Treatments in 2009
C801	Weedy control	C901	Weedy control
C802	terbut. + mezot. + EO + CDMP in slightly hard water	C902	terbut. + mezot. + EO + CDMP in slightly hard water
C803	terbut. + mezot. + EO + CDMP in very hard water	C903	terbut. + mezot. + EO + CDMP in very hard water
C804	terbut. + mezot. + EO + AN in slightly hard water	C904	terbut. + mezot. + EO + AN in slightly hard water
C805	terbut. + mezot. + EO + AN in very hard water	C905	terbut. + mezot. + EO + AN in very hard water
C806	terbut. + mezot. + EO + TEA in slightly hard water	C906	terbut. + mezot. + EO + TEA in slightly hard water
C807	terbut. + mezot. + EO + TEA in very hard water	C907	terbut. + mezot. + EO + TEA in very hard water
C808	terbut. + mezot. + EO in slightly hard water	C908	terbut. + mezot. + EO in slightly hard water
C809	terbut. + mezot. + EO in very hard water	C909	terbut. + mezot. + EO in very hard water
C810	terbut. + mezot. + TEA + CDMP in slightly hard water	C910	terbut. + mezot. + CDMP in slightly hard water
C811	terbut. + mezot. + TEA + CDMP in very hard water	C911	terbut. + mezot. + CDMP in very hard water
C812	terbut. + mezot. + TEA + AN in slightly hard water	C912	terbut. + mezot. + AN in slightly hard water
C813	terbut. + mezot. + TEA + AN in very hard water	C913	terbut. + mezot. + AN in very hard water
C814	terbut. + mezot. + TEA in slightly hard water		
C815	terbut. + mezot. + TEA in very hard water		

Terbut.: terbutilazine, mezot.: mezotrione

Phenological stages of weed species in the time of herbicidal treatments are shown in *Table 3*.

Table 3

Phenological stages of weed species in the time of herbicidal treatments

Weed species	Nicosulfuron treatments		Terbutilazine + Mezotrione treatments	
	2008	2009	2008	2009
Setaria glauca	3 leaves-1 branch	5 leaves-2(3) branches	2-5 leaves	5 leaves-2(3) branches
Echinochloa crus-galli	3 leaves-1 branch	5 leaves-2(3) branches	2-5 leaves	5 leaves-2(3) branches
Panicum miliaceum	2 leaves-2 branches	4 leaves-2 branches	-	-
Datura stramonium	-	2-6 leaves	1-4 leaves	2-6 leaves
Chenopodium album	-	4-10 leaves	-	4-10 leaves
Polygonum lapathifolium	3-8 leaves	-	-	-
Amaranthus retroflexus	2-7 leaves	4-8 leaves	-	-
Hibiscus trionum	-	-	-	1-2 leaves
Solanum nigrum	2-5 leaves	-	-	-
Ambrosia artemisiifolia	-	-	2-7 leaves	-
Abutilon theophrasti	-	-	1-4 leaves	-

Herbicidal treatments were made on 22 05 2008 and on 17 05 2009 in case of nicosulfuron an on 16 05 2008 and on 17 05 2009 in case of terbutilazine + mezotrione. Times of evaluations of herbicidal treatments were 06 06 2008, 21 06 2008, 03 06 2009, 20 06 2009 in case of nicosulfuron and 23 05 2008, 26 05 2009, 20 06 2009 in case of terbutilazine + mezotrione.

RESULTS

Affects of pH adjusters on efficacy of nicosulfuron

MPP, CDMP and AN stabilized spray mixture at a slightly acidic pH, DPP and TEA stabilized that at a slightly basic pH, so the herbicide was ionized in spray carrier.

Herbicidal efficacy of nicosulfuron was evaluated on *Echinochloa crus-galli, Setaria glauca, Panicum miliaceum, Polygonum lapathifolium, Solanum nigrum, Amaranthus retroflexus* in 2008 (*Table 4*). Minimal differences were observed in effects of the herbicide in case of monocotyledonous species (3-5%), and 8-10% differences in case of dicotyledonous species, expected *Amaranthus retroflexus*. Mixtures containing MPP, CDMP and AN were most effective, and mixtures without pH adjusters had a reduced activity. Water hardness had a slightly effect on efficacy.

In 2009, overgrown *Echinochloa crus-galli, Setaria glauca, Panicum miliaceum, Datura stramonium, Chenopodium album, Amaranthus retroflexus* were treated with nicosulfuron. Similar tendency was observed like in the previous year, but differences were found in wide range. pH adjusters enhanced biological activity of nicosulfuron by about 20-40% in case of *Echinochloa crus-galli* and *Setaria glauca*. In case of *Panicum miliacem* acidic pH adjusters enhanced efficacy significantly (by about 30%). In case of *Datura stramonium* pH adjusters increased weed control efficacy by 10-20%. *Chenopodium album* was controlled significantly by spray mixtures added AN. Water hardness had a slightly influence on efficacy of nicosulfuron (*Table 5*).

Table 4

Weed control efficacy of nicosulfuron influenced by pH adjusters in 2008

Abbreviations of treatments	Weed control efficacy (%)											
	ECHCR		SETGL		PANMI		POLLA		SOLNI		AMARE	
	1E	2E	1E	2E	1E	2E	1E	2E	1E	2E	1E	2E
M802	94.3	94.7	89	94.3	86	98	80.7	89.3	90	93.5	100	100
M803	94.3	91.7	86.7	91.7	81.3	98	80	89.3	82.3	86	100	100
M804	94.7	94.3	88.7	94.3	82.3	98	83	89.3	89	91.5	100	100
M805	88	93	81.3	90.3	82.3	98	80.7	89	85	88	100	100
M806	95.7	93.7	86.3	93.7	85	98	81.3	90	87.5	89.7	100	100
M807	95.7	95	88.7	94.3	81	98.7	83	90	89.3	92.3	100	100
M808	92	89.3	84	89.3	79	98	83	87	88.5	88	100	100
M809	86.7	90.3	84.7	90.3	77.5	97.7	77.7	84.7	86	89	100	100
M810	90	91	75	89.3	82	97.7	75.7	84.7	79.5	87.7	100	100
M811	91	88.7	80	88.7	80	97.7	55	83	75	88.3	100	100
M812	86.7	87	77.5	85.3	80	94.3	73.3	79.3	86.7	81.3	100	100
M813	82.7	89	80	89	73.3	95.3	74	80	60	73.3	100	100

ECHCR: *Echinochloa crus-galli*, SETGL: *Setaria glauca*, PANMI: *Panicum miliaceum*, POLLA: *Polygonum lapathifolium*, SOLNI: *Solanum nigrum*, AMARE: *Amaranthus retroflexus*, 1E: 1st evaluation (06 06 2008), 2E: 2nd evaluation (21 06 2008)

Table 5

Weed control efficacy of nicosulfuron influenced by pH adjusters in 2009

Abbreviations of treatments	Weed control efficacy (%)											
	ECHCR		SETGL		PANMI		DATST		CHEAL		AMARE	
	1E	2E	1E	2E	1E	2E	1E	2E	1E	2E	1E	2E
M902	87	93	85	93	82	85	90	94	37	40	100	100
M903	85	89	83	87	76	84	86	88	18	20	100	100
M904	89	93	86	92	82	87	90	94	20	28	100	100
M905	83	91	82	89	76	80	85	91	22	37	100	100
M906	90	93	86	93	82	88	87	95	47	64	100	100
M907	90	92	87	92	79	88	88	94	60	61	100	100
M908	84	89	82	85	76	65	88	91	7	3	100	100
M909	80	83	79	81	72	66	88	94	3	5	100	100
M910	80	75	81	75	60	35	87	83	2	3	100	100
M911	82	78	80	78	79	65	89	95	5	7	100	100
M912	81	60	81	60	59	51	84	73	7	0	100	100
M913	80	48	82	48	63	45	80	69	0	7	100	100

ECHCR: *Echinochloa crus-galli*, SETGL: *Setaria glauca*, PANMI: *Panicum miliaceum*, DATST: *Datura stramonium*, CHEAL: *Chenopodium album*, AMARE: *Amaranthus retroflexus*, 1E: 1st evaluation (03 06 2009), 2E: 2nd evaluation (20 06 2009)

Affects of pH adjusters on efficacy of terbutilazine + mezotrione

Differences in efficacy of treatments were observed in case of monocotyledonous weeds (*Setaria glauca, Echinochloa crus-galli*) in 2008. Acidic pH adjusters increased activity of herbicides compared to mixtures without pH adjusters, however, unfavourable effects of basic spray mixtures were found. Water hardness had a slightly influence on efficacy (*Table 6*).

In 2009, overgrown weeds were treated by several mixtures. Control of dicotyledonous weeds was similar with all mixtures, however, efficacy ranged from 33% to 95% in the time of 2nd evaluation depending on added pH adjuster and spray water hardness in case of *Echinochloa crus galli*, and ranged from 23% to 93% in case of *Setaria glauca.* In slightly hard water acidic pH adjusters enhanced efficacy against monocotyledonous weeds by about 50-60 % (2E), but in very hard water only AN had similar effects. Basic pH of mixtures had a harmful affect on biological activity of herbicides (*Table 7*).

Table 6

Weed control efficacy of terbutilazine + mezotrione influenced by pH adjusters in 2008

Abbreviations of treatments	Weed control efficacy (%)				
	SETGL	ECHCR	AMBEL	DATST	ABUTH
C802	92.3	98	100	100	100
C803	91	97.6	100	100	100
C804	93.3	97	98.3	100	100
C805	95.6	97.3	100	100	100
C806	87.3	98	100	100	100
C807	87	97.6	98.6	100	99.6
C808	88	98	100	100	100
C809	85.6	97.3	98	100	100
C810	91.6	97	96	100	100
C811	85	96.6	99	100	99.3
C812	93	97.6	100	100	99.6
C813	93.6	97.6	99.6	100	100
C814	82.6	93.3	100	100	100
C815	79	90	97.3	100	99.6

ECHCR: *Echinochloa crus-galli*, SETGL: *Setaria glauca*, AMBEL: *Ambrosia artemisiifolia*, DATST: *Datura stramonium*, ABUTH: *Abutilon theophrasti*

Table 7

Weed control efficacy of terbutilazine + mezotrione influenced by pH adjusters in 2009

Abbreviations of treatments	Weed control efficacy (%)									
	ECHCR		SETGL		CHEAL		DATST		HIBTR	
	1E	2E	1E	2E	1E	2E	1E	2E	1E	2E
C902	98	94	91	89	100	100	100	100	100	98
C903	63	53	69	50	100	100	100	100	100	98
C904	100	94	93	89	100	100	100	100	100	98
C905	99	95	96	93	100	100	100	100	100	98
C906	56	37	50	25	100	100	100	100	97	98
C907	62	33	57	23	100	100	100	100	98	98
C908	62	47	62	45	100	100	100	100	100	98
C909	70	53	66	53	100	100	100	100	100	98
C910	99	94	89	86	100	100	100	100	100	98
C911	63	47	58	45	100	100	100	100	100	98
C912	99	92	94	90	100	100	100	100	100	98
C913	97	94	94	89	100	100	100	100	100	99

ECHCR: *Echinochloa crus-galli*, SETGL: *Setaria glauca*, CHEAL: *Chenopodium album*, DATST: *Datura stramonium*, HIBTR: *Hibiscus trionum*, 1E: 1. evaluation (26 05 2009), 2E: 2. evaluation (20 06 2009)

CONCLUSIONS

Favourable or unfavourable effects of pH and hardness of spray water could be observed under field conditions. Evaluation of weed control efficacy is suitable for examination of affects of spray water pH and hardness on herbicides.

Sensitivity of the three herbicides was significantly different against the pH and hardness of spray carrier: mezotrione did not respond to hardness significantly and was only slightly affected by pH, the efficacy of nicosulfuron was influenced moderately by pH and hardness, and terbutilazine responded to either pH or hardness spectacularly.

Certain pH adjusters (e.g. AN) can lessen harmful affects of water hardness effectively.

Significant loss of efficacy of sensitive herbicide was found in hard water (by about 50-60%), and surfactants was not able to eliminate that harmful affect. However, biological activity was the same as in soft water with AN which can overcome antagonism of salts. That pH adjuster had a more significant affect on the efficacy of the herbicide than surfactant had in that experiment.

Choosing adequate adjuvants has significant role in efficacy of herbicides, especially under unfavourable conditions (existing weeds after optimum phenological stage, unfavourable weather conditions and spray carrier properties).

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The Invasion of the Town Hódmezővásárhely interior Areas by the Creeping Woodsorrel (*Oxalis corniculata)*

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SUMMARY

As a consequence of urbanisation, our built-in environment is taking away larger and larger space from natural living sites. Changes in flora are taking place paralelly to globalisation, the rate of adventives is increasing. Not only natural living sites are endangered by the advancement of invasive plants but the flora of built-in settlements is changing as well. The purpose of our work was to take stock of the range of spread of creeping woodsorrel (Oxalis corniculata) on the inner territory of Hódmezővásárhely town. Survey for data on spreading range of this weed species was carried out in 2005-2007, on the territory of Hodmezovasarhely, on mown turf and lawn premises, public squares and streets. In the course of my studies, the inner site of the town was divided into 15 grid squares, each of 1 km^2. The received 15 map units were surveyed in July-August of 2005-2007, aiming at creeping woodsorrel infestation. As a result of these studies, we could establish that all surveyed units were infested and further slow advancement of the weed could be observed.

Keywords: creeping woodsorrel, native vegetation, invasion

INTRODUCTION

Fighting against undesirable weeds is a task originated back to the times mankind started producing crop. The creation of cultivated plant stocks and the transformation of the habitat in general have led to the upset of the former balance in the vegetal co-existence, as well as to a constant struggle between mankind and the vegetation for the earlier soil. As a result of the activity of transforming nature, certain abiotic ecological factors seem to be changing, and at the same time, the climatic factors of the built environment also divert significantly from their originals. The development of commerce of plants and plant products entailed the appearance of species in certain areas that had not existed there before. These newcomer plants – especially from the 20th century – have started to have a more and more significant influence on the native vegetation (Kovács and Priszter 1974). Changes within the natural flora occur in line with globalization and thus the proportion of adventive weeds increase (Solymosi 2002). According to the findings of Czimber and his colleagues (Czimber et al. 2004) the number of species known to be earlier or lately immigrated has increased in Central-Europe. These mainly derive from warmer regions, especially from the Mediterranean. Gumption, persistency and a great ecological plasticity are generally typical for adventives that are capable acclimatization (Hódi 2006). The anthropogenic environment of settlements often serves as a starting point for the acclimatization of certain newcomer species (Brandes and Schlender 1999).

In our country, the invasive spread of the creeping woodsorrel in the last decades concerns mainly urban environments. Its gradual expansion is undesirable also within the built environment, therefore, it is essential for us to get acquainted with its biology to organise the effective protection from it. Balogh and his colleagues referred to it as one of the most important invasive species in Hungary (Balogh et al. 2004). The objective of our work was to examine the spread of the creeping woodsorrel within interior areas of the town Hódmezővásárhely.

MATERIALS AND METHODS

Data concerning the spread of the weed species was recorded between 2005 and 2007 in the interior areas of Hódmezővásárhely, the mowed lawn and turf areas of the town as well as in public squares and streets. For the data collection we used a regular street catalogue grid map available in commerce trade. For the record of infected areas found we used a Magellan Gold GPS device.

During our research we split up the interior area of the town on the grid map to 15 meshes, 1 square-kilometre each. These 15 meshes covering almost the whole town were first examined for wood sorrel infection in July and August 2005. Data record with the same method was repeated both in 2006 and 2007.

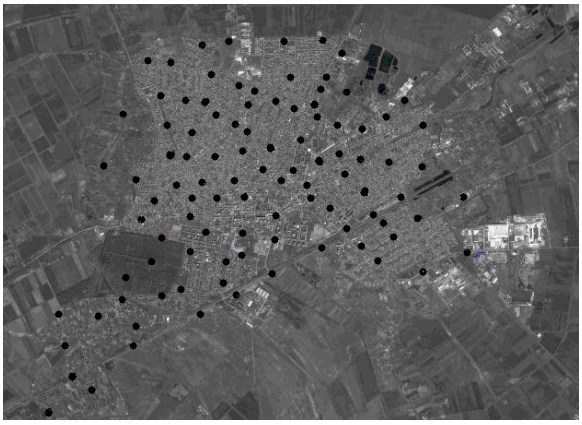
RESULTS AND DISCUSSION

During data record of the population of Hódmezővásárhely we also carried out phenological observations. We found out that the observed plant stock's morphological characteristics, habit and the conditions of the habitat itself were identical to those described by the reference literature. The fold of the fruited penduncle is clearly observable on the inflorescence (*Figure 1*).

Figure 1: ***Oxalis Corniculata* – Fruited penduncle. (Photo by Anna Mária Hódi)**

The grid-based systematic floristic exploration cannot be considered as usual in Hungary. Research of towns' and cities' flora carried out this way is rather unique (Bán 2008). During the first year (2005) of the data record of the plant's spread it became clear that all of the 15 meshes were infected. Habitats are presented on the Google Earth Satellite photograph below (*Figure 2*).

Figure 2: **The spread of *Oxalis corniculata* within Hódmezővásárhely between 2005 and 2007 (Map: Google Map)**



CONCLUSIONS

It became obvious already in the first year of examination that the interior area of Hódmezővásárhely is heavily infected by the creeping woodsorrel, especially the parts and streets where there is a regular mowing. In less frequently mowed areas the weed's probability of occurrence reduces. However, the probability of the weed's occurrence increases in areas where the mowing is a task of the local government, including cycling paths parallel to public roads and main roads. The heavy-duty mowing machines contribute to the spread of the creeping woodsorrel as the rate of infection in these areas is especially high. The occurrence of the weed in the outer regions of the town that were excluded from the research is almost insignificant. The primary infection always develops at the margin areas of lawn and turf and expands from there. There is quite a high chance for this weed to appear in areas it has not appeared before.

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Research on the results of the allelopathic effect between the allergenic species *Iva xanthiifolia* Nutt. and other crop plants

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SUMMARY

Iva xanthiifolia Nutt., popularly known as "ierboaie", is a neophyte invasive species notorious for being an allergenic weed, identified in the west of Romania, in two locations near Oradea, in Bihor County, near the border with Hungary. This species belongs to the allergenic weeds, being considered by some even more dangerous than Ambrosia artemisiifolia L., the two representing in summer the primary source of allergies, or diseases like hay fever, due to the pollen released in the atmosphere.

The research is about the results of the allelophatic effect upon the germination and growth of plants, immediately after springing, viewed as the interaction between the species of Iva xanthiifolia and five other crop plants: wheat (Triticum aestivum L.), rye (Secale cereale L.), barley (Hordeum vulgare L.), rape (Brassica napus L.) and lucerne (Medicago sativa).

The experiments that were performed consisted in applying treatments with aqueous extracts obtained from different vegetative organs (roots, leaves, stems and seeds) harvested from Iva xanthiifolia plants. In all cases, the results indicate a rather large inhibitor effect, no matter if the aqueous extracts were obtained from green plants or dehydrated ones.

Keywords: allelopathy, Iva xanthiifolia, wheat, rye, barley, rape, lucerne

INTRODUCTION

De Condolle stated the idea regarding the influence of some chemical substances released in the environment by certain organisms upon other neighbouring organisms in 1832. He concluded that all the plants secrete through their roots certain substances that can stimulate or, on the contrary, can inhibit the growth of other plants. Molisch (1937) published the results of the research concerning the action of ethylene on some superior plants, phenomenon that he later had named "*Allelopathie"*. He was the one who defined for the first time the term of allelopathy, and it meant, at that time, the biochemical interactions established between all types of plants, including both harmful and stimulating interactions. A real breakthrough occurred once Rice had published his book, Allelopathy, in 1974, in which the author defined allelopathy as being the toxic effect of a plant upon another one by producing some chemical compounds that are released and diffused in the environment (Hodişan et al. 2009).

The allelopathy describes the field which studies the relationships between different plant species and at the same time between the individuals coming from that same species. The chemical compounds that take part in the interaction between plants are generally called allelopathic substances, having an important part in the primary metabolic processes, essential for the survival of plants (Rice 1974).

The species *Iva xanthiifolia* has its origin on the North American Continent, but it has been spread across the countries in Central Europe, too. In the neighbouring country, Hungary, the species was identified in various locations, mainly in the South-Eastern counties, Békés and Csongrád, where it is to be found in small number, in weed controlled crops, especially in those of sunflower (Hódi 2005).

This species was signaled in Serbia, too, where it is to be found along the roads as well as in some agricultural crops like maize, sunflower, sugar beet and soya, showing an important potential for spreading (Marisavljevic et al. 2005).

According to the bibliographical data belonging to the American researchers, *Iva xanthiifolia* is as harmful as *Ambrosia artemisiifolia* L., because of the allergenic affections caused to our peers, during blossoming (Juhász and Juhász 2006).

In Romania it has been identified in the West part of the country, in two locations near Oradea, in Bihor county, near the border with Hungary. This species belongs to the allergenic weeds, being considered by some even more dangerous than *Ambrosia artemisiifolia* L., the two representing in summer the primary source of allergies, or diseases like hay fever, due to the pollen released in the atmosphere.

The allelophatic effect of the species *Iva xanthiifolia* was accurately studied by the Hungarian researchers, who concluded that the extracts obtained from the seeds have mutual effects on the germination in the interaction of the latter with some species of weeds and crop plants (Hunyadi et al. 1998).

After laboratory studies where aqueous extracts obtained from roots, stems, leaves, seeds or whole plants of *Iva xanthiifolia* were used on the germination of some crop plants, observed a slight stimulating effect in the germination of maize seeds in all cases, except the treatments with stem aqueous extracts, an insignificantly allelophatic effect in the germination of sunflower seeds in all cases, and a significantly inhibiting effect on white mustard seeds (Hódi and Torma 2000).

MATERIALS AND METHODS

There were harvested both green *Iva xanthiifolia* plants and dehydrated mature plants, from which there were collected roots, stems, leaves and seeds.

It was the allelophatic effect upon the germination and growth of plants, immediately after springing, that was studied on different species cultivated in autumn or late summer: wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), barley (*Hordeum vulgare* L.), rape (*Brassica napus* L.) and lucerne (*Medicago sativa*).

To prepare the aqueous extracts, 100 g of vegetal material (roots, stems, leaves and seeds) were scaled, minced and macerated in 500 ml of distilled water for 48 hours at room temperature, more precisely at 22-25°C. The preparation obtained was decanted and filtered and then preserved until usage in the dark, at 4-5°C.

The seeds of the studied crop species were put to germinate, by 100 grains, in ceramic pots, in sand. The germination substratum was obtained by totally dehydrating the sand in the drying oven at 180-200°C. After that it was sifted through 0,3 mm sieve in order to standardize the granulation and then rehydrated by adding 100 ml of aqueous extract to 1000 g of sand, waiting then to homogenized. There were used equal quantities of sand, carefully scaled for each germination pot in order to maintain the depth laying of the seeds at 1 cm.

The same method was applied to the variant, control sample, with the difference that only distilled water was used for the rehydrating of the sand.

The germination pots were kept at 22-25°C, in natural light, with the specific diurnal variations, without being rehydrated.

Three recurrences were made for each experimental variant.

The reading of the results took place four days after the germination of the seeds and consisted in counting the seeds that sprang, but for the biometrical determinations, the reading took place only after seven days by measuring the height of the plants.

The data obtained through observation and measurements was interpreted statistically.

RESULTS AND DISCUSSIONS

Results obtained with wheat *(Triticum aestivum L.)*

Having in mind these results, a significantly inhibiting effect of the treatments with *Iva xanthiifolia* extracts on the germination of the wheat seeds (*Triticum aestivum* L.) can be established, no matter if these came from green plants or dehydrated ones (*Table 1*).

Table 1

The influence of *Iva xanthiifolia* Nutt. extract on the germination of wheat seeds (*Triticum aestivum* L.)

Symbol	Type of extract	Number of germinated seeds	Percent of germinated seeds (%)	Difference	Significance of the differences	The Duncan classification
E1	Control	96.67	100.0	0.00	Mt.	F
E2	Roots	95.00	98.3	-1.67	-	F
E3	Leaves	31.67	32.8	-65.00	000	B
E4	Stems	90.67	93.8	-6.00	000	E
E5	Dehydrated Roots	95.67	99.0	-1.00	-	F
E6	Dehydrated Leaves	35.00	36.2	-61.67	000	C
E7	Dehydrated Stems	41.33	42.8	-55.33	000	D
E8	Seeds	20.00	20.7	-76.67	000	A
	DL (p 5%)			2.94		
	DL (p 1%)			4.08		
	DL (p 0.1%)			5.67		

Differences of germination significantly negative compared to the control sample, with values from –55.33 to –65.00 under the limits of experimental errors were observed in the case of leaf, dehydrated leaf and dehydrated stem extract treatments (variant E3, E6 and E7), while in the case of E8 variant a –76.67 value was obtained after using seed extract. In what concerns variants E2 and E5 (roots and dehydrated roots), the germination differences were insignificantly negative compared with the control sample (*Table 1*).

Regarding to the influence of the *Iva xanthiifolia* extracts upon the height of the wheat plants (*Triticum aestivum* L.) after springing, influences were observed in all test variants with negative differences, less significant for E5 variant (dehydrated roots) and significant for E2 and E4 variant (roots and stems).

Real significant negative differences of height, with rate values between 58.3% and 41.7% compared to the control sample were obtained for E3, E6 and E7 variants (leaves, dehydrated leaves and dehydrated stems) and of 37.5% for the treatment with seed extract, E8 variant (*Table 2*).

As a result of the allelopathic effect of the extracts obtained from different organs of the species *Iva xanthiifolia,* the reduction of plant height can be reproduced in field conditions after the decomposition of

vegetal weed remnants with the most hindering effects on the evolution of wheat growth after springing in the field.

The slow growth of plants after springing can affect the twinning and preparation processes of the wheat crop for the winter through hardening and late optimal stage of vegetation at the beginning of winter.

Table 2

The influence of *Iva xanthiifolia* Nutt. extract on the height of wheat seeds (*Triticum aestivum* L.) after springing

Symbol	Type of extract	Height of plants (cm)	Height of plants (%)	Difference	Significance of the differences	The Duncan classification
E1	Control Sample	4.00	100.0	0.00	Mt.	D
E2	Roots	3.50	87.5	-0.50	00	C
E3	Leaves	2.33	58.3	-1.67	000	B
E4	Stems	3.50	87.5	-0.50	00	C
E5	Dehydrated Roots	3.67	91.7	-0.33	0	C
E6	Dehydrated Leaves	2.17	54.2	-1.83	000	B
E7	Dehydrated Stems	1.67	41.7	-2.33	000	A
E8	Seeds	1.50	37.5	-2.50	000	A
	DL (p 5%)			0.30		
	DL (p 1%)			0.41		
	DL (p 0.1%)			0.57		

Results obtained with rye (*Secale cereale* L.)

The effect of root and dehydrated root *Iva xanthiifolia* extracts observed in the variants E2 and E5 show an insignificant negative influence on the germination of rye seeds (*Secale cereale* L.) (*Table 3*).

The green leaf and dehydrated leaf extracts have a strong inhibiting effect on the germination of rye seeds. In this particular case, the data presented in table 3 shows accentuated negative differences of germination, with values between 51.6% and 58.2% compared to the control sample. Whereas in the case of seed extract (variant E8) really significant negative differences of germination are registered compared to the control sample, but with a much lower percentage of germinated seeds, 26.6% (*Table 3*).

With less effect, but worthy to be taken into consideration is the treatment with stem and dehydrated stem extracts (variant E4 and E7), the germination differences being significantly negative compared with the control sample (*Table 3*).

Table 3

The influence of *Iva xanthiifolia* Nutt. extract on the germination of rye seeds (*Secale cereale* L.)

Symbol	Type of extract	Number of germinated seeds	Percent of germinated seeds (%)	The difference	Significance of the differences	The Duncan classification
E1	Control Sample	81.33	100.0	0.00	Mt.	E
E2	Roots	78.67	96.7	-2.67	-	DE
E3	Leaves	42.00	51.6	-39.33	000	B
E4	Stems	77.33	95.1	-4.00	0	D
E5	Dehydrated Roots	79.33	97.5	-2.00	-	DE
E6	Dehydrated Leaves	47.33	58.2	-34.00	000	C
E7	Dehydrated Stems	77.33	95.1	-4.00	0	D
E8	Seeds	21.33	26.2	-60.00	000	A
	DL (p 5%)			3.35		
	DL (p 1%)			4.65		
	DL (p 0.1%)			6.46		

Regarding the height of rye plants (*Secale cereale* L.) immediately after springing (*Table 4*), the results obtained for the variants E2, E4 and E5, variants in which root, dehydrated root and stem extracts were used, indicated insignificant negative differences compared to the control sample. On the other hand, real significant negative differences of rye plant height, compared to the control sample, were obtained in variants E3 and E6, in which green leaf and dehydrated leaf extracts were used and thus a percentage between 48.3% and 27.6% was registered, the same situation repeating itself in the treatment with seed extract, where the percent of rye plant height was of 20.7% compared to the control sample (*Table 4*).

Table 4

The influence of *Iva xanthiifolia* Nutt. extract on the height of rye (*Secale cereale L.*) plants after springing

Symbol	Type of extract	Height of plants (cm)	Height of plants (%)	Difference	Significance of the differences	The Duncan classification
E1	Control Sample	4.83	100.0	0.00	Mt.	C
E2	Roots	4.67	96.6	-0.17	-	C
E3	Leaves	2.33	48.3	-2.50	000	B
E4	Stems	4.67	96.6	-0.17	-	C
E5	Dehydrated Roots	4.67	96.6	-0.17	-	C
E6	Dehydrated Leaves	1.33	27.6	-3.50	000	A
E7	Dehydrated Stems	4.33	89.7	-0.50	-	C
E8	Seeds	1.00	20.7	-3.83	000	A
	DL (p 5%)			0.76		
	DL (p 1%)			1.05		
	DL (p 0.1%)			1.46		

Results obtained with barley (*Hordeum vulgare* L.)

The treatments made with aqueous extracts of *Iva xanthiifolia* on the germination of barley seeds (*Hordeum vulgare* L.), indicated significant negative effects when it comes to green leaf, dehydrated leaf, dehydrated roots, dehydrated stems and seed extracts. The germination differences ranged between –12.67 and –52.00 under the limits of experimental errors (*Table 5*).

In what concerns the treatments with root and stem extracts (E2 and E4), insignificant negative differences in germination compared to the control sample were established.

Referring to the height of barley (*Hordeum vulgare* L.) plants, the root extracts, variant E2, had an insignificant negative effect, while the stem extract, variant E4, shows more significant negative differences, worthy to be taken into consideration (*Table 5*).

For the variants E3, E5, E6, E7 and E8, more precisely green leaf, dehydrated roots, dehydrated leaves, dehydrated stems and seeds, highly negative differences in height were registered.

Table 5

The influence of *Iva xanthiifolia* Nutt. extracton the germination of barley seeds (*Hordeum vulgare* L.)

Symbol	Type of extract	Number of germinated seeds	Percent of germinated seeds (%)	Difference	Significance of the differences	The Duncan classification
E1	Control Sample	96.67	100.0	0.00	Mt.	F
E2	Roots	95.33	98.6	-1.33	-	F
E3	Leaves	68.33	70.7	-28.33	000	C
E4	Stems	97.33	100.7	0.67	-	F
E5	Dehydrated Roots	89.33	92.4	-7.33	000	E
E6	Dehydrated Leaves	48.67	50.3	-48.00	000	B
E7	Dehydrated Stems	84.00	86.9	-12.67	000	D
E8	Seeds	44.67	46.2	-52.00	000	A
	DL (p 5%)			2.67		
	DL (p 1%)			3.70		
	DL (p 0.1%)			5.14		

Results obtained with rape (*Brassica napus* L.)

The germination of rape seeds (*Brassica napus* L.) is strongly affected by the leaf, stem, dehydrated roots, dehydrated leaves and seed extract treatments, with highly negative differences compared to the control sample, reaching values from 82.5% to 14.7% in E3, E5, E6, E7 and E8 variants and distinctly negative differences in variant E4, where the stem extract was tested (*Table 7*).

The *Iva xanthiifolia* root extract (variant E2) has insignificant negative germination differences compared to the control sample.

Table 6

The influence of *Iva xanthiifolia* Nutt. extract on the height of barley plants (*Hordeum vulgare* L.) after springing

Symbol	Type of extract	Height of plants (cm)	Height of plants (%)	Difference	Significance of the differences	The Duncan classification
E1	Control Sample	6.17	100.0	0.00	Mt.	E
E2	Roots	5.83	94.6	-0.33	-	DE
E3	Leaves	3.33	54.1	-2.83	000	B
E4	Stems	5.50	89.2	-0.67	0	CD
E5	Dehydrated Roots	5.17	83.8	-1.00	000	C
E6	Dehydrated Leaves	2.50	40.5	-367	000	A
E7	Dehydrated Stems	5.00	81.1	-1.17	000	C
E8	Seeds	3.00	48.6	-3.17	000	B
	DL (p 5%)			0.49		
	DL (p 1%)			0.68		
	DL (p 0.1%)			0.95		

Table 7

The influence of *Iva xanthiifolia* Nutt. extract on the germination of rape seeds (*Brassica napus* L.)

Symbol	Type of extract	Number of germinated seeds	Percent of germinated seeds (%)	Difference	Significance of the differences	The Duncan classification
E1	Control Sample	84.00	100.0	0.00	Mt.	F
E2	Roots	82.00	97.6	-2.00	-	EF
E3	Leaves	28.00	33.3	-56.00	000	B
E4	Stems	79.33	94.4	-4.67	00	E
E5	Dehydrated Roots	69.33	82.5	-14.67	000	D
E6	Dehydrated Leaves	37.33	44.4	-46.67	000	C
E7	Dehydrated Stems	67.33	80.2	-16.67	000	D
E8	Seeds	12.33	14.7	-71.67	000	A
	DL (p 5%)			3.05		
	DL (p 1%)			4.23		
	DL (p 0.1%)			5.87		

In all experimental variants (*Table 8*), statistical results show halting in the growth of rape plants immediately after springing.

In variants E2, E4 and E5 in which root, stem and dehydrated roots were used, the height differences were significantly negative compared to the control sample, with percentual differences ranging from 88.9% to 86.1%. Really significant negative differences compared to the control sample, represented by values between 72.2% and 16.7%, were obtained in green leaf, dehydrated leaf, dehydrated stems and seeds, a particular difference of height being noticed in variant E8 where a growth of 16.7% compared to the control sample was registered.

Table 8

The influence of *Iva xanthiifolia* Nutt. extract on the height of rape (*Brassica napus* L.) plants after springing

Symbol	Type of extract	Height of plants (cm)	Height of plants (%)	Difference	Significance of the differences	The Duncan classification
E1	Control Sample	6.00	100.0	0.00	Mt.	E
E2	Roots	5.33	88.9	-0.67	0	DE
E3	Leaves	2.17	36.1	-3.83	000	B
E4	Stems	5.33	88.9	-0.67	0	DE
E5	Dehydrated Roots	5.17	86.1	-0.83	0	D
E6	Dehydrated Leaves	1.33	22.2	-4.67	000	A
E7	Dehydrated Stems	4.33	72.2	-1.67	000	C
E8	Seeds	1.00	16.7	-5.00	000	A
	DL (p 5%)			0.64		
	DL (p 1%)			0.89		
	DL (p 0.1%)			1.23		

In the case of the results obtained with rape, the fact that *Brassica napus* L. is a highly sensitive plant in what concerns the majority of herbicides (substances that inhibit in various ways the growth and multiplication of vegetal cells) is once more confirmed, so that the present results are no longer a surprise.

Results obtained with lucerne (*Medicago sativa*)

The tests on germination of lucerne seeds (*Medicago sativa*) show in all variants a negative influence (*Table 9*), with significant negative differences only in the case of root extract treatment (variant E2) and really significant negative ones in the case of the rest, with percentual values up to 73.4% compared to the control sample (*Table 9*).

Table 9

The influence of *Iva xanthiifolia* Nutt. extract on the germination of lucerne seeds (*Medicago sativa*)

Symbol	Type of extract	Number of germinated seeds	Percent of germinated seeds (%)	Difference	Significance of the differences	The Duncan classification
E1	Control Sample	89.00	100.0	0.00	Mt.	E
E2	Roots	84.67	95.1	-4.33	0	D
E3	Leaves	71.33	80.1	-17.67	000	B
E4	Stems	82.00	92.1	-7.00	000	D
E5	Dehydrated Roots	77.33	86.9	-11.67	000	C
E6	Dehydrated Leaves	65.33	73.4	-23.67	000	A
E7	Dehydrated Stems	78.00	87.6	-11.00	000	C
E8	Seeds	68.00	76.4	-21.00	000	A
	DL (p 5%)			3.26		
	DL (p 1%)			4.52		
	DL (p 0.1%)			6.28		

The results referring to the height of lucerne (*Medicago sativa*) plants after springing indicate insignificant negative differences in the dehydrated root extract treatment (variant 5) and significant negative differences compared to the control sample in the variants E2 and E4, after applying root and stem extract treatments (*Table 10*).

Table 10

The influence of *Iva xanthiifolia* Nutt. extract on the height of lucerne seeds (*Medicago sativa*) plants after springing

Symbol	Type of extract	Height of plants (cm)	Height of plants (%)	Difference	Significance of the differences	The Duncan classification
E1	Control Sample	3.50	100.0	0.00	Mt.	E
E2	Roots	3.00	85.7	-0.50	0	CD
E3	Leaves	1.83	52.4	-1.67	000	B
E4	Stems	3.00	85.7	-0.50	0	CD
E5	Dehydrated Roots	3.17	90.5	-0.33	-	DE
E6	Dehydrated Leaves	1.50	42.9	-2.00	000	B
E7	Dehydrated Stems	2.67	76,2	-0.83	000	C
E8	Seeds	0.83	23.8	-2.67	000	A
	DL (p 5%)			0.36		
	DL (p 1%)			0.51		
	DL (p 0.1%)			0.70		

Really significant negative differences compared to the control sample were observed in green leaf, dehydrated leaf and seed extract treatments, the most low value being 23.8%, in E8 variant (seeds).

After the research on the influence of *Iva xanthiifolia* aqueous extracts on the germination of the seeds and the growth of plants immediately after springing in the case of wheat, rye, barley, rape and lucerne, an allelopathic effect of inhibition was established in all studied cases.

The results of the tests point out real low percentual values both in the case of germination and in the growth of plants, immediately after springing, when the treatments were made with seed and leaf aqueous extract from the species *Iva xanthiifolia,* phenomenon that can open new fields of usage in agriculture.

In *Iva xanthiifolia´*s case*,* the allelopathic chemical substances acted with the same intensity both in the aqueous extracts made from green plant organs and in those from dehydrated ones.

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Study of allelopathic effect between species *Xanthium strumarium* L. and some agricultural crops

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SUMMARY

This work shows the results of the allelopathic effect manifested on germination and growing of plants, immediately after emerging, in the interaction between the species Xanthium strumarium L. sin. X. italicum Moretti, and six species of cultivated plants: wheat (Triticum aestivum L.), sunflower (Helianthus annuus L.), barley (Hordeum vulgare L.), rape (Brassica napus ssp. oleifera L.), Spanish trefoil (Medicago sativa) and corn (Zea mays). The experiments consisted in the application of treatments with water extracts derived from roots, leaves, stems, dried leaves and fruits of X. strumarium on the seeds of the six cultivated plants. The obtained results show the presence of some chemical compounds with inhibiting allelopathic effect in all tested water extracts. The tested plants showed high sensitivity, the obtained results of the plants' germination and growing showed significantly negative differences and highly significant negative differences.

The conclusions of this work completely justifies the setting down of new research objectives for discovering the chemical compounds of the extracts of X. strumarium which are manifesting so powerfully and the possibilities of their using.

Keywords: allelopathy, Xanthium strumarium, wheat, sunflower, barley, rape, Spanish trefoil, corn

INTRODUCTION

The species *Xanthium strumarium* L. sin *X. italicum* Moretti is part of the dangerous weeds widespread in the whole world. One of the reasons that leads to its classification within this category is related to the presence of some chemical compounds with allelopathic character which manifested during several experiments made with water extracts on some species of cultivated plants and weeds (Dávid et al. 2005).

Allelopathy defines the field studying the contradictory (opposed) relations between different species of plants, but also between individuals from the same species. The chemical compounds taking part to the interactions between plants are generally called allelopathic substances, playing a role in the primary metabolic processes, essential for the plants surviving. (Rice 1974).

The allelopathic effects are produced by chemical substances released in the outer environment by the plant. Among the allelochemical products the predominant are the alkaloids, phenols, terpens and glycosids. Most of the allelochemical substances are initially found in the plants body, in inactive form. Following some subsequent transformations like hydrolisis, oxidoreduction, methylation or demethylation, etc. there are generated new products, with specific allelopathic properties (Whittaker and Feny 1971, quoted by Corbu 2007).

According to some authors (Calera et al. 1995; Einhellig 1995 and 1999; Blum et al. 1999; Reigosa et al.. 1999; Macias et al. 1999) the allelopathic compounds are affecting the cellular division of competitive plants, the phytohormones activity, the functional efficiency of chloroplasts and mitochondria, the enzymatic dynamics, the biomembranes function, the plant – water relationship and other various processes of plants. The idea of the influence exercised by some chemical substances released in the environment by some organisms, on other neighbouring organisms, has been given out by De Candolle in 1832 who reached to the conclusion that all plants excrete through their roots some substances that may stimulate or inhibit the other plants growing (Corbu 2007).

Molisch, in 1937, published the researches results related to ethylene action on some superior plants, phenomenon named by him *„Allelopathie"*. He is also the first person who gives a definition of the term allelopathy, which meant at that time, the biochemical interactions established between all types of plants, the same notion including the harmful as well as the stimulating interactions (Uludag et al. 2006).

A real progress in understanding the allelopathy phenomenon took place in 1974 as a result of publishing the book *„Allelopathy*" by Rice, where the author defines the allelopathy as the harmful effect exercised by a plant on other plant by producing some chemical compounds that are released and spread in the surrounding environment (Hodişan 2009).

Results of researches related to similar studies mentioned in this paper showed that the species *X. strumarium* excrets allelochemical compounds that are influencing both the seeds germination as well as the development of the vegetative mass of the cultivated plants and weeds (Bozsa and Oliver 1993; Sondhia and Saxena 2003; Sinha and Samart 2004; Dávid et al. 2005; Tanveer et al. 2008).

MATERIALS AND METHODS

There have been gathered plants of *X. strumarium* during the vegetation period, from which the vegetative organs, leaves, roots, stems and fruits have been separated, as well as dried plants from which only the leaves have been gathered in.

In order to prepare the water extracts there have been weighed 100 g of vegetal material (roots, stems, leaves, dried leaves and fruits) which has been minced and left to rest, to macerate, in 500 ml distilled water for 48 hours at room's temperature, that is 22-25°C. The obtained concoction was decanted and filtered, afterwards it has been kept in darkness at 4-5°C temperature until using it.

The allelopathic effect of the prepared water extracts was studied on the seeds germination and plants growing immediately after their emerging at different species cultivated in the spring, autumn or late summer: wheat (*Triticum aestivum* L.), sunflower (*Helianthus annuus* L.), barley (*Hordeum vulgare* L.), rape (*Brassica napus* L.), Spanish trefoil (*Medicago sativa*) and corn (*Zea mays*).

The seeds of the studied cultivated species were left to germinate, that is 100 grains, in ceramic vessels, in sand. The germinating sublayer was obtained by sand calcination in the drying stove at the temperature of 180-200°C, afterwards is has been passed through sieves of 0,3 mm in order to uniform the grains and it has been rehydrated by adding 100 ml water extract to 1000 g of sand. For each vessel of germination there have been used equal quantities of sand, in order to maintain the laying depth of the seeds to 1 cm.

For the untreated control variant, the procedure was identical excepting that for the sand rehydration there has been used distilled water.

The seeds germination took place at the temperature of 22-25°C in conditions of natural luminosity with specific diurnal variations.

There have been carried out three repetitions for each experimental variant.

The results reading for determining the germinative energy was carried out after four days from leaving the seeds for germination, by counting the sprung grains, and for the biometric determinations, the results reading was carried out after seven days by measuring the plants' height.

The data obtained following the observations and measurements have been statistically interpreted.

RESULTS AND DISCUSSIONS

Results obtained for the wheat (*Triticum aestivum* L.)

It has been observed a powerful effect of inhibition of wheat seeds germination after the application of water extracts treatments, in all experimented cases. The percentage differences of germination are significantly negative, having been registered values of 69,1% up to 42,4% against the untreated control. Our attention has been drawn by the values registered in the cases where the treatments were carried out with extracts derived from dried leaves of *X. strumarium* when the percentage of germinating grains was of 7,9% against the untreated control (*Table 1*).

The germination differences are wholly verified by the assessment according to multiple comparison test, Duncan test.

Concerning the influence of water extracts derived from different organs of *X. strumarium*, on the wheat plants' height after emerging, there have been found significant negative differences in all experimented cases (*Table 2*).

Table 1

Influence of the extract of *Xanthium strumarium* L.on the wheat seeds germination (*Triticum aestivum* L.)

Symbol	Extract Provenience	Germinated Grains (pcs.)	Germinated Grains (%)	Difference	Differences significance	Duncan Classification
E1	Untreated control	92,67	100,0	0,00	Mt.	E
E2	Roots	54,00	58,3	-38,67	000	C
E3	Leaves	39,33	42,4	-53,33	000	B
E4	Stems	60,00	64,7	-32,67	000	D
E5	Dried leaves	7,33	7,9	-85,33	000	A
E6	Fruits	64,00	69,1	-28,67	000	D
	DL (p 5%)			4,79		
	DL (p 1%)			6,81		
	DL (p 0.1%)			9,86		

Table 2

Influence of the extract of *Xanthium strumarium* L.on the wheat plants' height (*Triticum aestivum* L.) after emerging

Symbol	Extract Provenience	Plants Height (cm)	Plants Height (%)	Difference	Differences significance	Duncan Classification
E1	Untreated control	2,83	100,0	0,00	Mt.	C
E2	Roots	1,00	35,3	-1,83	000	A
E3	Leaves	1,17	41,2	-1,67	000	AB
E4	Stems	1,67	58,8	-1,17	000	B
E5	Dried leaves	1,17	41,2	-1,67	000	AB
E6	Fruits	1,17	41,2	-1,67	000	AB
	DL (p 5%)			0,50		
	DL (p 1%)			0,71		
	DL (p 0.1%)			1,03		

The height differences show percentage values of 35,3% increasing up to 58,8% against the height of the untreated control. The plants' height decreasing as a result of the allelopathic effect of the extracts resulted from various vegetative organs of species *X. strumarium* may be repeated in field conditions as a result of decomposition of the weed's vegetal remaining with most unfavorable effects for the evolution of wheat vegetation after emergence in the field.

The plants' growth slowdown after emerging may alter the processes of union and preparing of wheat crops for wintering through hardening and delay the optimum stage of vegetation when going into winter.

Results obtained for sunflower (*Helianthus annuus* L.)

The allelopathic effect of extracts of roots, leaves, stems, dried leaves and fruits of *X. strumarium* is also powerfully manifested on germination of seeds of *H. annuus*. According to the data mentioned in table 3, the germination due to the extracts' inhibiting effect ranges between 86,1% and 28,7% representing significant differences from the untreated control variant (table. 3).

Table 3

Influence of the extract of *Xanthium strumarium* L.on the sunflower seeds germination (*Helianthus annuus* L.)

Symbol	Extract Provenience	Germinated Grains (pcs.)	Germinated Grains (%)	Difference	Differences significance	Duncan Classification
E1	Untreated control	81,33	100,0	0,00	Mt.	E
E2	Roots	58,00	71,3	-23,33	000	C
E3	Leaves	35,33	43,4	-46,00	000	B
E4	Stems	70,00	86,1	-11,33	000	D
E5	Dried leaves	23,33	28,7	-58,00	000	A
E6	Fruits	57,33	70,5	-24,00	000	C
	DL (p 5%)			3,89		
	DL (p 1%)			5,52		
	DL (p 0.1%)			8,00		

In the case of E5 variant when the treatments were performed with extracts derived from dried leaves, the germination against the untreated control is of 28,7%. The germination differences are wholly verified through the assessment according to multiple comparison test, Duncan test.(*Table 3*).

Likewise, the obtained values of *H. Annuus* plants' height immediately after emerging, show highly significant negative differences against the untreated control (*Table 4*).

E5 Variant shows again the biggest difference, the sunflower plants' height treated with extract derived from dried leaves is only of 24,0% against the height of the untreated control.

Results obtained for barley (*Hordeum vulgare* L.)

The treatments performed with water extracts derived from vegetative organs of *X. strumarium* on the germination of barley seeds, have significant negative effects on all tested variants.

It comes back to our attention the E5 variant where there has been registered the biggest difference against the untreated control, the percentage of the germinated grains was only 6,8% (*Table 5*).

Table 4

Influence of the extract of *Xanthium strumarium* L. on the sunflower plants height (*Helianthus annuus* L.) after emerging

Symbol	Extract Provenience	Plants Height (cm)	Plants Height (%)	Difference	Differences significance	Duncan Classification
E1	Untreated control	4,17	100,0	0,00	Mt.	D
E2	Roots	2,17	52,0	-2,00	000	B
E3	Leaves	2,00	48,0	-2,17	000	B
E4	Stems	2,83	68,0	-1,33	000	C
E5	Dried leaves	1,00	24,0	-3,17	000	A
E6	Fruits	3,00	72,0	-1,17	000	C
	DL (p 5%)			0,36		
	DL (p 1%)			0,51		
	DL (p 0.1%)			0,74		

Table 5

Influence of the extract of *Xanthium strumarium* L. on the barley seeds germination(*Hordeum vulgare* L.)

Symbol	Extract Provenience	Germinated Grains (pcs.)	Germinated Grains (%)	Difference	Differences significance	Duncan Classification
E1	Untreated control	78,00	100,0	0,00	Mt.	E
E2	Roots	67,33	86,3	-10,67	000	D
E3	Leaves	35,33	45,3	-42,67	000	B
E4	Stems	67,33	86,3	-10,67	000	D
E5	Dried leaves	5,33	6,8	-72,67	000	A
E6	Fruits	57,33	73,5	-20,67	000	C
	DL (p 5%)			3,95		
	DL (p 1%)			5,61		
	DL (p 0.1%)			8,12		

On the barley plants' height the extracts derived from roots, leaves, stems, dried leaves and fruits of *X. strumarium* show values whose percentage difference against the untreated control is highly significant negative (*Table 6*).

The height differences are wholly verified through the assessment according to multiple comparison test, Duncan test.

Table 6

Influence of the extract of *Xanthium strumarium* L.on the barley plants height (*Hordeum vulgare* L.) after emerging

Symbol	Extract Provenience	Plants Height (cm)	Plants Height (%)	Difference	Differences significance	Duncan Classification
E1	Untreated control	4,00	100,0	0,00	Mt.	F
E2	Roots	3,32	83,2	-0,66	000	E
E3	Leaves	2,33	58,3	-1,67	000	B
E4	Stems	3,33	83,3	-0,67	000	D
E5	Dried leaves	1,50	37,5	-2,50	000	A
E6	Fruits	3,00	75,0	-1,00	000	C
	DL (p 5%)			0,32		
	DL (p 1%)			0,45		
	DL (p 0.1%)			0,66		

Results obtained for rape (*Brassica napus* L.)

The rape seeds' germination is also strongly affected after the treatments with extracts derived from different organs of the species *X. strumarium*. In all tested variants the obtained values show significant differences against the untreated control (*Table 7*).

Table 7

Influence of the extract of *Xanthium strumarium* L.on the rape seeds germination (*Brassica napus* L.)

Symbol	Extract Provenience	Germinated Grains (pcs.)	Germinated Grains (%)	Difference	Differences significance	Duncan Classification
E1	Untreated control	72,33	100,0	0,00	Mt.	E
E2	Roots	17,67	24,4	-54,67	000	D
E3	Leaves	7,67	10,6	-64,67	000	B
E4	Stems	13,67	18,9	-58,67	000	C
E5	Dried leaves	0,00	0,0	-72,33	000	A
E6	Fruits	7,33	10,1	-65,00	000	B
	DL (p 5%)			3,18		
	DL (p 1%)			4,52		
	DL (p 0.1%)			6,55		

This time, in the case of the treatment with extract derived from dried leaves, variant E5, the germination was of 0%, the allelopathic effect has been manifesting very strongly.

The rape plants, immediately after emergence, prove to be very sensitive to the allelopathic effect of all used extracts (*Table 8*).

The statistical results show a stopping in height increase of rape little plants, immediately after emerging, their height is only 17,6% against the height of the untreated control, the differences being very significant. (*Table 8*).

Otherwise, the rape is known as a very sensitive plant to most of the herbicides (substances that inhibit in various ways the development and multiplication of the vegetal cells) this is why the present results are not such a big surprise. It must be further studied, through the modern means available nowadays, which of the chemical compounds of extracts of *X. Strumarium* do so strongly manifest on the rape.

Table 8

Influence of the extract of *Xanthium strumarium* L.on the rape plants height (*Brassica napus* L.) after emerging

Symbol	Extract Provenience	Plants Height (cm)	Plants Height (%)	Difference	Differences significance	Duncan Classification
E1	Untreated control	2,83	100,0	0,00	Mt.	C
E2	Roots	0,50	17,6	-2,33	000	B
E3	Leaves	0,50	17,6	-2,33	000	B
E4	Stems	0,50	17,6	-2,33	000	B
E5	Dried leaves	0,00	0,0	-2,83	000	A
E6	Fruits	0,50	17,6	-2,33	000	B
	DL (p 5%)			0,21		
	DL (p 1%)			0,31		
	DL (p 0.1%)			0,44		

Table 9

Influence of the extract of *Xanthium strumarium* L.on the Spanish trefoil seeds germination (*Medicago sativa*)

Symbol	Extract Provenience	Germinated Grains (pcs.)	Germinated Grains (%)	Difference	Differences significance	Duncan Classification
E1	Untreated control	84,67	100,0	0,00	Mt.	F
E2	Roots	61,33	72,4	-23,33	000	D
E3	Leaves	38,67	45,7	-46,00	000	B
E4	Stems	70,67	83,5	-14,00	000	E
E5	Dried leaves	6,00	7,1	-78,67	000	A
E6	Fruits	46,67	55,1	-38,00	000	C
	DL (p 5%)			5,90		
	DL (p 1%)			8,39		
	DL (p 0.1%)			12,14		

Results obtained for Spanish trefoil (*Medicago sativa*)

The tests performed on the Spanish trefoil seeds' germination (*Table 9*) show a negative influence of the extracts of *X. strumarium*. The differences values are between 83,5% and 45,7% and in the case of variant E5 these are reduced to only 7,1% against the untreated control, which show significant negative differences.

The results obtained after the treatments with extracts of *X. strumarium* on the Spanish trefoil plants' height after emerging (table 10) show distinctly significant negative differences in the case of variants E2, E4 şi E6. The highly significant negative differences were obtained in the case of the treatments with extracts derived from leaves (E3) and dried leaves (E5).

Table 10

Influence of the extract of *Xanthium strumarium* L.on the Spanish trefoil plants height (*Medicago sativa*) after emerging

Symbol	Extract Provenience	Plants Height (cm)	Plants Height (%)	Difference	Differences significance	Duncan Classification
E1	Untreated control	3,17	100,0	0,00	Mt.	D
E2	Roots	2,31	73,5	-0,82	00	C
E3	Leaves	1,17	36,8	-2,00	000	B
E4	Stems	2,33	73,7	-0,83	00	C
E5	Dried leaves	0,33	10,5	-2,83	000	A
E6	Fruits	2,17	68,4	-1,00	00	C
	DL (p 5%)			0,52		
	DL (p 1%)			0,73		
	DL (p 0.1%)			1,06		

Results obtained for corn (*Zea mays*)

The results obtained after the treatments with extracts of *X. strumarium* on the corn seeds' germination (*Table 11*) show distinctly significant negative differences in the case of variants E2, E4 and E6. Highly significant negative differences were obtained in the case of the treatments with extracts derived from leaves (E3) and dried leaves (E5).

Table 11

Influence of the extract of *Xanthium strumarium* L. on the corn seeds germination (*Zea mays*)

Symbol	Extract Provenience	Germinated Grains (pcs.)	Germinated Grains (%)	Difference	Differences significance	Duncan Classification
E1	Untreated control	94,00	100,0	0,00	Mt.	D
E2	Roots	85,32	90,7	-8,66	00	C
E3	Leaves	59,33	63,1	-34,67	000	B
E4	Stems	84,67	90,1	-9,33	00	C
E5	Dried leaves	34,67	36,9	-59,33	000	A
E6	Fruits	85,33	90,8	-8,67	00	C
	DL (p 5%)			5,65		
	DL (p 1%)			8,03		
	DL (p 0.1%)			11,63		

The corn plants' height immediately after emerging has been influenced after the treatment with extracts of *X. strumarium*, distinctly significant negative differences have been obtained in the case of variants E2, E4 and E6 (*Table 12*).

The treatments performed with extracts derived from leaves and dried leaves showed significant negative differences against the untreated control.

The results of the experiments with water extracts derived from vegetative organs (roots, leaves, stems, dried leaves and fruits) of species *X. strumarium* show the presence of some chemical compounds with inhibiting allelopathic effect in all tested variants.

The six cultivated plants species that were tested: wheat (*Triticum aestivum* L.), sunflower (*Helianthus annuus* L.), barley (*Hordeum vulgare* L.), rape (*Brassica napus ssp. oleifera* L.), Spanish trefoil (*Medicago sativa*) and corn (*Zea mays*) had manifested sensitivity to the treatments applied in all tested variants.

The seeds' germination was inhibited in all tested variants, the differences against the untreated control had been significant, registering percentage values of 86,3% up to 6,8% and 0,0% for rape when the treatment had been performed with extract from dried leaves. The corn showed a better tolerance in all variants of treatment with extracts from roots, stems and fruits, in these cases the differences have been distinctly significant.

Table 12

Influence of the extract of *Xanthium strumarium* L. on the corn plants height (*Zea mays*) after emerging

Symbol	Extract Provenience	Plants Height (cm)	Plants Height (%)	Difference	Differences significance	Duncan Classification
E1	Untreated control	3,50	100,0	0,00	Mt.	D
E2	Roots	2,67	76,2	-0,83	00	C
E3	Leaves	2,33	66,7	-1,17	000	B
E4	Stems	2,68	76,3	-0,84	00	C
E5	Dried leaves	1,50	42,9	-2,00	000	A
E6	Fruits	2,67	76,2	-0,83	00	C
	DL (p 5%)			0,46		
	DL (p 1%)			0,65		
	DL (p 0.1%)			0,95		

The plants growing was inhibited, as well as the germination, in all tested variants, the percentage value of the little plants height against the untreated control was between 83,3% and 10,5%. These percentage values showed highly significant differences at wheat, sunflower, barley and rape, whereas at the Spanish trefoil and corn there had been registered highly significant differences in the case of treatment variants with extracts from leaves and dried leaves and distinctly significant differences in the variants of treatment with extracts from roots, stems and fruits.

Among the tested plants, the rape showed the highest sensitivity both at seeds' germination as well as at plants' growing, and the corn showed a better tolerance both at seeds' germination as well as at little plants' vegetative development.

Among the tested water extracts, those derived from green leaves and dried leaves had the highest inhibiting effect both in germination as well as in little plants growing, the differences against the untreated control were highly significant in all experimented variants.

There must be further studied, through the modern means available nowadays, which of the chemical compounds of extracts of *X. Strumarium* do so strongly manifest and the possibilities of their using.

From agrotechnical point of view, the obtained results, confirm the dominant character of the species *X. strumarium* and clarifies the reason why this species highly dominates significant areas of uncultivated lands, as well as its presence in the agricultural crops. Therefore, it is recommended that the lands infested with *X. strumarium* should be preserved clean at least for one year before cultivating the land, and in crop-rotation it is recommended that the first crop should be the corn, taking into consideration the increasing of the seed standard per hectare to 15-20%.

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Distribution of weed seeds in sugar beet and maize crops

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SUMMARY

Seed weed bank provides unique data on potential population of particular weed species at various soil depths. Seeds of weeds can initiate their invasion on agricultural areas, which makes timely control of this weedy vegetation in different crops important. Seed bank of weeds distributes horizontally and vertically in arable soil level. In autumn, after harvesting of the crops by machines, weed of weeds is brought out to the soil surface and represents potential weed seed bank. Crop varieties, as well as the type and tillage have the greatest influence on the quantity of weed seeds. In 2008 at locality Backi Maglic studies on distribution, quantity and expansion of weed seeds at soil depths of 0-10, 10-20 and 20-30 cm were performed according to methods of Conn, (1987) and Shartt, (1998). Soil samples were taken from sugar beet and maize crops after sowing and before emergence of crops and after lifting of the beet, i.e. maize harvest. Data obtained from the soil depth of 20-30 cm, after sowing and before shooting sugar beet crop showed greater presence of Amaranthus retroflexus seeds in the quantity of 728 seeds per m^2, Chenopodium album seeds in the quantity of 450 seeds per m^2, and lower quantity of Solanum nigrum seeds in the quantity. In fields under maize, after sowing and before emergence, at soil depth of 10-20 cm, the greater quantity of Amaranthus retroflexus seed 181 seeds per m^2 was established, as well as Chenopodium album seeds (225 seeds per m^2), Datura stramonium (262 seeds per m^2) and Solanum nigrum (275 seeds per m^2) at soil depth of 0-10 cm. After harvest, i.e. lifting of the beet, significant reduction in number of weed seeds was recorded in all studied soil layers.

Keywords: weed seeds, sugar beet, maize, seed bank

INTRODUCTION

Weed seed bank represents essential part of plant communities for they significantly contribute to ecological processes. The ability of vegetation regeneration is mainly contributed to seeds of weed populations in soil (Davis, 2006). Control of weed seed bank includes herbicide application, timely soil cultivation, as well as the forecast of weed occurrence. Monitoring of weed seed bank is of extraordinary significance for more reasons, primarily for weed seedlings can be easily observed, they are very susceptible to timely soil cultivation and can be very concurrent toward crop if uncontrolled (Gunsolus and Buhler 1999). From that reason, forecast of weed occurrence, their timely determination and control are extremely important, especially in regimes of reduced herbicide use (Barberi, 2002).

Seed bank is the main cause of weed persistence on agricultural areas (Cousens and Mortimer, 1995). During harvest at agricultural areas weed seed remains on the plant and machine cultivation brings it to the soil surface where it represents future weed bank (Konstantinovic, 2008). Quantity and density of weed seeds in greatest measure depend upon soil type, previous pre-crops, soil tillage, as well as from herbicide use (Konstantinovic et al., 2008). Weed seeds density can influence to crops, but herbicide use in the phases of weeds' germination and emergence can reduce size of the seed bank (Taylor and Hartzler, 2000). Weed seed bank influence to distribution of annual (Steinmann and Klingebiel, 2004), as well as perennial weed species (Blumenthal and Jordan, 2001), which causes the spreading of weed community during years. In the last years, at the territory of Serbia studies of seed bank under different crops were performed. During 2006 and 2007, at localities Vajska and Kikinda, under maize greater presence of certain weed species such as *Polygonum lapathifolium, Amaranthus retroflexus, Sinapis arvensis, Solanum nigrum, Chenopodium album* and *Abutilon theophrasti* was established (Konstantinovic et al., 2008).

MATERIALS AND METHODS

In 2008 from the locality Backi Maglic soil samples from sugar beet and maize crops were taken. At the same locality soil was sampled twice, for the first time after sowing and before emergence of crops, and the second time after lifting the beets, i.e. maize harvest. Samples were taken from different arable soil layer depths, from 0-10 cm, 10-20 and 20-30 cm by methods of Conn (1987) and Shartt (1998). In the laboratory conditions, soil samples were sieved through sieves of various diameters. This was followed by seed abstraction and determination of weed seeds (Skender et al., 1998).

RESULTS AND DISCUSSION

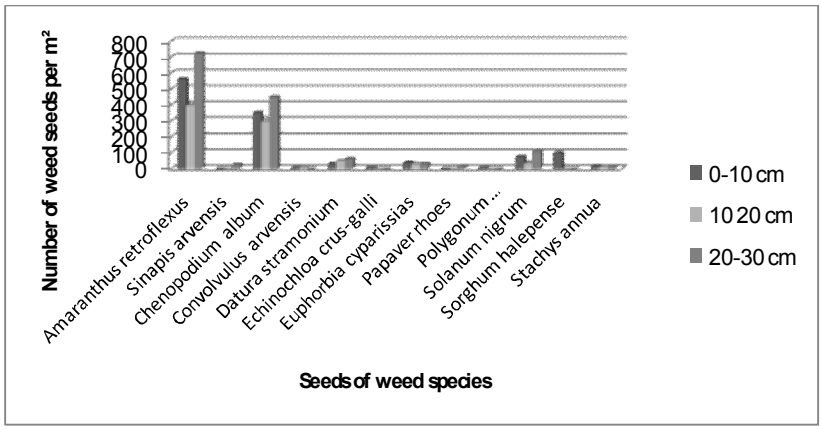
In the Republic of Serbia the basic row material in sugar processing industry is sugar beet that is grown in Vojvodina on 80-10 000 ha annually. Sugar beet is broadcast summer raw crop and it gives the highest yield per unit of area. Therefore, it is human labor and machine work demanding, especially in weed control. Typical

weeds that occur in sugar beet crops are *Abuthilon theophrast, Ambrosia artemisiifolia, Amaranthus retroflexus, Chenopodium album,Datura stramonium, Echinochloa crus-galli, Setaria glauca, Setaria viridis* and *Sorghum halepense* (Konstantinovic, 1999).

Maize is also broadcast crop with great distance between rows and plants in the raw. In early development phases it grows slowly which enables emergence and development of weeds and early establishment of weed comunity in maize (Konstantinovic, 1999). Weed comunity in maize is typical for row crops and floristically very rich. It comprises of 150 weed species that do not have equal significance in weed infestation. In building of the characteristic weed comunity participate only small number of species such as *Abuthilon theophrast, Ambrosia artemisiifolia, Amaranthus retroflexus, Chenopodium album, Cirsium arvense, Convolvulus arvensis, Cynodon dactylon, Digitaria sanguinalis, Hibiscus trionum, Rubus caesius, Echinochloa crus-galli, Polygonum aviculare, Polygonum lapathifolium, Polygonum persicaria, Setaria glauca, Setaria viridis, Solanum nigrum* and *Sorghum halepense* (Konstantinovic, 1999).

At all studied localities seeds of 17 weed species were determined, of which in maize crop 13 and sugar beet crop 15 weed species. In both crops occur seeds of the following weeds: *Amaranthus retroflexus* L, *Calystegia sepium* L, *Chenopodium album* L, *Datura stramonium* L, *Euphorbia cyparissias* L, *Hibiscus trionum* L, *Sinapis arvensis* L, *Solanum nigrum* L, *Sorghum halepense* L, *Polygonum lapathifolium* L. and *Stachys annua* L. In maize crop seeds of weed species *Convolvulus arvensis* L, *Echinochloa crus-galli* L, *Papaver rhoeas* L. and *Capsicum annum* L. and in sugar beet crop *Panicum capilare* L. and *Veronica hederifolia* L. were also determined.

Figure 1: **Seeds of weed species at locality Backi Maglic after sowing and before emergence in sugar beet crop at various soil depths in 2008**



* Studied soil depths (0-10 cm, 10-20 cm and 20-30 cm) are given in different shadows

In sugar beet crop, after sowing and before emergence of the crop at the locality Backi Maglic at soil depth of 0-10 cm greater number of seeds of the weed species *Amaranthus retroflexus* L (562 seeds per m^2) and *Chenopodium album* L. (350 seeds per m^2) were determined; in the soil layer at depth of 10-20 cm seeds of weed species *Amaranthus retroflexus* L. (400 seeds per m^2) and *Chenopodium album* L. (300 seeds per m^2) were established, while in the soil layer at depth of 20-30 cm the highest number of seeds of weed species *Amaranthus retroflexus* L. (725 seeds per m^2) and *Chenopodium album* L. (450 seeds per m^2) were found (*Figure 1*).

At the same locality, after lifting of the beets at the soil depth of 0-10 cm, greater presence of seeds of weed species *Amaranthus retroflexus* L. (339 seeds per m^2) and *Chenopodium album* L. (214 seeds per m^2) was determined. At the soil depth of 10-20 cm higher quantity of seeds of weed species *Amaranthus retroflexus* L. (340 seeds per m^2) and *Chenopodium album* L. (228 seeds per m^2) was established, while at soil depth of 20-30 cm seeds of weed species *Amaranthus retroflexus* L. proved to be dominant with 368 seeds per m^2 and *Chenopodium album* L. with 223 seeds per m^2 (*Figure 2*). Although seeds of *Amaranthus retroflexus* and *Chenopodium album* were dominant at this locality before, as well as after lifting the beets, significant reduction in seed quantity was noticeable at all studied soil layers.

Figure 2: **Seeds of weed species at locality Backi Maglic after lifting the beets at various soil layers in 2008**

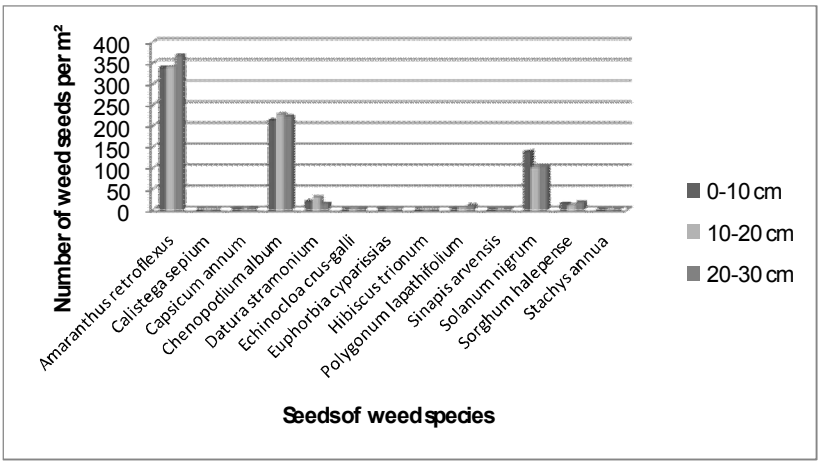


Figure 3: **Seeds of weed species at locality Backi Maglic after sowing and before emergence in maize crop at various depths in 2008**

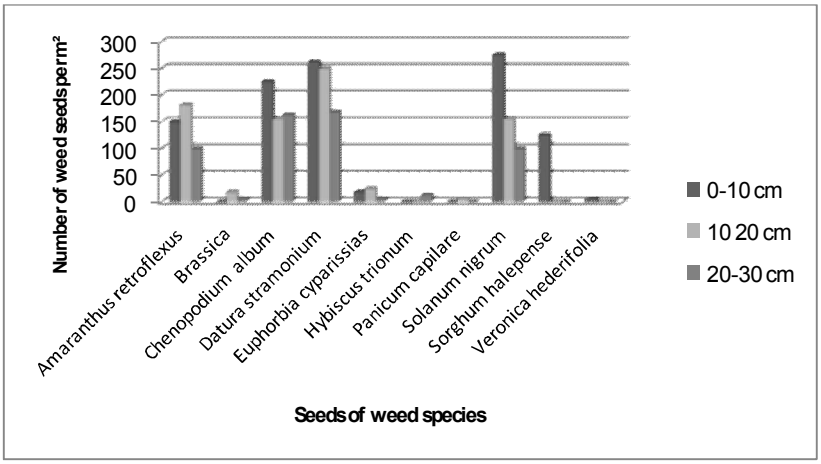
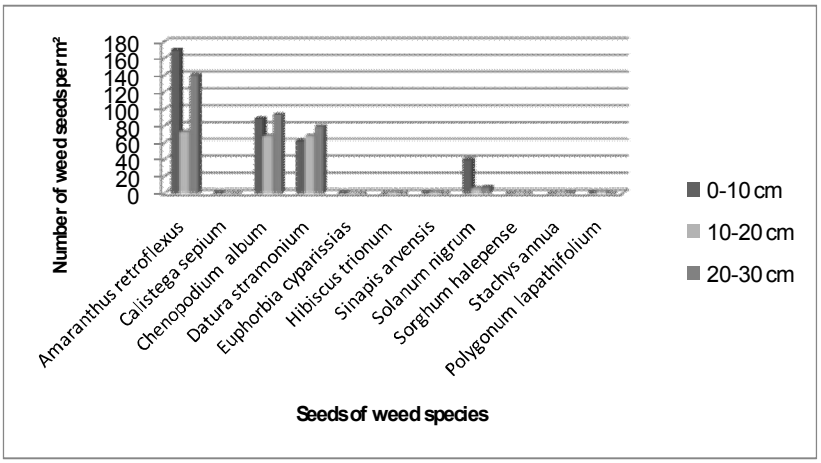


Figure 4: **Seeds of weed species at locality Backi Maglic after harvest in maize crop at various soil depths in 2008**



The analysis of the soil samples that were taken from the same locality after sowing and before maize emergence at soil depth of 0-10 cm showed that the highest number of seeds had weed species *Solanum nigrum* L with 275 seeds per m^2 and *Datura stramonium* L. with 262 seeds per m^2. At the soil layer of 10-20 cm greater number of *Datura stramonium* L. seeds (250 seeds per m^2) and *Amaranthus retroflexus* L. seeds (181 seeds per m^2) were found, and at soil layer of 20-30 cm great number of weed species *Datura stramonium* seeds was determined (168), as well as *Chenopodium album* L. in the quantity of 162 seeds per m^2 (*Figure* 3).

At the same locality, after harvest of the maize, the following results were obtained: the highest number of seeds per m^2 at soil depth of 0-10 cm was found for weed species *Amaranthus retroflexus* L. (170) and *Chenopodium album* L. (89). At soil depth of 10-20 cm higher number of seeds of weed species *Amaranthus retroflexus* (73 seeds per m^2), and the identical number of seeds, i.e. 68 seeds per m^2 were found for weed species *Chenopodium album* L. and *Datura stramonium* L. At soil depth of 20-30 cm the highest number of seeds of weed species *Amaranthus retroflexus* L. m^2 (140 seeds per m^2) and *Chenopodium album* L. (93 seeds per m^2) was determined (*Figure 4*). The observed significant reduction in seed quantity of all determined weed species, after harvest of maize can be assigned to efficient weed control measures during vegetation, i.e. proper soil cultivation and other cultural practices such as weeding, optimal sowing density, herbicide application, etc.

Figure 5: **Soil sampling**

CONCLUSIONS

Seed bank represents potential weed community. By production of higher seed number, weeds ensure their survival in changeable and inconvenient environmental conditions, securing by this efficient dispersal. Studies of weed seeds and determination of their number is very important for it is useful in their control, as well as for timely determination of weed control measures, as well as herbicide use. Data processing during experimental period showed that there exist certain potential of weed seed bank in soil. Application of cultural practice and chemical measures in weed control has great impact to reduction of weed seed bank. After maize harvest and lifting the beets, significant reduction of weed seed bank in soil was evidenced.

At locality Backi Maglic, in maize crop, after sowing and before crop emergence by determination of weed seeds in various soil layers, significant number of weed species *Solanum nigrum* L. seeds was established (275 seeds per m^2) at soil depth of 0-10 cm, while seeds of weed species *Datura stramonium* L. in greater number was found at layers of 10-20 and 20-30 cm. In autumn, after maize harvest, domination of weed species *Amaranthus retroflexus* L. seeds was determined in all three soil layers, but in lower extent than before sowing. In sugar beet crop, after sowing and before crop emergence, as well as after lifting of the beet, seeds of weed species *Amaranthus retroflexus* L. and *Chenopodium album* L. at all studied soil layers proved to be dominant. However, after vegetation significant reduction in seed quantity in soil was again established.

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The impacts of the effective herbicide treatments on the flowering and some morphological parameters of the culinary type sunflower

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SUMMARY

The treatments of the experiment were: 1./ hoeing 2 times; 2./hoeing once + ridged once; 3./ Weed control; 4./Dual Gold +Racer; 5./ Wing EC + Racer; 6./ Dual Gold+ Pledge; 7./ Wing EC + Pledge. We employed the herbicides by the proposed modes of the firms, that are: (Dual Gold 1.2 l/ha, Racer 2,2 l/ha, WingEC 3,5 l/ha preemergent; Pledge 50WP 0,080kg/ha postemergent). The employed sunflower was (Helianthus annuus var. macrocarpus L.) cultivar. Kisvárai). The row and plant distances were 70x35cm. The results are: weed status of the treatments - the hoed and ridged controls, Dual+Racer és Wing+Racer treated plots were practically free of weeds during the vegetation period. The weedy of plots treated with Dual+Pledge and Wing+Pledge were 2nd and 3rd degrees, the weedy of untreated plot was 9th degree. The most intensively flowered the ridged plot, the flowering intensity of herbicid treated plots were smaller than the mentioned, generally. The least intensively flowered treatment was the Wing+Racer. The plant heights were the best of untreated and earth up plots, the treatment of Dual+Racer was a bit smaller. At the rest ones the value of that parameter was smaller than the mentioned before. The order of the plate heights was same as the plant one. The diameter of the plate was the best of the hoed treatment, the values of the rest ones were practically the same. The least stalk breakage values were measured at the hoed and ridged treatments. Values of the weedy and herbicides treatments were more than twice as the ones mentioned before.

Keywords: herbicide treatment, sunflower

INTRODUCTION

The goal of the study to show, what kind of consequences come into being with the wide scale used herbicides in the case of an open pollinated sunflower variety. Weeds and its hazards in the sunflower production: *Amaranthus retroflexus* is well-known in Hungary because of its herbicide sensitivity doesn't cause many problems (Ulinici, 1977). The *Xanthium italicum* is relatively rarely, but very dangerous because of its relative herbicide resistance. The *Ambrosia artemisiifolia*, the *Datura stramonium* and the *Avena fatua*, are the most resistant weeds in the sunflower fields. The hazards of the *Xanthium italicum* and the *Ambrosia artemisiifolia* are the host plant of some sunflower diseases, (Rátainé, 2005). Against the perennial dicotyledons as *Rubus caesius, Cirsium arvense, Convolvulus arvensis, Symphytum officinale, Calystegia sepium and Persicaria amphibia* and especially some perennial monocotyledons *as Elymus repens*, syn. *Agropyron repens, Cynodon dactylon, Phragmites australis* there are many selective herbicides, but they can be employed only in the fore crop, or they are very expensive by the cause of their height prices and doses proposed (Németh, 2002). General facts of the sunflower weed controlling. Great part of herbicides posses positional selectivity, (Ulinici, 1977; Benécsné, 2005). Some active ingredient, e.g. fenuron, propizochlor, linuron depend of the organic material content of the soil. Other herbicides e.g. Pledge 50WP, Goal 2E, Goal 4F, Galigan 240EC need to work out soil surface well. For postemergent purpose there are only two chemicals for this purpose in Hungary, the Modown 4F and the Pledge 50WP, both of them have some phytotoxic effect on the plant. For the purpose stubble or preventive type of weed control against the perennial monocotyledons herbicides can be employed the a.i. glyphosate (Medallon Premium, Roundup Premium, Roundup Bioaktiv). To enhance their effects, previously is proposed to put 6–10 kg/ha ammonium-sulfate, or ammonium-nitrate type fertilizer in to the spraying solution. By the basic of the experiences, if the previous year there were no weed controlling, proposed to use the mechanical types controlling methods in the sunflower too (Benécsné, 2005). Some special aspects of the weed control: if in the sunflower fields, are only the next weeds: *Amaranthus-, Chenopodium- , Poligonum* species, *Sinapsis arvensis, Raphanus raphanistrum, Matricaria inodora, Setaria-* and *Echinocloa* species and some another late summer type weeds (Ulinici, 1977; Frank, 1999; Németh, 2002; Benésné, 2005) propose the next herbicides:

- presowing : with a.i. benefin, *Flubalex, Benefex 6,5-9,5 l/ha, or* trifluralin, Olitref 480EC, Triflurex 48EC, Treflan 48EC, Triflurex 26EC 2,3-3,5 l/ha selfly or frequently with a.i. fluorchloridon (Racer) 2,5–3 l/ha. Trifluralin gives no perfect effect if there are *Cruciferae, Compositae, Solanaceae and Malvaceae* species in the fields.
- preemergent: with a.i. linuron (Afalon Dispersion, Linurex 50WP) 1,5–2,0 illetve 1,5-3,0 l/ha, oxifluorfen, Goal 2E 0,8-1,0 l/ha, Goal 4F 0,4-0,5 l/ha, Galigan 240EC 0,8–1 l/ha, bifenox (Modown 4F), 1,8–2, 0l/ha, flumioxazin Pledge 50WP 0,08kg/ha, fluorchloridon (Racer) 2–3 l/ha, S-metolachlor (Dual Gold 960EC) 1,4–1,6 l/ha, dimetenamid (Frontier 900EC) 1,4–1,6 l/ha, pendimetalin (Stomp 330EC, Stomp 400EC, Panida 330EC) 3–5 l/ha, flufenacet (Tiara 60WG) 1,0l/ha, dimetenamid (Frontier 900EC) 1,4-1-6l/ha, acetochlor (Harness, Guardian Max, Trophy) 1,0-1,5-2,0l/ha. When there are a lot of *Datura stramonium* in the field, must use herbicide combination, e.g. presowing herbicides + any of the a.i. linuron (Afalon Dispersion), bifenox

(Modown 4F), or flumioxazin (Pledge 50WP). If there will *Ambrosia artemisiifolia* the fluorkloridon (Racer) is the most convenient herbicide. Against *Xanthium* species the most useful the next triplicate combinations: Racer + S-metolachlor or pendimetalin + propizochlor or dimetenamid.

-postemergent mode*:* - against the annual dicotyledons weeds - pre-post: bifenox (Modown 4F) 1,5 l/ha, or flumoxazin Pledge 50WP 0,08kg/ha. Against the annual and perennial monocotyledons weeds: can be used the fluazifop-P-butil (Fusilade Forte) 0,8–2,8 l/ha, quizalofop-P-etil (Targa Super) 0,7–3,5 l/ha, haloxifop-R-metilesther (Perennal) 0,4–1,5 l/ha, propaquizafop (Agil 100EC) 0,4–1,5 l/ha, quizalofop-p-tefuril (Pantera) 0,8–3,5 l/ha, cycloxidim (Focus Ultra) 1–4 l/ha.

By the idea of Hoffmann (2003) to spray the herbicides under the leaf is count a very effective methods in Hungary, because by this method can be control the dicotyledons type of weeds.

MATERIALS AND METHODS

Soil: Braun wood; pH: 4,6; humus-0,9%; K_A- 30-32, thick of the fertile lay- 45-50cm. Climate data: precipitation mm (mean of 57years): April 15-30: 26,4 (25,8); May 1-May 9: 4,4(15,8); May 10-31: 33,8 (41,3); vegetation-416,7(310). Temperature C^0 (mean of 57 years): April 15-30: 12,1 (11,8); May 1-May 9: 12,9(14,8); May 10-31: 17,7 (16,7); vegetation-18,6(17,8)

Net plots: length- 10 m, with- 2,1 m (3 row). Sowing date: 5 May. Arrangement: randomized block in four replication Treatments: controls: hoed-, ridged-, weedy; herbicide treated in : Dual Gold +Racer (1,2l/ha+2,2l/ha), Wing EC + Racer (3,5l/ha+2,2l/ha) Dual Gold +Pledge 50WP (1,2l/ha+0,08kg/ha), Wing EC +Pledge 50WP (3,5l/ha+0,08kg/ha). Time- and mode of treatments: preemergent- 7 of May; hoeing and ridging: 24, 31 of May; postemergent - 2. of June: Pledge 50WP. Measurements and its dates: phytotoxicity and the weeds two weeks after the preemergent treatment, two weeks after the postemegent treatment and just before the harvesting. Plant number counting in the beginning of the flowering. Checking the flowering: July of 23, 28, 31 and 2 of August. Desiccation before the harvest, was not employed. Plant and plate height was measured before the harvest. Harvest date: 17. of September. Samples: 4x5 piece plate/treatments. Labor activities: stalk parameters of 9-10 plant/ treatment, was measured during November by precision instrument – mortise gauge.

RESULTS AND DISCUSSION

Flowering

There were not significant differences among the treatments. The flowering of the control treatments was more intensive as the herbicide treated ones. The differences at the last two dates were about 4%. Among the control the most intensively flowered among the herbicide treated the Dual+Racer did so. By the basic of treatment groups can be stated that the preemergent and Dual typed ones flowered more quickly than the pre. + post or Wing EC types ones, *Table 1*.

Table 1

The weedig and flowering parameters by the treatments, Kisvárda 2008

Treatment	Weed	Flowering %			
		july			august
	1-9	23	28	31	2
Hoed	1	5,93	27,54	50,42	70,76
Ridged	1	8,39	37,23	57,30	70,07
Weedy controll	9	5,12	21,26	49,21	70,87
Dual+ Racer	1	9,50	30,58	49,59	69,83
Wing+Racer	1	5,26	25,44	43,42	61,40
Dual+ Pledge	3	6,46	29,28	51,71	67,30
Wing+ Pledge	2	7,00	27,24	52,14	67,32
Mean total		**6,81**	**28,37**	**50,54**	**68,22**
Ds 5%		**Ns.**	**Ns.**	**Ns**	**Ns.**
Mean of controll		**6,48**	**28,68**	**52,31**	**70,57**
Mean of treated		**7,06**	**28,13**	**49,21**	**66,46**

Morphological parameters

There were not significant differences among the treatments. At the control types treatment the plant and plate height were more than by the herbicide treated ones. The extent of the stalk breakage and the stoop were more in the herbicide treated ones than the control ones. Among the control the highest plant- and plate height belong to the weedy one, the biggest plate diameter and the lowest stalk breakage belongs to the hoed one. The plate stoops were the same by all of the control. Among the herbicide treated stand the highest plant and plate height belongs to the Dual+Racer one, but the stalk breakage was one of the worst by this treatment almost reached the value of the weedy control one' value. By the extention of the stoop the more outstanding was the

Wing +Racer treatment. Exception of the stalk stoop the Dual type treatments were better in all the parameters than the Wing EC types. By the basic of height types values the preemergent treatments were better than the pre-post types ones. In the aspect of other parameters the differences are not outstanding, *Table 2*.

Table 2

Some important morphological parameters by the different treatments, Kisvárda 2008

Treatment	Plant	Plate		Stalk breakage %	Stalk stoop cm
	heigth cm		Diameter cm		
Hoed	349,5	334,7	20,0	13,6	14,8
Ridged	356,0	340,8	19,7	17,0	15,3
Weedy control	358,5	343,5	19,2	34,2	15,0
Dual+ Racer	357,8	343,0	19,5	32,5	14,8
Wing+Racer	334,8	316,5	19,0	30,3	18,3
Dual+ Pledge	352,3	335,0	19,3	29,6	17,3
Wing+ Pledge	346,8	331,0	19,1	27,5	15,8
Mean total	**350,79**	**334,92**	**19,39**	**26,39**	15,9
Ds 5%	**Ns**	**Ns**	**Ns**	**Ns**	Ns
Mean of control	**354,67**	**339,65**	**19,62**	**21,60**	**15,01**
Mean of treated	**347,88**	**331,38**	**19,22**	**29,98**	**16,50**

Root mass and the thickness of the plant part at the bottom of the stalk

There were significant differences among the treatments, e.g. between the ridged and weedy control and the Dual+Pledge and Wing+Pledge. By the treated stands both of the values were higher than by the control ones. Among the controls the least value of the root mass posses to the weedy. In the view of the thickness of the rind the lowest plant part the ridged control had the lowest value. By the herbicide treated stands the most outstanding values belong to the Dual+ Pledge and the Wing+ Pledge variants. In the Dual – Wing version the differences were most outstanding by the rind to the advantage of the Wing. To the premergent+postemergent treatments belongs better values both parameters, *Table 3*.

Table 3

Root and stalk parameters by the treatments, Kisvárda 2008

Treatment	Air dried root mass g/ plant	Rind thickness direct above the root mm
Hoed	57,0	10,40
Ridged	47,0	7,69
Weedy control	**41,0**	**10,98**
Dual+ Racer	49,0	9,61
Wing+Racer	54,0	11,02
Dual+ Pledge	70,0	10,45
Wing+ Pledge	66,0	12,59
Mean total	**54,9**	**10,39**
Ds 5%	17,7	2,19
Mean of control	**48,33**	**9,69**
Mean of treated	**59,75**	**10,92**

By the diameter of the four undermost plant parts it is difficult to say clean cut results. Among the control treatments all instances the hoed control gives the most outstanding values. Among the herbicide treated stands the results were almost the same by the Wing+Pledge. Generally by the control treatments the first and the third part were thicker the second and the fourth were thinner than by the herbicide treated ones. In relative of Dual – Wing EC and preemergent-preemergens+postemergent the situation is more clean cut, since the Wings and preemergens+postemergent treatments showed the better values. By the rind thickness of the four undermost plant part at the control treatments the hoed ones were the best by the basic of the first-, second- and the third parts' results. Clean cut values belong in the direction of Dual-Wing EC, because the stand of the last one's the rind were all instances (at all plant parts) were the thicker. By the stated differences of the direction of preemergent-preemergent+postemergent the values of the first two part were better by the preemergent, the values of the third and fourth part were better by the premergent+postemergent treatments.

CONCLUSIONS

By the instances of the variety of the Kisvárdai**,** the herbicide treatments cause the next significant differences: root mass of the Dual+Pledge and the Wing+Pledge were bigger than the root mass of the weedy control. The thickness of the bottom and the middle plant part were by all the herbicide treated stands than the one of the ridged' one. Absolutely the same situation can experience by the third plant part of the Wing + Racer and the Wing+Pledge treatments. The cause of the ridged treatments lower values is the surface transference in the time of the tillage creating. Notable phenomenon's, by the weedy control: decreasing of root mass, the diameter of the bottom plant part, flowering intensity and stalk breakage % increasing. Other phenomenon, at the weedy control the first three part' rind thickness values are smaller than at the hoed control.
Also notable that the values of stalk breakage apart from weedy control are big by the preemergent and especially the Dual treatments. These differences to the hoed and ridged control are not significant.

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