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Neuer Weg 22/23
06484 Quedlinburg
Tel. 0 39 46 / 47-244
Fax 0 39 46 / 47-202
E-Mail: bafz-al@bafz.de
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Contents

Investigation to evidence of *Polymyxa graminis* Led. in different *Hordeum* – species

Ute Kastirr
Federal Centre for Breeding Research on Cultivated Plants, Institute for Resistance Research and Pathogen Diagnostics, Theodor - Roemer- Weg 4, 06449 Aschersleben

Introduction

Polymyxa graminis is an obligate parasite of cereal roots and a vector for transmission of plant viruses of different genera of Potyviruses. This fungal vector transmits for example the Bymoviruses BaMMV, BaYMV and WSSMV and the Furoviruses SBCMV and SBWMV to barley, wheat and rye.
The controlling of these pathogens is possible with the help of resistance breeding only. The screening of several origins of resistance to the viruses and to the fungus is very important for a successful breeding. By means of multiple investigations of resistance to viruses we have a good knowledge. But the origins of resistance to *Polymyxa graminis* still remains unknown.
For this reason we have established different methods for *Polymyxa graminis* detection and compared the susceptibility of various *Hordeum* – species to this fungal vector.

Material and Methods

1. Production of inoculum

The inoculum for infection tests was produced by using resting spores from infected field plants. These *Polymyxa* isolates were multiplicated on *Hordeum* seedlings. The root flour was produced from dry roots 8 weeks after inoculation and used for inoculum of direct root inoculation.

2. Assessment of infection rate

The assessment of resting spores concentration in the roots by the microscopical analyse was monitored with the help of a scoring scale marked from 1 (1% up to 25%) to 3 (75% up to 100%).
The serological detection of the fungus was compared from 3 to 29 days after inoculation (dpi) by PTA-ELISA.
Molecular biological proof of *Polymyxa* was carried out using specific primers (Ward, E. and Adams, M.J.; 1998). The product of amplification by *Polymyxa* specific primers is an DNA fragment with the length of 300 bp.

3. Methods of *Polymyxa* evidence

Microscopically analysis

The classical method of fungus detection is the light microscopical analysis of different fungal thalli (resting spores, plasmodia, zoosporangium, zoospores) in plant roots. The microscopical analysis could be performed easily for the assessment of resting spores, which are formed 4 up to 8 weeks after infection. Other fungal thalli (plasmodia, zoosporangium) must be coloured for a reliable detection. The zoospores are very mobile and difficult to discover.

Serological proof

Moreover the fungus can be proofed using serological methods. The serological proof of *Polymyxa* is very important for an early and precise detection of different fungal thalli. For this reason polyclonal antiserums were produced. For the antigen preparation resting spores of *P. graminis* were separated, homogenised, pelleted and the supernatant was injected in rabbits. The immunisation followed by 3 injections at weekly intervals intramuscular and 3 measurements of antiserum titer weekly. One booster injection was given 5 months after the first immunisation. For the antiserum production the serum supernatant was precipitated , the sediment was dialysed and the antiserum was saturated with roots of healthy plants.
The cross reaction with other fungal pathogens was tested.
The antiserum was employed in the PTA – ELISA. The antigen was prepared in PBS and 0,2%dry milk (dm) and incubated for 4 h at 37°C. The blocking reaction was carried out in PBS and 5% dm for 30 min. at 37 °C
The antiserum was incubated in PBS and 2% dm for 2,5 h at 37°C. The conjugate incubation was carried out overnight at 4°C. The substrate reaction was performed for 1 h at room temperature.

Molecular biological evidence

Polymyxa specific primers for the molecular biological evidence of fungus specific DNA in inoculated plants different *Hordeum* species were used.

Results

Differences between the *Hordeum* species were observed in the susceptibility to the virus vector *Polymyxa graminis*. Compared to *H. vulgare* , the number of resting spores of *P. graminis* in the plant roots was lower in *H. spontaneum* and undetectable in *H. bulbosum* (Proeseler, G. et al., 1999). To confirm the result obtained

microscopically, the serological analyse and the *Polymyxa* specific DNA evidence in the course of 29 dpi were proofed to estimate the infection rate of *P. graminis* in these *Hordeum* species. A reliable serological detection of *Polymyxa* infection is possible 19 days after inoculation. After this time the fungus specific DNA fragment was found in the total DNA, isolated from roots of inoculated plants too (Figure).

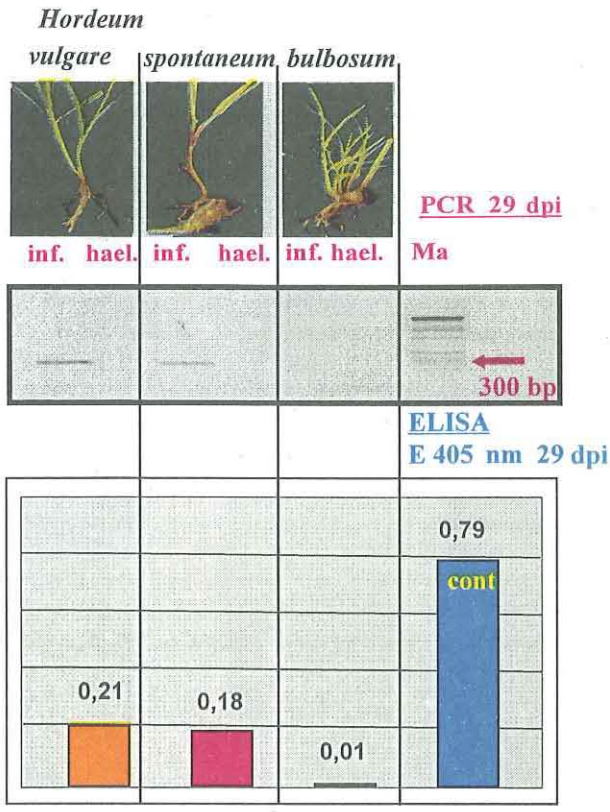

Figure: Investigation to evidence of *Polymyxa graminis* Led. in different *Hordeum* – species by serological analyse and PCR with *Polymyxa* specific primers in the course of 29 dpi
cont - infection standard of *P. graminis*, inf.- infected plants, heal.- healthy plants, Ma - marcer

The infection rate of *P. graminis* increased continuously in *H. vulgare* and *H. spontaneum* but not in *H. bulbosum*. It was impossible to detect a serological reaction and the specific *Polymyxa* - DNA - fragment in roots of *H. bulbosum* plants up to 29 dpi and in repeated investigations with these plants 6 months after inoculation.
These results indicate that the proofed genotypes of *Hordeum bulbosum* developed a resistance reaction to *Polymyxa graminis*. The reason of resistance and it's inheritance in the breeding process is still to be investigated.

Summary:
Serological and molecular biological methods of *P. graminis* detection have been established.
The evidence of *P. graminis* in *H. vulgare* and *H. spontaneum* is possible using PTA – ELISA and PCR 19 days after inoculation.
Inoculated *H. bulbosum* plants had not been infected with *P. graminis* up to 6 months after inoculation. The origin of resistance in *H. bulbosum* and it's inheritance is still to be investigated.

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FACTORS AFFECTING RED THREAD DISEASE OF TURF GRASSES

Alexander Berestetski [1,2], Ute Kastirr [1]
[1] Federal Centre for Breeding Research on Cultivated Plants, Institute for Resistance Research and Pathogen Diagnostics, Theodor-Roemer-Weg 4, 06449 Aschersleben, Germany; [2] All-Russian Research Institute of Plant Protection, Podbelski sh. 3, Pushkin, St.-Petersburg, 196608, Russia

Abstract
In order to determine standardized conditions for artificial infection of turf grasses (*Lolium perenne* and *Festuca rubra*) with *Laetisaria fuciformis* effects of 4 factors as temperature, wind, periodical wetness and plant age on red thread disease development were studied in controlled environment. The most important factor significantly (P≤0,001) affecting the disease development was temperature. The optimums for leaf colonization (22°C) and for subsequent development of the disease (14°C) were different; it supposes that temperature should be alternated during a day. Wind created by a fan caused significantly (P≤0,05) negative effect on red thread disease intensity on perennial ryegrass. Interactions between the disease intensity and periodical wetness (at 18 and 25°C) or plant age were insignificant (P≤0,05).

Key words: *Laetisaria fuciformis*, *Lolium perenne*, *Festuca rubra*, artificial infection, epidemiology

Introduction

In Europe in addition to rusts red thread disease caused by the basidiomycetous fungus, *Laetisaria fuciformis* (McAlpin) Burdsall is the most serious foliar disease of turf grasses (Gondran, 1994; Feuerstein, Roulund, 1994; Jensen et al., 2000). The most promising method to control red thread disease is introduction of resistance cultivars because cultural practices have temporary effect and fungicide application is constrained in public and rural places. However, the progress in this perspective direction was delayed by a lack of method of artificial infection of turf grasses with *L. fuciformis* (Feuerstein, Roulund, 1994). Though a number of inoculation techniques were compared and proposed (Berestetski, Kastirr, 2001), also it fs necessary to determine standardized conditions for the artificial infection to compare experimental results properly. This study was aimed to search for factors favorable for red thread disease development under controlled conditions.

Material and Methods

Plants and their maintenance

Seeds of susceptible cultivars of perennial ryegrass ("Juwel") and red fescue ("Olivia") were sown in a mixture of sand and soil (1:3) in boxes (28x18x5 cm) or multi-pot trays with holes of 5,5 cm in diameter at a rate of 30-40 g/m^2. Prior infection plants were maintained in greenhouse and cut 1-2 times in a week. Plants were inoculated and incubated under controlled conditions at temperatures given below and 16/8 h day/night regime.

The fungus and inoculation

For experiments an isolate of *L. fuciformis* (M-24) was used. Inoculum was produced on malz-peptone agar in 60-mm plastic dishes for 3 weeks. Inoculation was performed using "replica" technique described earlier (Berestetski, Kastirr, 2001). A central part of the turf was covered with a dish for determined time, then the dish with the fungal culture was accurately divided from grass leaves.

Effect of temperature, wind and periodical high wetness

Plants of ryegrass in the boxes were inoculated with the fungal culture during 7 days and incubated at two temperature regimes of 18 and 25°C, wind was proved by a fan, high relative humidity created by polyethylene bags for 16 h at once in every 3 days through the experiment. The experiment was performed twice.

Effect of temperature on leaf colonization

Plants of ryegrass in the trays were inoculated at 5, 14, 18, 22, and 26°C during 2, 4, 6, and 8 days, then moved in a chamber with constant temperature of 16°C.

Effect of temperature on red thread disease development

Plants of ryegrass in the boxes were inoculated at 16°C during 7 days, then moved in chambers with permanent temperature of 5, 14, 18, 22, and 26°C.

Red thread disease development depending on plant age

Plants of ryegrass and red fescue of age 2, 4, 6, and 8 weeks (after seedlings emerging) in the boxes were inoculated as described above, then incubated under the same temperature. The experiment was performed twice.

Data analysis
The disease severity was assessed visually by a scale: 0 = 0, 1 = up to 1%, 2 = 1-6, 3 = 6-12, 4 = 12-25, 5 = 25-50, 6 = 50-75, 7 = 75-87, 8 = 87-94, 9 = 94-100% plants covered with pink or red mycelium of the fungus or with specific symptoms like coral red sclerotia. In the cases of the disease assessment in the boxes, it was constructed a frame with net of 2x2-cm cells accordingly to recommendation of Hims et al. (1984). All experiments were carried out in 5 replications per variant. Data were analyzed by ANOVA and means were compared by Fisher's LSD test (SAS program).

Results
The highest infection level by 30 day after inoculation occurred at 18°C without wind and did not depend on the periodical wetness (table 1). In this experiment the wind of a fan played a role of additional factor of dryness negative for the disease development. At both relatively high temperatures wetness promoted growth not only *L. fuciformis* but also saprotrophic fungi which could constrain the infection development. The data given in the table 2 show high significant effect of temperature and wind on red thread disease development. Periodical high humidity had effect only on the disease incidence. This experiment demonstrated great effect of temperature on the disease development, and it was decided to study this factor in more detail.

As shown in the figure 1 *L. fuciformis* needs relatively high temperatures from 18 to 26°C for fast colonization of ryegrass leaves. But the most successful establishing occurred at 18-22°C because at 26°C the infection caused leaf soaking and plant death by 8th day of inoculation. The fungus was not practically able to colonize leaves of ryegrass at 5°C and colonized them significantly ($P \leq 0,05$) weaker and slowly at 14°C (fig. 1).

The optimum temperature for red thread development (after successful colonization) lays between 14 and 22°C, the higher level of the infection occurred at 14°C (fig. 2). Temperature of 5°C was not extreme for red thread development, because the fungus was able to survive and grow slowly on leaf surface or fast after replacing at higher temperatures. Temperature of 26°C was found to be the extreme as it was demonstrated before (table 1). At this temperature the fungus disappeared during a course of time (40 days after inoculation).

The data of figure 3 show that young fast growing plants are affected with red thread disease in lower extent than older plants, but these results did not demonstrate significant effect of plant age on red thread disease development (P=0,059 and 0,217 for *L. perenne* and *F. rubra*, respectively.). However, plant age had significant effect on leaf colonization (data not shown, P=0,028 and 0,0075).

Table 1: Effect of temperature, wind and wetness on red thread disease development

Temperature, °C	Wind (+/-)	Wetness (+/-)	I	S	IxS
18	+	+	10,5	3,16	41,1
		-	12,2	2,99	46,6
	-	+	12,5	3,39	53,6
		-	13,2	3,09	52,8
25	+	+	1,8	1,25	3,0
		-	3,0	1,47	5,4
	-	+	4,4	1,61	9,0
		-	3,4	1,97	13,6
LSD $_{0,05}$			*2,3*	*0,50*	*13,3*

I - disease incidence - percentage of the cells with infected plants; S - severity of the disease (0-9); IxS - intensity of disease - expressed as sum of severity of the disease in the all cells with infected plants

Table 2: Significance (P) of the studied factors on components of the disease development

Factors	I	S	IxS
Temperature	0,0001	0,0001	0,0001
Wind	0,0009	0,0221	0,0120
Wetness	0,0479	0,8245	0,3530

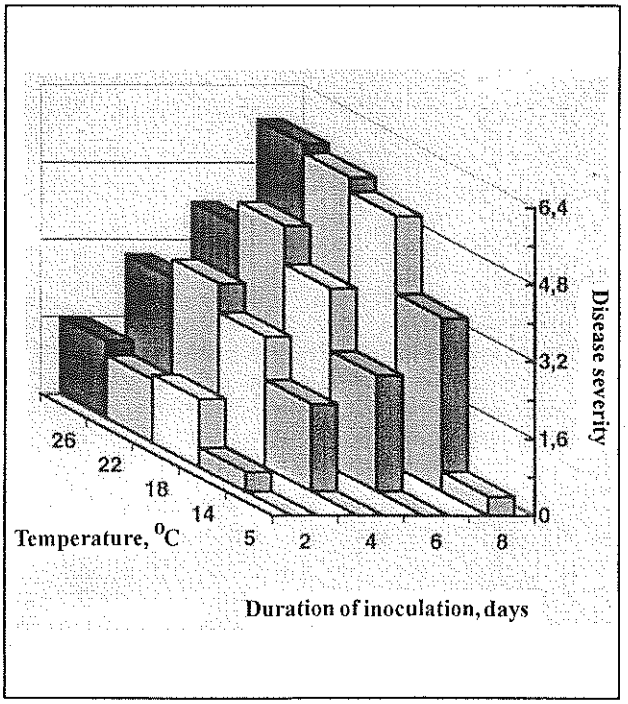

Figure 1: Effect of temperature on colonization of leaves of *Lolium perenne* by mycelium of *Laetisaria fuciformis*, 10 days after inoculation. LSD $_{0,05}$ = 0,8

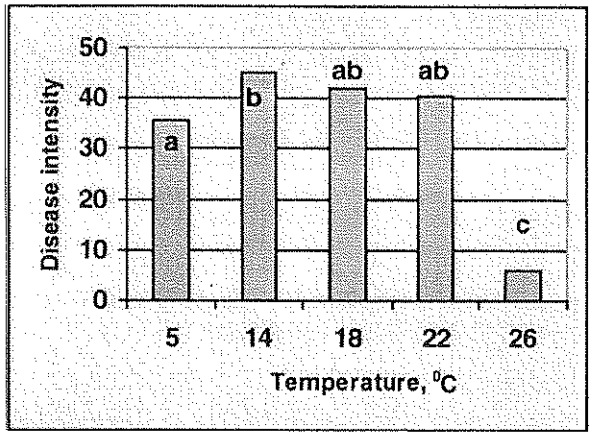

Figure 2: Effect of temperature on red thread disease development on *Lolium perenne*, 30 days post inoculation

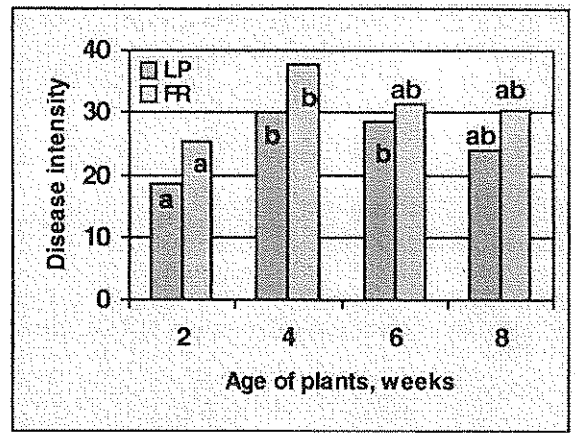

Figure 3: Effect of plant age on red thread disease development on *Lolium perenne* (LP) and *Festuca rubra* (FR), 30 days post inoculation

Discussion

Among three climatic factors which could affect red thread disease development as temperature, wetness and wind, the first one appears to be the most important. The detailed study showed that the optimal temperature for leaf colonization lays between 18 and 26°C, and it is about 14°C for the disease development. The data are corresponding with reports of Erwin (1941) and Bahuon (1985) who had studied growth of *L. fuciformis* in pure culture. They found that minimum temperature is 1°C, optimum 14-20°C, maximum 25-30°C, at 6°C the fungus was able to grow. Because there is a contradiction with different temperature optimums for plant colonization by the pathogen and the disease development it may suppose that temperature should be alternated during a day from 14 (or below) to 18-22°C to induce both colonization of leaves and the red thread development. Bahuon (1985) had come to the same conclusion empirically and she obtained the typical infection symptoms at 18°C on days and 6°C on nights under 12/12h day/night light regime and 80-90% relative humidity. In our previous study (Berestetski, Kastirr, 2001), where different types of the inoculum were used, the higher infection level was observed at 18/14°C temperature alternation than at permanent 20°C under 16/8 h day/night regime. Indeed, such conditions of contrast temperatures during a day are normal in springs and autumns when the symptoms of red thread disease are usually observed. Also, based on our data it may predict that, in general, under warm springs and rainy summers, like in the season of year 2000 in Germany, the disease will progress, but under cold springs and hot summers red thread disease will decrease or not appear at all.

Susceptibility of plants to diseases can vary in their different growth stages. It seems that plant age has no significant effect on red thread disease development (Erwin, 1941), but young fast growing plants of perennial ryegrass and red fescue of 2-weeks age were less susceptible to *L. fuciformis* than older plants of the age of 4-8 weeks (fig. 3). Possibly, effect of active consumption of nutrients by seedlings and their growth was rather observed than direct effect of plant age on the disease development because it is well known that any factors promoting growth of turf grasses like high temperatures and nitrogen fertilizers cause the same effect of the disease reducing (Metz et al., 2000).

Further studies of factors affecting red thread disease development as effect of air and soil humidity or factors forming special microclimate in the turf grass stand as plant density and frequent cutting could prove information for predicting epidemics of the disease and answer on such curious question why has red thread disease been never found in fodder grasses (E.Czembor, H.Schamp, personal communications) belonging to the same plant species or genera though *L .fuciformis* seems has wide host range (Couch, 1995)?

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HEAD BLIGHT OF CEREALS IN RUSSIA: PROGRESS AND PROBLEMS

LEVITIN, M.M.

All-Russian Institute of Plant Protection, St. Petersburg – Pushkin, Russia

Fusarium head blight (FHB) is presently one of the most important fungal diseases affecting cereal crops in Russia. Only in the Krasnodar district (North Caucasus) there were three large epidemics of head blight. The loss of wheat crop reached 25-50% and the contamination of cereal grain by mycotoxins increased more than 25 times (Levitin et al., 1994a). During 1989-1992 on average about 23% samples of cereals (wheat, barley, rye) in Russia were contaminated by DON. Amongst them, 9% of samples contained DON in concentrations exceeding the permissible level. In 0,4% of samples of bread and groats products concentrations of mycotoxins exceeded hygienic standards (Tuteljan, 1995). The high degree of damage by FHB claim to develop collaborative researches to protect cereals. The researches included: 1) the study of species composition of *Fusarium* fungi and their toxigenicity, 2) the study of biology of the most dangerous species, 3) development of cultural control techniques, chemical and biological methods of disease control, 4) breeding of resistant cultivars.

MATERIAL AND METHODS

Samples of cereal grains have been collected in the European and Asian parts of Russia. In each zone three regions were researched and in each of them 25-30 samples were selected. Fungal cultures have been prepared from infected grains according to common isolation procedures with prior surface sterilization and plating of infected material on solid media. Identification of species was carried out according the classification systems proposed by Gerlach and Nirenberg (1982) and Nelson et al., (1983).

The inoculation of plant was carried out according to the methods described by Mesterchasy, Rowaished (1977) and Stack and McMulen (1985). The cultivar estimation on resistance to FHB was carried out after artificial inoculation by conidial suspension (10^5 conidia/ml) using 5-point scale.

RESULTS AND DISCUSSION

The study of *Fusarium* species present on cereals of Russia (Ivaschenko et al., 1997; Levitin et al., 1998; Ivaschenko et al., 2000) has allowed to identify 17 species (tabl.1).

Table 1. Fusarium species on cereals in Russia

Species	Region									
	North Caucasus	Central	Central Black Earth	Volga	Volgo-Vyatski	North-West	Ural	West Siberia	East Siberia	The Far East
F. acuminatum	-	-	-	+	++	-	-	+	+++	+
F. avenaceum	++	+++	+++	++	+++	+++	++	++	+++	+++
F. culmorum	+	+++	-	-	++	++	-	+	+	-
F. equiseti	+	++	+	+++	++	+	+++	++	++	-
F.graminearum	+++	-	++	-	-	-	-	-	-	+++
F.heterosporum	+	-	-	++	-	-	-	+	+	-
F. moniliforme	+++	-	+	+++	+	-	-	+++	+	+
F. nivale	+	-	+	+	+	+	-	-	-	-
F. oxysporum	++	++	++	++	+	+	+	+	++	+
F. poae	+++	+++	+++	+++	+++	+++	+++	+	-	+++
F. proliferatum	++	-	-	-	-	-	-	-	-	-
F. sambucinum	+	+	+	+	+	+	+	+	+	+
F. semitectum	+	-	+	-	-	-	-	-	-	++
F. solani	+	+	+	+	+	+	+	-	-	+
F.sporotrichioides	+++	+++	+++	+	+++	+++	++	+++	++	++
F. subglutinans	+	-	-	-	-	-	-	-	-	-
F. tricinctum	+	++	-	+	++	++	-	-	-	-

(+++) - high frequency; (++) - medium frequency; (+) - low frequency; (-) - non-existent

North Caucasus is one of the main regions producing grain. The warm and humid climate of this district is very favorable for the development of fusariosis. The dominant species causing *Fusarium* head blight on cereals in the North Caucasus region are *F. graminearum, F. moniliforme, F. poae* and *F. sporotrichioides.* But *F. graminearum* is a more serious and dangerous pathogen.

The Central region is characterized by a moderately continental cool climate. Amongst the *Fusarium* species, *F.avenaceum, F. culmorum, F. poae, F. sporotrichioides* are dominant.

The other large grain producing region is the Central "Black Earth" region where *F.avenaceum, F. poae* and *F. sporotrichioides* on winter wheat are dominant.

The Volga region is characterized by dry climate, therefore fusariosis develops weakly. The dominant species is *F. equseti,* but other species such as *F. poae and F.moniliforme* are often observed.

In the Volgo-Vyatski region *F. avenaceum, F.poae and F. sporotrichioides,* are dominant.

The North-West region of Russia is characterized by a moderately continental humid climate with cool summer and a short growing period. Ten *Fusarium* species were isolated from cereal grain. Amongst them *F. avenaceum, F. poae and F. sporotrichioides* are dominat.

The dominant species in the Ural are *F.equiseti* and *F. poae*, but such as *F.avenaceum* and *F.sporotrichioides* are often observed.

In West Siberia, *F. moniliforme* and *F. sporotrichioides* are dominant; in East Siberia, *F. acuminatum* and *F. avenaceum* are dominant. Some *Fusarium* species which infect wheat in the European part of Russia (e.g. *F. tricinctum, F. graminearum* and *F. semitectum*) were not observed in Siberia.

.The biggest diversity of *Fusarium* species on cereals was observed in the Far East. The South of the Far East is characterized by a warm and very humid climate. Such an environment as well as that in North Caucasus is very favorable for the development of fusariosis. In this zone the dominant species causing an epiphytotium is *F. graminearum.*

All these data show that *Fusarium* species composition is very diverse and that the dominance of some species in different climatic conditions is observed. So, *F. graminearum* is dominant in the warm and humid conditions of North Caucasus and the Far East; *F. culmorum* is more spread in ecological conditions of the Central part of Russia. However there are species characterized by wide adaptability. For example, *F. avenaceum* and *F. sporotrichioides* are found in all cereal-producing zones of Russia.

Fusarium graminearum is most dangerous pathogen causing head blight on wheat. We carried out the study of biology of F. graminearum and showed, that the ascogenous stage of this fungus – *Gibberella zeae* can be remained on plant debris and during whole vegetative period ascogenous stage produces ascospores which infect plants (Ivaschenko, Nazarovskaya, 1990). The main reservation of infection is maize. The spread and the development of fusariosis after mize is 3 fold increased than after winter wheat. The intensity of perithecia formation on wheat after maize 5 fold higher than after other precursors. The correlation between resistance of maize to stalk rot and the number of perithecia G. zeae was established (Ivaschenko et al., 1994).

The problem of wheat resistance to FHB has the first priority in the researches of Russian immunologists and breeders. Using the infectional phones we estimated the resistance of 252 samples from 26 species of Triticum. It was shown (Gagkaeva et al., 1993), that the ploidy does not influence the resistance to FHB. But, some constitutional characters of plants can influence the resistance to Fusarium head blight. So the species T. durum, T. turanicum, T. urartu characterizing the open type of flowering were very succeptible to the disease. The species from humid regions (T.timopheevii, T. persicum) - were tolerant, whereas from arid steppe regions of Central Asia (T. vavilovii, T. dicoccoides, T. sphaerococcum) - are susceptible. Among Triticum species some wheat samples were resistant to FHB (tabl.2)

Table 2. Samples of *Triticum* spp. Resistant to *Fusarium graminearum*

Samples of *Triticum* sp.	Origin of samples	N of samples in VIR catalogue	Healthy kernels, %
T. dicoccum	Germany	7501	96.3
T. spelta	Switzeland	19097	95.4
T. militinae	Georgia	46007	97.2
T. karamyschevii	-"-	38549	92.4
T. timopheevii	-"-	29539	94.0
T. persicum	Dagestan	26828	93.2
T. persicum	-"-	32487	92.9
T. persicum	-"-	40307	93.2

We estimated the resistance of different Aegilops species to FHB. Among Aegilops species Ae. Tauschi was the most heterogeneous on resistance. From 56 samples 32% are highly resistant to FHB.

During last years Krasnodar breeders created the cultivars of winter wheat tolerant to FHB: Dakha, Yuna, Demetra, Kroshka, Echo. The cv. Kincso, Nung Ta 173, lee, Frontana, WSP96.6, Livius, Ringo Star has been used as a sources of FHB resistance in breeding program. Using these resources the new tolerant cultivars were bred (Ribalkin et al., 2000). They combine moderate resistance with high productivity, excellent grain quality and good adaptability. They have also very low mycotoxin contamination.

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Biodiversity of *F.graminearum* isolates from different geographical locations

GAGKAEVA T.[1], KOOPMANN B.[2], WOLF G.A. [2]

[1] All-Russian Institute of Plant Protection, 196608 St.-Petersburg-Pushkin, Russia
[2] Institute of Plant Pathology and Plant Protection, University of Gottingen, W-3400 Gottingen, Germany

Fusarium head blight is an economically important plant disease, not only because it causes significant reduction in yields, but also because infected seeds are often contaminated with mycotoxins that rose a serious threat to human and animal health. *Fusarium graminearum* is the principal pathogen responsible for head blight in many countries. A wide distribution this fungus in the whole word to attractive from point of view population studies of a geographically distinct group of *F.graminearum* (Bai G-H., Shanar.G.,1996; O'Donnell et al., 2000).

The objective of study was to investigate the biodiversity of *F.graminearum* populations on base of different markers relevantly to wide range of the geographical conditions of Russia, China, Germany and Finland. The next characters were used in this study for accession of variation in F.*graminearum* populations: pathogenicity; enzymes activities, vegetative compatibility and molecular- genetics markers.

MATERIALS AND METHODS

Fungal strains*

52 single-spore isolates of *F.graminearum* were used in the study. These included 48 isolates that were obtained from seeds or ears of commercial wheat cultivars in the wheat-growing areas - 11 isolates from the Far East part of Russia (Primorskiy [Pr.] and Khabarovsk kray [Hab.]), 16 isolates from the South of European part of Russia (The North Ossetia [CO.], Krasnodarsk kray [Kr.]), 9 isolates from China [Ch.], 12 isolates from Germany [G.]. Since these isolates were collected from naturally infected plants and selected by chance from a large number of independent isolates according to their morphological properties, they are reckoned to be representatives of a general populations from different geographical locations. Four isolates of *F.graminearum* from Finland were derived from root and stem base of wheat, barley and oat.

Pathogenicity

Isolates of *F. graminearum* were tested for pathogenicity on wheat seedlings (cv. Leningradka). Seeds were surface disinfected in 70% alcohol, rinsed with sterile water and placed in a little volume of water containing streptomycin (1 mg/L). After 1 day the germinated seeds, selected for uniformity, were disposed on 10 week-old cultures of isolates (Czapek-Dox agar). Three replicate Petri dishes were examined for each isolate. The control seeds were placed on uninfected Czapek-Dox agar. Disease reaction was assessed after 7 days of growth at temperature 22°C in the darkness. Each plant was assessed by severity of the disease symptoms and the seedling length. It was used 5 rating scale according to percentage of necrosis on the seedling (0-completely healthy; 1-less than 25% necrosis; 2- 25-50% necrosis; 3- 51-75% necrosis; 4- more than 75% necrosis and 5-blind seed). All isolates in dependence on their ability for reducing of length were classified into four groups: high aggressive - the length of seedlings was 5% and less percentage of control; aggressive - from 5 till 10 %; middle aggressive - from11 till 20% and low aggressive - the length of seedlings was more than 20% of control.

Enzymes activities

Wheat stems (cv. Bussart) of 3-weak-old plants were inoculated by isolates of *F. graminearum*. Inoculum was prepared as 5 mm disc of 1 week-old culture on PDA. Mycelium disc was placed into a small wound under the basal leaf prepared by a needle. In total for each isolate 16 plants were inoculated. Plants with sterile agar discs served as a controls. High relative humidity was maintained by plastic bags for 3 days. Plants were harvested one week after inoculation. Cuttings of stems (3 cm length) were used for enzyme extraction and estimation of the activity of cellulase, chitinase, xylanase, 1,3-ß-glucanase, amylase according to S.J.Wirth and G.Wolf (1992).

Vegetative compatibility

The procedures employed for obtain and phenotypes detect of *nit*-mutant carry out by methods described previously (Puhalla, 1985; Correl *et al.*, 1987). Mutants detected as *nit*M were used as testers for VC. One of *nit*M testers was set in the centre of a Petri dish containing MM. Around the tester were placed 4 other n*it*–mutants for joint growing. Heterokaryon formation appeared as an area of dense, wild-growth mycelium. Isolates that formed heterokaryons were placed in the same vegetative compatibility group.

Molecular- genetics markers

Mycelium for DNA extraction was grown on liquid medium in 250 ml Erlenmeyer flasks for 1 week. Mycelium was harvested and frozen at - 20° C.

Total DNA was isolated using two methods: as described by Lee and Taylor (1990) and by Möller with colleagues (1992), but in both cases the fresh-frozen mycelium (0.2 g) was taken. The DNA pellet was dissolved in 100 µl TE buffer and quantified spectrophotometrically.

The primers were used: BOX A 1R (CTACGGAAGGCGACGCTGACG) and complex ERIC 1R (ATGTAAGCTCCTGGGGATTCAC) and ERIC 2 (AAGTAAGTGACTGGGGTGAGCG). Amplification reactions was carried out in volume of 30 µl containing 3 µl of primers, Taq buffer (x10) and dNTP; 2,4µl of 25 mM $MgCl_2$; 1 U of *Taq* polymerase; fungal DNA and water. Reaction mixture was overlain with mineral oil prior to amplification.

DNA was amplified in HYBAID thermal cycler with the following programme: ERICs - 94°C for 7 min, 52°C of annealing temperature for 1 min, 65°C for 8 min (37 cycles) and BOX - 1 min at 94°C , 1 min at 52°C and 3 min 72°C (30 cycles) and with final extension step for 3 min at 72°C.

The amplification product (10µl) was mixed with loading buffer (1 µl) and then electrophoresed through 1,7% agarose gel in 1x TAE buffer (pH 8.5) and run at 52 V for 9 h. In total 31 samples of *F.graminearum* and one F. culmorum (as overcontrol) and molecular marker (1 kb DNA Ladder) were analysed under identical conditions. The gel was stained with ethidium bromide and photographed in UV light. The bands were detected visually from photographs of the gels and recorded in a 1/0 (present/absent) matrix, regardless of their intensity.

Statistical analysis

The standard statistical programmes EXEL, SigmaPlot were used for evaluation of variation into groups of isolates. Genetic distances between isolates, claster and principal component analysis were carried out using computer Programs SYN-TAX-pc for Multivariate Data Analysis in Ecology and Systematics (Podani, 1993).

RESULTS

Pathogenicity

The non-pathogenic isolates were not detected in our study. The pathogenic isolates caused necrotic lesions on the seedlings. The development of seedling blight ranged from score 4,8 to 2,3. There were no disease symptoms in control seedlings growing on uninfected agar. There was significant difference between groups in rating disease severity. The China and Germany isolates were less aggressive and clearly distinct from other groups studied (Table 1).

All seedlings treated by *F.graminearum* had a reduced length. It ranged from 1% to 43 % of the length of seedlings in the control; the more aggressive isolates caused much higher decrease of the length. The investigation of the variability within the groups shown that isolates from Germany and China were less aggressive towards seedlings than isolates from the rest groups. High aggressive isolates were not represented into groups from Germany and China.

Table 1

Aggressiveness of *F.graminearum* isolates originated from different geographical locations to wheat seedlings (cv.Leningradka)

Origin of isolates	Number of isolates	Rating of disease severity	Isolates with different level of aggressiveness, %			
			HA	A	MA	LA
Russia- the Far East						
Prymorskiy kray	5	4,40 ± 0,17	0	60	40	0
Khabarovsk kray	6	4,48 ± 0,13	33	50	0	17
China	8	3,42 ± 0,19	0	0	50	50
Russia - the South European part						
The North Ossetia	10	4,42 ± 0,06	20	50	30	0
Krasnodar kray	6	4,42 ± 0,03	50	33	17	0
Finland	4	4,46 ± 0,06	75	25	0	0
Germany	10	3,45 ± 0,21	0	10	40	50

(HA) -high aggressive; (A)- aggressive; (MA) - middle aggressive; (LA) - low aggressive isolates.

Enzymes activities

The results of the production of cell wall-degradation enzymes by *F.graminearum* and the presence of these enzymes in the infected wheat stem tissue are given in table 2. Dates show that activity of cellulase,

chitinase, xylanase and 1,3-β glucanase in inoculated stems was significantly higher than it is in control. In contrast of these enzymes the activity of amilase was equal to control or generally lower.

It was revealed thai cellulase, xylanase and amylase activity of isolates from Germany and China was significantly lower as and their aggressiveness. These observations allowed to propose that cell wall-degradation the cellulase, xylanase and amylase are the most important and have more relationship with infection efficiency. Coefficients of correlation were estimated for paired comparisons of the activity enzymes and pathogenicity in groups of isolates and it was shown the significant connection between these date: for cellulase – 0,85; xylanase- 0,86; amylase- 0,90.

Table 2.

Enzymes activity of *F.graminearum* isolates originated from different geographical locations in inoculated wheat stems (cv. Bussart)

Origin of isolates	Number of isolates	Enzyme activities, % to control				
		Cellulase	Chitinase	Xylanase	Amylase	1,3-ß-Glucanase
Russia- the Far East						
Primorskiy kray	5	252	122	282	87	140
Khabarovsk kray	6	303	133	332	106	145
China	9	239	142	258	48	150
Russia - the South European part						
The North Ossetia	10	339	166	278	77	159
Krasnodar kray	6	298	179	336	69	159
Finland	4	310	137	379	108	157
Germany	10	201	136	207	56	150

Vegetative compatibility (VC)

From 52 isolates used in this study only 31 isolates (61,1%) produced *nit*-mutants. Analysis of mutant phenotypes shown that 6 isolates was *nit*M mutants: two from Germany - G.1-15 and G.34-1, one from China – Ch.39-1, one from the North Ossetia – CO.41-1, one from Krasnodarskiy kray – K.1-1 and one from Primorskiy kray – Pr.82-2. They were used in our experiments as test-isolates.

Our results demonstrated that *nit*M mutants G.1-15 was complementary with Chinese isolates Ch.324 and Ch.31-3 and formed heterokaryons with each other to compose single VC group.

The rest isolates did not form heterokaryons with testers. In most of cases there was no contact between the hyphal fronts of the paired colonies to demonstrate the antagonistic interactions. In some combinations hyphal contact was observed, but complementary growth was absent to indicate vegetative incompatibility between isolates. In total the 29 VCGs were found amoung 31 isolates.

Molecular- genetics markers

The results revealed that the isolates could not be differentiated by BOX primer, so all isolates *F.graminearum* produced the similar profile of bands consisted of 15 bands range from 2500 till 300 br in size. One isolate of *F.culmorum* yielded 11 bands (10 from these were common with *F.graminearum*).

Specific polymorphic patterns produced by complex ERICs primers were presented in 20-27 distinct bands which distributed in the molecular weight range from 3200 to 220 bp.

Dendrogram was constructed by using coefficient of genetic distance (Fig.). The coefficient of genetic distance between *F.graminearum* isolates and one isolate of *F. culmorum* was 0,55.

The study has revealed that all detected isolates of *F.graminearum* are congregated in 2 major molecular types according to the coefficient of genetic distance - 0,24. The first type consists of 17 isolates and isolates originated from Finland, the Far East and Krasnodarsk kray of Russia put into this claster.

All isolates from China and Germany disposed in the second molecular type consisting of 13 isolates.

The ERICs praimers used in this study detected 10 haplotypes (single clonal lineages based on 100% similarity among 30 isolates. These haplotypes consisted of 1-6 members independently of their geographical origin.

DISCUSSION

Our results shown that the aggressiveness of *F.graminearum* isolates varied in all geographical groups, but aggressiveness of Germany and Chinese isolates was lower than one of the rest isolates. Along with the similarly of isolates from China and Germany in low pathogenicity, the means enzyme activities of xylanase, cellulase and amylase in inoculated wheat plants were low, too.

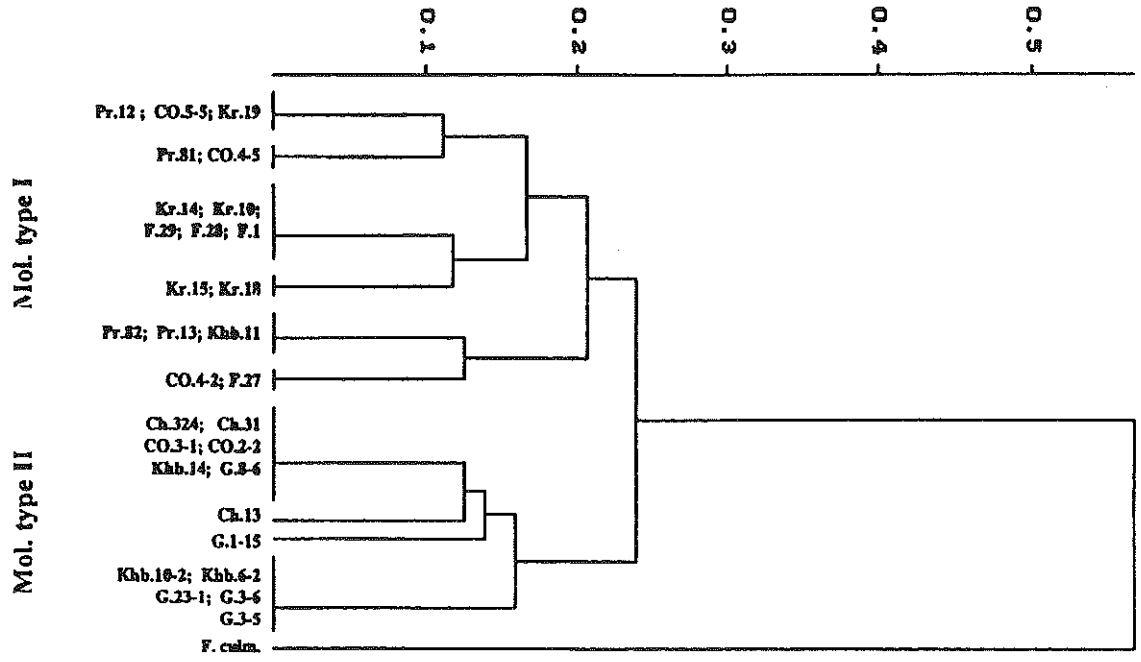

Fig. Claster analysis of genetic distances of 30 *F.graminearum* and one *F.culmorum* isolates obtained by ERICs primers

Moreover, this information agree with results which were produced by VCG method. It has been found one complicated VCG which is consisted of isolates originated from far distant locality (Germany, China) and similar by their low aggressiveness.

Above all, the members one VCG, *nit*-mutants from China Ch.324 and Ch 31-3, were generated from isolates which were placed into one haplotypes by PCR analysis and the isolate from Germany G.1-15 has the high level similarity with them (86%). These three mutants formed one subgroup clearly proving evidence of close genetic relationship between these isolates.

It was revealed the correlation between aggressiveness and obtained molecular markers. In table 3 the significant difference between two clasters in means of aggressiveness and pathogen related xylanase, cellulase and amylase was demonstrated. This observation let us to make the assumption about the direct relationship between molecular criteria generated by ERICs-PCR , VCGs and aggressiveness of *F.graminearum* isolates.

Table 3

Aggressiveness and enzymes activity of *F.graminearum* isolates belong to differ molecular types (obtained by ERICs primers according the coefficient of genetic distance 0,24)

Molecular type	Aggressiveness, % to control	Enzyme activities, % to control		
		Cellulase	Xylanase	Amylase
I	6,7 ± 1,2	303,1 ± 23,3	320,4 ± 19,8	93,3 ± 9,9
II	19,3±3,07	252,2 ± 25,4	258,2 ± 35,7	63,2 ± 16,5

At present it is difficult to comment with certain the nature of similarity in pathogenicity between groups of isolates from Germany and China. The dates of molecular analyse did not show clear clustering of isolates into geographical groups on base ERICs-PCR patterns. In our researches, VCG and DNA fingerprint patterns were common for isolates derived from various clonal lineages from several geographic localities. These results suggest that geographical distance does not correlate with genetic distance and presence of convergent evolution of populations under the similar conditions and through it pathogenicity characteristics of isolates develop independently.

We suppose that it is necessary to undertake more extensive testing to determine the real variation between fungus populations. We have the plan to detect variation among *F.graminearum* isolates using next characters: aggressiveness of isolates to ears of wheat, toxigenicity, isozymes patterns and another molecular-genetics markers. This information will improve the understanding of the population dynamics of *F.graminearum* and strategy of plant breeding.

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*The detailed information about strains is possible to obtain corresponding the author T.Gagkaeva (E-mail address: tug@MN1780.spb.edu).

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OCCURRENCE AND VIRULENCE OF WHEAT LEAF RUST IN HUNGARY

Manninger, K.
Plant Protection Institute, Hungarian Academy of Sciences
H- 1525 Budapest, P.O. Box 102, Hungary
Email: sman@nki.hu

Summary
Wheat leaf rust in Hungary has occurs annually and even in the past decade in 1994, 1995 and 1999 was strongly widespread and reached epidemic levels.
No. 20, No. 77, No. 61 and No. 12 dominant races were determined from wheat leaf rust populations during 1956-1999. In the past decade pathotypes of the races were also detected annually.
Isolates about 60% to 100 % were virulent on near-isogenic lines with Lr2b, Lr2c, Lr3, Lr3bg, Lr3ka, Lr11, Lr12, Lr13, Lr14a, Lr14b, Lr15, Lr16, Lr17, Lr18, Lr21, Lr22, Lr26, Lr30, Lr33, Lr34, Lr35, Lr37, LrB/C and LrB resistance genes at seedling stage. The isogenic lines with Lr2b, Lr2c, Lr3, Lr3ka, Lr14a, Lr15, Lr16, Lr26, Lr30 Lr B/C, resistance genes were susceptible or moderately susceptible at adult plant stage. The Hungarian rust population/isolates were avirulent on the isogenic lines with resistance genes Lr9, Lr19, Lr24, Lr28, Lr29, Lr38, LrW, which are originated from *Aegilops umbellulata, A. squarrosa, and Agropyron elongatum,* at booth plant stage.
It was shown that the virulence of leaf rust populations changed continuously in Hungary in the past 40 years. In this period the virulence change in wheat leaf rust population in Hungary was mostly influenced by the use of new varieties with known (Lr3, Lr26) or unknown resistance genes, but also by abiotic factors (temperature, rain), too. Lr3, Lr26 and unknown resistance genes lost effectiveness against Hungarian leaf rust population, while Lr1, Lr2a became close to effective.
In comparison to data from other countries of Europe, our data show similarity and difference to virulence values found in various counties.

Introduction

The most general diseases of wheat are caused by rust fungi all over the world. The damages caused by wheat stem, leaf, yellow rust have been well known in Hungary for a long time.
In 1932 heaviest rust epidemic was in Hungary, when only the Bankuti wheat varieties could avoid the losses, because of their early spring type. At this time the conception of breeding for resistant varieties against this disease was composed. Thank so the successful work of the Hungarian plant breeding institutes, most of the cultivated varieties belong to earlier type and are resistant to stem rust at the present time. So the stem rust epidemic is not so overhanging as it was when the new varieties are taken in cultivation [8].
Nowadays the leaf rust (*Puccinia triticina* Erikss. syn. *P. recondita* Rob. es. Desm. f. sp. *tritici*) is one of the three rust diseases of wheat in Hungary which occurs most regularly. This pathogen causes more or less losses each year. The occurrence of leaf rust depends on interactions of plant and pathogen genetic systems with environment. The temperature is one of the most important factors which control growth, development and spread of epidemic. The most reasonable way of controlling this disease is the development of resistant varieties. To improve the resistance of wheat varieties against rust diseases has been an important part of the work in the Hungarian wheat breeding institutes in the past 30 years. The resistance to diseases is still an essential feature of a new variety. The only disadvantage of host resistance is that it may in time become less effective due to increased virulence in the pathogen. This may result in resistant varieties becoming susceptible (break down of resistance) [8]. Therefore the plant breeders have to know up to date the virulence change of wheat leaf rust.
The aim of this paper was:
-to determine the Hungarian occurrence of wheat leaf rust
-to study the virulence changes in the wheat leaf rust population
-to characterize the virulence of the wheat leaf rust population
-to determine the genes which can be efficient in case of epidemic.

Materials and Methods

Collection of samples
Samples of rusted wheat leaf were collected in May, June and July annually. Infected leaves were obtained at different locations in wheat nurseries and fields.

Identification of race and pathotype
International standard differential varieties were used to identity the races of leaf rust [7]. .Pathotypes of leaf rust were identified using near isogenic backcross lines with resistance genes as differentials [9].
Characterize the virulence
The determination of virulence genes in rust population and finding out the effective resistance genes against leaf rust in Hungary were done by using 41 near isogenic backcross lines at seedling stage and adult plant stage.

Results and Discussion
Wheat leaf rust occurred in all Hungarian wheat grown areas annually. The severity of leaf rust was variable. That means that sometimes there was an epidemic and sometimes the infection of wheat leaf rust was very low. Yearly from the collected samples about 80-150 isolates were cultured from 1956 to 1999. From 10 to 15 races per year were identified without sporadic races which they occur ones or twice in the population. Among the important races only few were dominant in the populations. [3, 4, 8]
During the 40 years in the Hungarian leaf rust population races No. 20, No. 77, No .61 and No. 12 predominated following one another (Table 1).

Table 1

Prevalent wheat leaf rust races in Hungary, 1956-1999

Year	Race			
	No. 20	No. 77	No. 61	No. 12
	%			
1956				
1961.	73.8	18.5		
1966.	12.0	**40.7**		
1970.	1.0	**52.0**		
↓				
1984		**57.4**	35.4	
1989		**56.7**	28.7	
1994		32.5	**41.7**	3.1
1998		12.5	15.0	**35.0**
1999		9.0	9.0	**65.4**

In the middle of the sixties and the seventies the race No. 77 first virulent on Lr3, prevailed over the race No. 20, which was avirulent on Lr3, and was important in the fifties and the sixties (Table 2). Replacement of race No. 20 race by race No. 77 in middle sixties could have been caused by high cultivated area of varieties possessing Lr3. In these years the most common cultivated variety was Besostaya-1 possessing Lr3 (80% of wheat cultivated area in Hungary in 1972).
Between 1975 and 1993 the most important wheat leaf rust race was No.77, too. This is a new pathotype (No. 77/S) of this race virulent on Lr3 and Lr26 resistance genes. This pathotype of race No. 77 probably increased due to the growing members of new varieties, which have 1B/1R translocation with genes Lr26, Yr9 and Sr31.
However it was shown, that the virulence of leaf rust population changed again considerably in Hungary since 1990. Our data showed that first No. 61 and later No.12. prevailed from 1990 to 1999. No. 61 was 41.7 % in 1994 and race No.12 in 65.4 % in 1999 (Table 1).We suggest that in this period the virulence change in wheat leaf rust population in Hungary was mostly influenced by the use of new varieties with unknown resistance genes, but also by abiotic factors. Between 1994-1995 and in 1999 was more rain from April to May and the temperature was colder than in other previous years.
It seems that currently important leaf rust races in Hungary (No. 12, 61 and 77) can also be found in European countries (e.g. Czech Republic, France, Italy), too, but the proportion of races in populations differs from one another [1].

Table 2

Relationship between wheat varieties and prevalent wheat leaf rust races in Hungary, 1956-1999

Year	Variety	Resistance gene	Race	Virulence of race
1956			No. 20	A V Lr3
1961				
	Besostaya-1	Lr3		
1966				
			No. 77	V Lr3
1971				
	Kavkaz, Aurora	Lr26		AV Lr26
1976				
			No. 77/S	V Lr3, V Lr26
1981				
1986				
	Mv16	Lr26+?		
1991				
	GK Góbé, Fatima-2	?	No. 61	V Lr3, V Lr26
1996				
			No. 12	V Lr3, V Lr26 V Lr?

V = virulent AV = avirulent

By use of Thatcher lines in virulence surveys it become possible to distinguish isolates that differed in only a single virulence. Out of the important races there were 3-5 pathotypes determined yearly. Pathotypes were differentiated into groups according to their reactions on lines with Lr15, Lr17, Lr21, Lr23 and Lr26.
The frequencies of virulence to lines with Lr1, Lr2a decreased considerably between 1990 and 1999 (Table 3)

Table 3

Frequency of virulent isolates on near isogenic lines with Lr resistance genes in Hungary, 1990-1999

Lr gene	Virulence of isolates %				
	1990	1992	1995	1997	1999
Lr1	82	80	10	27	10
Lr2a	73	70	10	29	14
Lr2b	73	80	70	67	85

The virulence of wheat rust populations has been characterised and the effective resistance genes of the 41 leaf rust resistance genes examined were determine (Table 4, 5).
The leaf rust populations were virulent on Lr2b, Lr2c, Lr3, Lr3bg, Lr3ka, Lr14a, Lr15, Lr16, Lr26, Lr30, LrB and LrB/C at seedling and adult plant stage. No virulence were found to resistance genes Lr9, Lr19, Lr24, Lr28, Lr29, Lr38, Lr44, LrW. At adult plant stage Lr12, Lr13, Lr17, Lr18, Lr20, Lr21, Lr22, Lr34, Lr35, Lr37 resistance genes were effective or moderately effective. Lr1, Lr2a resistance genes can also help in the protection against Hungarian leaf rust population. These resistance gene are effective against the isolates in 85% to 90 %.

Table 4

Virulence of Hungarian wheat leaf rust isolates on isogenic lines with Lr genes at seedling stage, 1995-1998

Type of resistance	Virulent		Resistant	Partly virulent
Infection type	3, 3+, 4		0, ;, 1, 1+	0, ;, 1, 1+, 3
Isogenic line with Lr resistance gene	Lr2b	Lr17	Lr9	Lr1
	Lr2c	Lr18	Lr19	Lr2a
	Lr3	Lr21	Lr24	Lr10
	Lr3bg	Lr22	Lr28	Lr20
	Lr2b	Lr26	Lr29	Lr23
	Lr11	Lr30	Lr38/K	Lr25
	Lr12	Lr33	Lr38/TMR	Lr32
	Lr13	Lr34	LrW	Lr44/T.sp.
	Lr14a	Lr35		Lr44
	Lr14b	Lr37		
	Lr15	LrB/C		
	Lr16	LrB		
Frequency of virulence %	60-100		0	10-30

Table 5

Resistance of isogenic lines with Lr genes against wheat leaf rust at adult plant stage, 1995-1998

Type of resistance			
MS-S	R	MR	R-MR-MS
Isogenic line with Lr resistance gene			
Lr 2b	Lr9	Lr13	Lr1
Lr2c	Lr12	Lr22	Lr2a
Lr3	Lr19	Lr34	Lr10
Lr3bg	Lr21	Lr35	Lr11
Lr3ka	Lr24	Lr37	Lr14b
Lr14a	Lr28		Lr17
Lr15	Lr29		Lr18
Lr16	Lr38/K		Lr20
Lr26	Lr38/TMR		Lr23
Lr30	LrW		Lr25
LrB/C			Lr32
LrB			Lr33
			Lr44/T.sp
			Lr44

It is very important to know the resistance genes in cultivated varieties, too. Lr1, Lr3, Lr13, Lr26, Lr34 leaf rust resistance genes were postulated to be present in the cultivated Hungarian varieties [2, 6, 8, 10], however the mean cultivated varieties carry only Lr3 or Lr26, and they were susceptible to leaf rust in the last year (Table 6).

Table 6

Resistance of the mean cultivated Hungarian wheat varieties against leaf rust

Variety	Postulated resistance gene	Year	
		1995-1997[5].	1999
		Wheat area (%)	Infection type
Fatima-2 (1992)	Lr26+	10.2	60 S
GK Öthalom (1985)	Lr3, Lr34	10.1	40 S
Jubileynaya 50 (1970)	Lr3, Lr34	8.1	70 S
Mv23 (1991)	Lr26	7.2	100 S
Mv Optima (1993)	Lr3	6.3	90-100 S
Mv Emma (1994)	Lr26	4.3	80 S
GK Csörnöc (1994)	Lr26	3.4	75-80 S
Mv Vilma (1994)	Lr26	3.0	40-100 MR, MS, S
Mv Pálma (1994)	Lr26	4.0	80-100 S
GK Göbé (1992)	?	3.5	R
Mv Magdaléna (1996)	?	3.4	R
GK Zugoly (1993)	?	3.0	5-10 MS, S

It is a fact, that the virulence frequency of isogenic lines with Lr3 and Lr26 was very high in the past years (90% to 100 %) Although the virulence frequency of isogenic line with Lr1 decreased (10%), Lr13, Lr34 were ineffective at seedling stage and moderately effective at adult plant stage (Table 4, 5). In this way currently Lr1, Lr13, and Lr34 can play an important role in the protection against leaf rust in Hungary. Although we discovered lot of resistance genes, which could help in the protection of wheat against leaf rust, that means the opportunity of protection of wheat by resistance genes is unexploited in Hungary at present time.

Conclusion

It was shown that the virulence of leaf rust populations changed continuously in Hungary in the past 40 years. This history of breeding for leaf rust resistance in winter wheat has shown that is a never ending battle. With the release of each new resistance gene, a new virulence can be expected to emerge in the near future. In spite of this fact we think that protection could be increased against leaf rust by utilization of many different kinds of resistance genes in breeding programs. Therefore Hungarian breeders have to find new sources which carry effective resistance genes against Hungarian leaf rust populations. Nowadays it is possible to identify effective resistance genes with the help of molecular markers. The effectiveness and safety of protection can be increased by using the combination of several types of effective resistance genes, too.

Acknowledgement

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ROOT ROT AGENTS OF CEREAL AND THEIR INTERRELATION

MAČKINAITĖ R.

Institute of Botany, Žaliųjų ežerų 49, LT – 2021 Vilnius, LITHUANIA

Introduction

Cereal are one of the most important agricultural crops not just in Lithuania but in the whole world as well. Wheat (*Triticum aestivum* L.), barley (*Hordeum distichon* L.), rye (*Secale cereale* L.), oats (*Avena sativa* L.) are extensively cultivated in Lithuania. Their crops amount to 1.2 mil ha, and make up 43 – 48 % of the total sown area. The yield of cereal and its quality is determined by a lot of conditions, including the spreading of diseases. Big damages to cereal are caused by root rots. They are spread in sown areas of all cereals, and can injure 20–30 % of plants. In some areas 60–70 % or even 100 % of plants were found injured (Špokauskienė, 1986). The spreading of root rots and the damage caused by them depend on many ecological factors (temperature fluctuations, precipitation, preparation of soil, forecrop, fertilisation) and especially on the species composition of disease agents (Leisse, Puhl, 1992; Bateman, Coskun, 1995; Smiley et al., 1996; Turkington, Clayton, 1997; Hall, Sutton, 1998; Damm, 1998; Dymina, 1998). The mycoflora of injured cereal roots is abundant and diverse. O. Špokauskienė (1991) identified 59 species and 15 varieties of micromycetes belonging to 31 genera. In this group there are parasites, which actively participate in the process of root injury, as well as saprotrophes, which affect the roots not being the primary cause of the process. According to the opinion of numerous authors, root rots are caused by a complex of micromycetes where most important are *Fusarium*, *Bipolaris*, *Rhizoctonia*, and *Pythium* genera. However, in various regions different species of fungi predetermining the severity of a disease dominate (Rossi et al., 1995; Smiley, Patterson, 1996; Damm, 1998; Gonzalez, Trevathan, 2000). While investigating the agents of root rots and their harmfulness, it is very important to ascertain the dominant species of pathogen complexes characteristic for a particular territory, and factors determining the dominance of those species.

The objectives of this study were to: i) determine most important root rot agents of cereals (wheat, barley, rye, oats) and wild plants growing nearby in a natural biotope; ii) research into their interaction; iii) ascertain more aggressive species or isolates that are able to influence this pathological process.

Materials and methods

The injured plants were collected in 26 localities of different regions of northern Lithuania. Root rot agents were isolated from the roots of cereals (*Secale cereale* L., *Triticum aestivum* L., *Hordeum distichon* L., *Avena sativa* L.) and 26 wild plants growing beside (*Agropyron repens* (L.) P. B., *Arthemisia vulgaris* L., *Centaurea cyanus* L., *Cirsium arvense* Scop., *Dactylis glomerata* L., *Chenopodium album* L., *Ch. hybridum* L., *Erigeron annuus* (L.) Pers., *Euphorbia helioscopia* L., *Matricaria maritima* L., *Medicago lupulina* L., *Melilotus albus* Med., *Mentha arvensis* L., *Myosotis arvensis* (L.) Hill, *Plantago major* L., *Phleum pratense* L., *Rumex acetosella* L., *Taraxacum officinale* Weber, *Sonchus arvensis* L., *Trifolium arvense* L., *T. hybridum* L., *T. pratense* L., *T. repens* L., *Tussilago farfara* L., *Vicia angustifolia* Grufb., *V. sativa* L.). In every locality five plants of each species were collected before the harvest time of cereal.

Pure cultures of fungi were isolated employing the common methods (Bilai, 1977; Metod. ukaz. ...,1985; Dudka et al., 1982). The fungal species were identified on the basis of their cultural and morphological characteristics, according to Ellis (1976), Bilai (1977), Arx (1981), Gerlach, Nirenberg (1982), Nelson et al. (1983). The distribution frequency (DF) of separate fungi species and the percentage they made up of the total number of isolates were calculated.(Mirchink, 1976). Single spore cultures of selected isolates were transferred on malt extract agar medium (MEA) in tubes for preservation and were used in the interrelation survey.

119 isolates of 10 micromycete species most widespread in the roots of tested plants were selected for the investigations on their interaction: *Fusarium avenaceum* (Fr.) Sacc. (19 isolates), *F. culmorum* (Wm. G. Sm.) Sacc. (21), *F. oxysporum* (Schltdl.) W. C. Snyder et H. N. Hansen (13), *F. sambucinum* Fuckel var. *minus* Wollenw. (35), *Bipolaris sorokiniana* (Sacc.) Shoemaker (6), *Rhizoctonia* sp. (5), *Aspergillus ochraceus* K. Wilh. (3), *Chaetomium globosum* Kunce:Fr. (2), *Gliocladium catenulatum* J. C. Gilman et E. V. Abbott (9), *Talaromyces flavus* (Klöcker) Stolk et Samson (6).The research on the interaction of micromycetes was carried out *in vitro* in dual-plate assay on the MEA, evaluating them after 5, 10, 15, and 20 days of growth. The interaction between different isolates of the same species, and different isolates of separate species was tested.

While investigating different isolates of one species, the experiments with isolates from the same species of host-plant growing in different localities and with isolates from host-plants of different species growing in the same locality were carried out. Most aggressive isolates were selected and used in the research on the interaction between different species of root rot agents.

For evaluation of the interaction between micromycetes, forms of the microorganisms interaction proposed by I. Babushkina (1974) were applied.

Results and discussion

Our research data, as the data of other researchers, ascertained that the root rots are caused by a complex of fungi. In the roots of the investigated cereals (wheat, barley, rye, oats) 41 taxa of micromycetes belonging to 22 genera (*Acremonium* Link:Fr., *Alternaria* Nees, *Apiosordaria* Arx et W. Gams, *Arthrinium* Kunze:Fr. in Kunze et J. C., *Aspergillus* P. Michel ex Link:Fr., *Bipolaris* Shoemaker, *Cylindrocarpon* Wolllenw., *Chaetomium* Kunze:Fr., *Cladosporium* Link:Fr., *Fusarium* Link:Fr., *Gliocladium* Corda, *Nigrospora* Zimm., *Penicillium* Link:Fr., *Periconia* Tode:Fr., *Phoma* Sacc., *Rhizoctonia* DC, *Sepedonium* Link:Fr., *Stemphyllium* Wallr., *Ulocladium* Preuss, *Talaromyces* C.R. Benj., *Zygodesmus* Corda, *Zygorrhynchus* Vuill.) were identified. Micromycetes of the *Fusarium* genus predominated among them and made up 39.7 % of the total number of isolates. 14 species and 1 variety belonging to this genus were determined. *F. sambucinum* var. *minus* (DF 30.2 %), *F. culmorum* (DF 29.4 %), *F. avenaceum* (DF 13.5 %), and *F. oxysporum* (DF 7.9 %) prevailed. These four species made up 70.3 % of all *Fusarium* isolates. Among the fungi of other genera *B. sorokiniana* (DF 27.8 %), *Phoma* spp. (DF 31.7 %), *T. flavus* (DF 26.2 %), *Rhizoctonia* sp. (DF 16.7 %) were most widespread. Species of the *Gliocladium* and *Chaetomium* genera were frequent as well. Their distribution frequency was 13.5 and 9.5 %, respectively.

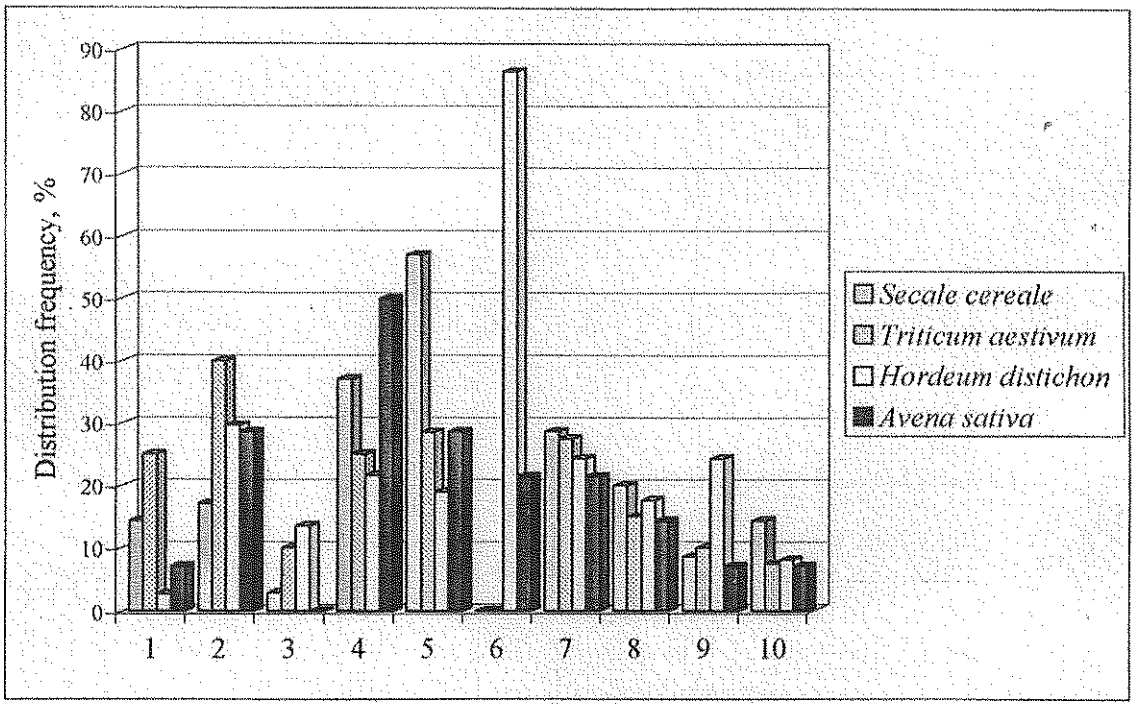

Fig. 1. Distribution frequency (%) of most widespread root rot agents in cereal crops (1 – *Fusarium avenaceum*, 2 – *F. culmorum*, 3 – *F. oxysporum*, 4 – *F. sambucinum* var. *minus*, 5 – *Phoma* spp., 6 – *Bipolaris sorokiniana*, 7 – *Talaromyces flavus*, 8 – *Rhizoctonia* sp., 9 – *Gliocladium catenulatum*, 10 – *Chaetomium* spp.)

The distribution of these species varied in roots of various cereals. *F. sambucinum* var. *minus* was widespread in roots of all investigated cereals, however its highest distribution frequency was revealed in the crops of rye and oats (37.1 and 50.0 %, respectively). *F. culmorum* was most abundant in the roots of wheat, barley, and oats. Its distribution frequency made up 40.0, 29.7, and 28.6 %, respectively. *F. avenaceum* was most frequent in the roots of wheat (DF 25.0 %), *F. oxysporum* – in the roots of barley (DF 13.5 %).The latter was not found in oats roots. Its distribution frequency in the roots of rye was only 2.9 % (Fig. 1).

Together with these fungi *F. graminearum* Schwabe was frequently found in the roots of rye (DF 11.4 %), *F. sambucinum* Fuckel - in the roots of wheat (DF 12.5 %), *F. graminum* Corda - in the roots of oats (DF 14.3 %). Distribution frequency of other identified *Fusarium* species in crops of different cereals amounted to 2.5 – 8.6 %.

Among the fungi of other genera B. sorokiniana was most widespread. In the roots of barley its distribution frequency amounted up to 86.5 %, in the roots of oats – up to 21.4 %, however in the roots of rye and wheat it was not revealed. Fungi of the *Phoma* genus were widespread in all cereals, but the highest distribution

frequency was noted in the roots of rye (DF 57.1 %). *G. catenulatum* was most frequent in the roots of barley (DF 24.3 %), while *Chaetomium* spp. – in the roots of rye (DF 14.3 %). *Rhizoctonia* sp. (DF 14.3 –20.0 %) as well as *T. flavus* (DF 21.4 – 28.6 %) were numerous in the roots of all investigated cereals (Fig. 1).

Some other widespread fungi should also be mentioned. *Alternaria alternata* (Fr.:Fr.) Keissl. was frequent in the roots of rye and wheat (DF 17.1 and 10.0 %, respectively); *Cylindrocarpon didymum* (Harting) Wollenw. was frequently detected in the roots of barley (DF 13.5 %). Distribution frequency of other root rot agents in different cereals varied from 2.5 to 8.7 %.

Fungi mostly spread in cereal roots were also identified in roots of wild plants. After the analysis of 285 roots of 26 wild plant species collected in 26 localities situated next to cereal fields, it was noticed that the complex of root rot agents of wild plants in natural biotope is similar to that of the cereal. Fungi were not so widely spread in natural biotope, however dominant species in roots of the investigated cereals and in wild plants were equal. *Fusarium* dominated among the root rot agents of wild plants forming 49.4 % of the total number of isolates. *F. sambucinum* var. *minus* (DF 9.1 %) was most frequent, equally as in the roots of cereals. On the contrary, *F. culmorum* was less frequent in natural biotope (DF 4.9 %), *F. avenaceum* (DF 8.1 %), and especially *F. oxysporum* (DF 6.7 %), were detected more frequently in roots of wild plants than in cereal roots if compared with other agents of root rot (Fig. 2).Among other fungi identified in the roots of wild plants *Rhizoctonia* sp. (DF 7.4 %), *Phoma* spp. (DF 5.3 %), *A. alternata* (DF 4.9 %) and *C. didymum* (DF 4.6 %) prevailed

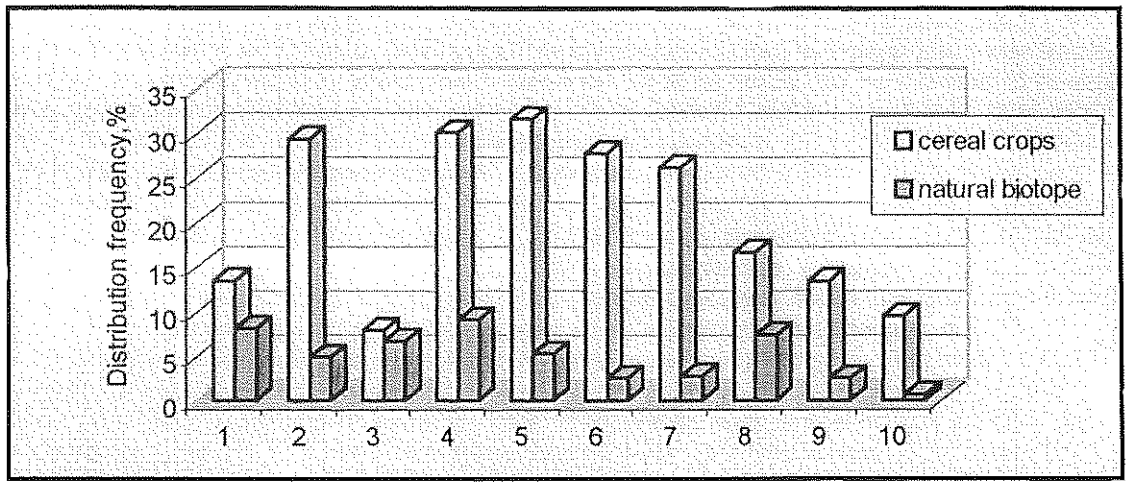

Fig. 2. The distribution of most widespread root rot agents in biotopes of different antropogenisation level (1 - *Fusarium avenaceum*, 2 – *F. culmorum*, 3 – *F. oxysporum*, 4 – *F. sambucinum* var. *minus*, 5 – *Phoma* spp., 6 – *Bipolaris sorokiniana*, 7 – *Talaromyces flavus*, 8 – *Rhizoctonia* sp., 9 – *Gliocladium catenulatum*, 10 - *Chaetomium* sp.)

Majority of root rot agents has a wide range of host-plants and were identified in roots of more than ten plant species. *F. sambucinum* var. *minus* was ascertained in the roots of 21, *F. avenaceum* – 17, *F. culmorum* – 15, *F. oxysporum* – 13, *Rhizoctonia* sp.- 14 plant species. *B. sorokiniana* was most frequently identified in the roots of barley and oats. It also was detected in roots of 7 wild plant species growing next to the barley and oats field.

The survey data indicated that root rot agents are not widely spread in natural biotopes and do not cause severe damages to wild plants. Nevertheless, they have a wide range of host-plants, that could act as infection reservers and under favourable conditions can become the source of infection for cultured plants.

Distribution of root rot agents and damage caused by them strongly depend upon the interrelations of micromycetes participating in the pathogenic process. Results of the research on the interaction of most widespread root – associated fungi demonstrated that relations among the micromycetes, which participate in the process of root injury and destruction, are diverse and strongly determined by the species and isolate of fungus. Four forms of micromycetes interaction were revealed: no impact, fungistatic trophic antagonism, territorial antagonism, and mutual antagonism.

By the interaction of investigated micromycetes the forms of fungistatic trophic and mutual antagonisms prevailed amounting to 46.0 and 40.0 %, respectively. Territorial antagonism was revealed in 5.8 % of the investigated cases, and no impact between the investigated micromycetes - in 7.8 % of cases. The interaction greatly depended upon the characteristics of the micromycete species and its separate isolates.

F. avenaceum inhibited the growth of mycelium of *F. culmorum*, *F. oxysporum*, *B. sorokiniana* and more or less overgrew some their isolates. All other investigated fungi were more aggressive than *F. avenaceum*. They inhibited the growth of investigated isolates of *F. avenaceum* overgrew them and separate isolates even destroyed the mycelium of *F. avenaceum*. *Rhizoctonia* sp., *Ch. globosum*, *A. ochraceus* were most aggressive

towards *F. avenaceum*. Evident antagonism was observed between *F. avenaceum* isolate 10053 and *B. sorokiniana* isolate 10276, as well as between all isolates of *F. avenaceum* and *G. catenulatum* isolate 10323.

The growth of *F. culmorum* was more intensive and abundant if compared with other investigated fungi, but its aggressiveness depended very much on the isolate and distinguished itself only towards certain isolates of *F oxysporum, F. sambucinum* var. *minus, B. sorokiniana*. More aggressive isolates of these fungi, equally as the isolates of *Rhizoctonia* sp., *G. catenulatum, A. ochraceus, Ch. globosum*, and *T. flavus*, inhibited the growth of *F. culmorum* and more or less overgrew its mycelium. *F. culmorum* isolate 10230 should be noted as it was more resistant towards the impact of many investigated fungi. Resistance and aggressiveness of other isolates of this micromycete varied greatly, depending on the species or even isolate of the interacting fungus.

All investigated root rot agents (except for *B. sorokiniana*) were more aggressive than *F. oxysporum*. They stopped the growth of *F. oxysporum* mycelium and more or less overgrew it. The mycelium of certain isolates even was destroyed. *F. oxysporum* was most resistant to the impact of *B. sorokiniana*. These fungi just inhibited the growth of each other, *F. oxysporum* even slightly overgrew *B. sorokiniana* at the edge of their contiguity. More distinguishable antagonism was observed between *F. oxysporum* isolates and the investigated isolates of *G. catenulatum, A. ochraceus, B. sorokiniana*, as well as *F. sambucinum* var. *minus* isolate 10415.

The investigated isolates of *F. sambucinum* var. *minus* grew more abundantly and inhibited the growth of *F. avenaceum* and *F. oxysporum* mycelium and more or less overgrew it. The interaction with *F. culmorum* and *Ch. globosum* depended upon their isolates: various isolates demonstrated different degree of aggressiveness towards the investigated isolates of *F. sambucinum* var. *minus. Rhizoctonia* sp., *G. catenulatum, A. ochraceum* and *T.flavus* were more aggressive than *F. sambucinum* var. *minus* .They inhibited the growth and overgrew the mycelium of F. sambucinum var. minus yet at the beginning of the growth of those fungi *F. sambucinum* var. *minus* grew more abundantly. For some isolates of *F. sambucinum* var. *minus* evident mutual antagonism with B. *sorokiniana, G. catenulatum, Ch. globosum* was characteristic.

The investigated micromycetes were more aggressive than *B. sorokiniana*, inhibited its growth and more or less overgrew it. Aggressiveness of *F. sambucinum* var. *minus, G. catenulatum*, and *Ch. globosum* depended upon the isolate. Evident antagonism was observed between *B. sorokiniana* and some isolates of *F. sambucinum* var. *minus, T. flavus*, as well as *Ch. globosum* isolate 10623.

Rhizoctonia sp. was one of the most aggressive among the investigated micromycetes and inhibited their growth. Only *A. ochraceus* stopped the growth of *Rhizoctonia* sp. and overgrew its mycelium.

G. catenulatum was more aggressive than other investigated micromycetes. Majority of micromycetes at the beginning grew more intensively and surrounded the colony of *G. catenulatum*, nevertheless *G. catenulatum* stopped their growth and, depending upon the species or isolate of the interacting fungi, more or less overgrew them. The growth of *G. catenulatum* was inhibited and it was overgrown only by more aggressive fungi *Rhizoctonia* sp., *T. flavus*, and *Ch. globosum* isolate 10623. Between some investigated micromycetes and isolates of *G. catenulatum* atnagonism was revealed (e.g. *A. ochraceus* with *G. catenulatum* isolate 10044).

A. ochraceus was considerably more aggressive comparing with other investigated fungi. It inhibited their growth and more or less overgrew them destroying the mycelium of some isolates. Only *T. flavus* was more resistant towards the impact of *A. ochraceus. A. ochraceus* grew more intensively, nevertheless it did not overgrow *T. flavus*, on the contrary, *T. flavus* stopped the growth of *A. ochraceus* and even started to grow on it. The impact of all investigated isolates of *A. ochraceus* was similar.

Investigated fungi of the *Fusarium* genus at the beginning grew more abundant, inhibited the growth and overgrew the colony of *T. flavus*. Later (on the 10th day of growth) the growth of *Fusarium* slackened. *T. flavus* was growing on, inhibiting the growth of *Fusarium* and growing over them. Mutual antagonism revealed itself, its intensity mostly depending on the isolate of *Fusarium*. The most evident antagonism was characteristic to the isolates of *B. sorokiniana* and *T. flavus*. Only *Rhizoctonia* sp. was more aggressive than *T. flavus*. The growth of all other fungi, depending on the isolate, was more or less inhibited and their mycelium overgrown by *T. flavus*. The impact of all investigated *T. flavus* isolates was similar.

The interaction of *Ch. globosum* with other fungi depended upon the isolate. *Ch. globosum* isolate 10623 was more aggressive towards many of the investigated micromycetes. Both the investigated isolates of *Ch. globosum* inhibited the growth of *F. avenaceum, F. culmorum, F. oxysporum*, more or less overgrew their separate isolates destroying them. Impact of the investigated isolates of *Ch. globosum* upon *F. sambucinum* var. *minus* and *B. sorokiniana* varied. *Ch. globosum* isolate 10623 was more aggressive than the isolates of *F. sambucinum* var. *minus* and *B. sorokiniana*, it inhibited the growth of their mycelium and overgrew them. On the contrary, *Ch. globosium* isolate 10604 was overgrown by the investigated isolates of *F. sambucinum* var. *minus* and *B. sorokiniana. Rhizoctonia sp., A. ochraceus, T. flavus* were more aggressive towards *Ch. globosum*. Both the investigated isolates of Ch. globosum were heavily overgrown, their growth was stopped.

The results of this research demonstrated that interaction of micromycetes, injuring roots of plants, vary and to a great extent depend on the species and isolate of fungus. Equal isolates being resistant to the impact of particular micromycetes can be very susceptible to the influence of the other, or could demonstrate aggressiveness or antagonism towards still other micromycetes.

The isolates of *Rhizoctonia* sp., *A. ochraceus* and *T. flavus* were distinguishing for the strongest aggressiveness, fungistatic, and in some cases even fungicidal impact *in vitro*. They inhibited the growth of a

number of root rot agents, some their isolates even destroyed the mycelium of pathogens, heavily overgrowing it. Among the *Fusarium F. avenaceum* and *F. sambucinum* var. *minus* were more aggressive. Aggressiveness and resistance of *F. culmorum* towards other micromycetes strongly depended upon the isolate. Most evident mutual antagonism was detected between the investigated isolates of *G. catenulatum* and *F. sambucinum* var. *minus*, as well as between the isolates of *B. sorokiniana* and *F. sambucinum* var. *minus*, *A. ochraceus*, *T. flavus*. The correlation between aggressiveness of separate isolates and biotope or a host-plant from which they were isolated were not ascertained. Aggressive isolates were detected in roots of cereal and wild plants growing nearby in various localities. Environmental conditions seem to be more important for the aggressiveness of pathogen than the host-plant.

The interaction among the root rot agents can influence the species composition of soil pathogens. Distribution of some fungi in roots is attributed to their ability to inhibit the growth of other pathogens (Lugauskas, 1988). Detection of such species among the root–associated fungi and understanding of their interactions in soil microbial community would make possible the rational development of biocontrol for agriculture.

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PRESENT STATUS OF FUSARIUM RESISTANCE IN WHEAT

Á. Mesterházy
Cereal Research non-profit Co, Szeged, Hungary

The FHB in wheat causes worldwide severe epidemics with significant yield losses and additionally toxin contamination being harmful for food and feed safety. As the results show, the possibilities of its control are especially moderate at highly susceptible cultivars by agrotechnical means, cropping system and chemicals. Numerous surveys prove all over the world that most of the grown cultivars are susceptible or highly susceptible to the disease, but a genetic diversity exists (Snijders b1990). This is the ultimate cause of the epidemics. Therefore a significant work was concentrated to breed more resistant cultivars.

Not only the breeding was a problem. For that we had to know what is resistance. What are the sources of resistance? How the disease is develops, what should be investigated, how the wheat-FHB system is working. We needed proper methods to identify resistance or susceptibility. We had to evaluate a breeding methodology by that the selection could be made effectively. And finally, we had to make research to find out, how durable the resistance to FHB is, how can we predict it.

As a result of the huge work made from China to US, in Europe, and other parts of the world, many questions have more or less solid answers. So a selection system can be formed and was formed on many parts of the world. With these numerous resistant genotypes were bred, however, most of them will come into commercial production in the coming years.

Now I would like to summarize some of the achievements.

Resistance to Fusarium spp. The wheat is attacked by many *Fusarium* species. *F. graminearum* dominates the warmer conditions, *F. culmorum* is the major pathogen in more northern areas and *F. avenaceum* can cause epidemics also in cooler areas. Beside them often can be isolated *F. sporotrichioides*, one of the most important toxin producer, F. *poae* and others. Even *F. nivale* (*Microdochium nivale*) was renamed, most of the breeders consider it as *Fusarium*, and therefore this species should also be considered. The question arises whether the resistance to these species is different or not. Additionally, in earlier literature races were described within *F. graminearum* indicating specific resistance against this pathogen. The data prove that the resistance to these pathogens is common. The data of the last 25 years show that there are no races within *F. graminearum* and *F. culmorum* (Mesterházy 1989, 1995, Mesterházy et al. 1999). The wheat plant cannot differentiate the two species; the resistance background against these species is the same (Table 1). These results were later supported also by other authors (Miedaner 1997, Parry et al. 1995, and Stack et al. 1997). Latest data (Mesterházy, unpublished) support this view for all *Fusarium* species listed. The conclusion is that a successful breeding program can be performed based on F. graminearum or F. culmorum and the plant will have resistance to all of the listed Fusarium species.

Resistance structure.

Components of physiologic head blight resistance: 1. Resistance to invasion (Schroeder and Christ. 1963), 2. Resistance to spreading (Schroeder and Christ. 1963), 3. Resistance to toxin accumulation, degradation (Miller et al. 1985, Mesterházy et al. 1999), 4. Resistance to kernel infection (Mesterházy 1989, 1995, 1999), 5. Tolerance (Mesterházy 1989, 1995, 1999), 6. Resistance to late blighting (Mesterházy, 1999, under progress), 7. Resistance to head death above infection site (Germany, under progress).

Morphologic resistance parameters: 1 Plant height (Mesterházy 1995), 2. Length of last internode below the head (Parry et al. 1995), 3. Presence of awns (Mesterházy 1987, 1995), 4. Compactness of the head (observation, no experimental proof), 5. Nutant or erect heads (observation, no experimental proof). 6. Erect leaves (Lienemann et al. 2000).

Resistance in other organs: 1. Resistance to stalk and crown rot (Butler 1962, 2. Resistance to seedling infection Mesterházy 1985), 3. Resistance to leaf infection (sporadic reports), 4. Resistance to nodal infection (under progress).

Of the 7 physiological resistance forms the resistance to spreading (No 2) is the most important, all resistant cultivars were selected to this trait and all genetic studies refer to this resistance parameter. The resistance to initial infection (No 1) seemed to be verified, but recently several authors question it. Mideaner thinks that this type of resistance occurs when a cultivar is susceptible according to single floret inoculation, but much more resistant when inoculated by spraying. Kang and Buchenauer (2000) did not find difference between initial infection processes among resistant and susceptible cultivars. So we think that the lists are not final, and we need for the other traits no effective selection method exist now.

Sources of resistance. The most used source is Sumey-3 (Soo-moo-3), beside this Frontana from Brazil is used more often. Nobeoka Bozu was used by several authors (Buerstmayr, et al., 1996, Yong-Fang, et al., 1997). All are spring wheats. However, different accesses from these have a considerable variation in resistance. As no immune source was found, new sources were screened from alien species. Sources with higher resistance than Sumey 3 were found in hybrids *T. aestivum-Leymus racemosus, T. aestivum-Roegneria komoji* and *T. aestivum-R. ciliaris*. The alien additions were identified also by RFLP (Chen, et al., 1997). *Rogneria* proved to be the best resistant genus of Triticeae where 1463 entries were tested from 85 species. Beside these the geni *Hystrix, Kengylilia, Agropyron (Elymus)* had good resistant species (Yong-Fang, et al., 1997). Lu and Wang (1991) found considerable resistance in *Haynaldia villosa*. Their breeding value will be tested later. Fedak et al., (1997) found high resistance *Thinopyrum intermedium* accessions where no spreading from the inoculation site occurred. They found also high level of resistance was identified in *Hordeum californicum* on chromosome 4. Ban (1997b) found in Japanese entries of *Agropyron humidus, A. tsukushiensis* and *A. racemifer* resistant accesses having higher resistance than Nobeoka Bozu. However, the spring wheat sources are agronomically poor, the alien species are even worse, and therefore an intensive prebreeding is needed to create adapted and highly resistant wheat genotypes suitable for crosses to produce commercial cultivars. The cloning of genes will be helpful in this process.

The best winter wheat sources are significantly less resistant, but much more adapted like in Arina, Bence, Ringo Star and others. They provide the possibility to build up a highly resistant winter wheat pool with different sources than spring wheat resistance sources.

Genetic analyses speak 2-3 genes in one resistant material. Recently intensive research is under way to find QTLs for resistance and the first QTLs have been identified. As resistance genes cooperate, and an additive effect was often found and transgressive segregations occur relatively often (Sumey 3), the possibility is there to combine several genes or QTLs to form even higher level of resistance. This can be an alternative strategy to the use of new alien resistance sources when molecular breeding will be introduced. We should stress, however, that by non-molecular methods very good resistance can be incorporated into the wheat lines, a successful breeding is possible. We are convinced that those breeding institutions will utilize best the molecular results that are well trained in non-molecular breeding and pathology work.

In Szeged the resistance research and breeding work has along tradition. Of the spring wheat sources the Sumey-3 and Notebook Bozu was extensively used. We started also to build up a winter wheat program, where higher resistant winter wheat genotypes were considered. From the large material I present the data for 1999.

The data were divided into two groups for early and medium maturity cultivars. Within the groups no significant correlations with inoculation date were found. The resistance sources are printed italics. Ranking was made according to FHB values.

Table 1. FHB resistance of early ripening wheat genotypes, 1999

Plot No.	Genotype	Trait				Inoculation
		DON ppm	Kernel inf.%	FHB %	Yield loss %	May
154	*Wuhan42B*	4.546	1.33	0.00	16.59	17
430	Zu/Ré-NB	0.586	4.00	0.00	2.15	20
386	Sgv-NB/MM-Sum3	0.801	0.25	0.19	2.12	21
183	*Frontana*	7.125	9.17	0.25	10.19	17
141	Sum3-81.60/Kő	1.157	0.35	0.58	7.63	19
155	*Wuhan6B*	0.877	0.09	0.69	3.20	19
188	RSt-MM/NB	1.545	0.01	1.25	2.13	20
139	*Wuhan2*	1.676	1.18	1.33	5.28	17
156	*Nobeoka Bozu*	2.069	0.25	5.12	24.10	20
100	Tiszatáj	8.491	2.92	5.17	26.14	21
149	Sum3-81.60/Kő	1.230	1.02	5.29	2.51	21
81	Répce	3.248	3.17	5.75	7.38	21
423	Sioxland	4.421	7.92	8.13	10.18	17
424	Ringo Star	2.629	1.59	8.13	8.02	20
187	RSt-MM/NB	2.814	0.42	10.54	20.29	20
387	China 93.103	3.608	1.08	12.33	15.55	17
35	Zu/Sgv-GT..	5.558	10.00	12.54	24.82	20
157	RSt/MM-NB	6.310	2.00	13.96	22.12	19
105	Tenger	8.718	8.10	15.00	30.93	20
30	Be-SK4821	6.898	3.61	17.88	22.56	19
128	Margit	6.515	3.33	19.25	35.01	21
131	Rába	16.983	18.92	19.79	32.73	20
106	Forrás	8.580	24.67	20.46	26.06	20
127	Tündér	5.212	6.17	21.21	21.26	21
113	Szálka	9.390	8.25	23.79	26.59	19
121	Csongrád	18.578	18.33	25.13	33.72	17
73	Jbj-50	9.170	19.33	25.96	36.36	20
75	Góbé	22.573	39.58	27.50	38.56	20
103	Jászság	25.835	39.17	27.88	25.15	19
93	Cipó	12.089	8.42	27.92	26.90	20
109	Bagoly	13.433	17.75	28.33	36.64	20
111	Fürj	16.058	17.93	29.00	35.50	20
86	Élet	10.765	45.42	29.04	37.94	19
151	Táltos	36.205	62.83	30.83	39.16	20
118	Héja	14.453	25.00	31.58	40.63	20
110	Sas	13.085	11.92	32.29	39.50	20
104	Verecke	15.335	24.17	33.54	47.24	20
92	Dávid	26.095	23.33	33.75	38.08	19
1	Öthalom	31.066	70.42	35.78	55.04	17
87	Kalász	36.896	45.92	36.17	38.36	20
91	Garaboly	19.533	25.25	38.42	53.22	17
124	Attila	11.918	24.17	44.00	26.52	17
78	Pinka	18.400	37.92	45.33	43.69	17
	Mean	**11.25**	**16.11**	**19.31**	**26.14**	
	LSD 5 %	10.77	2.11	3.6	8.84	

Inoculation May 17-21

Trait	DON ppm	Kernel inf.%	FHB %	Yield loss %
Kernel inf.%	0.8812***			
FHB %	0.7732***	0.7397***		
Yield loss %	0.7575***	0.71721***	0.8721***	
Inoculation day	-0.1154ns	-0.1757ns	-0.1600ns	-0.0891ns

*** P = 0.1 %, ns = non significant

In the early inoculated cultivars the infection severity was higher than in the later group due to cooler weather between 25-31 May. DON correlated best with kernel infection, the values are near 0,90, significant at P = 0,1 %. The tables show that many genotypes containing Sumey-3 and

Nobeoka Bozu reach the best sources, sometimes they are more resistant, even the differences are not always significant. Frontana is less resistant; its toxin contamination is relatively high. In most cases genotypes with no or very low kernel infection show DON contamination such are FHB 143 or Mérő. For visual notes they were free of infection, FHB numbers are also low, but it has white grains, therefore some of the Fusarium damaged kernels could be misjudged. Latent or light infection could be present and also toxin translocation could be occurred.

Table 2. FHB resistance of medium ripening wheat genotypes, 1999

Plot No.	Genotype	Trait				Inoculation
		DON ppm	Kernel inf.%	FHB %	Yield loss %	May
137	Sgv-NB/MM-Sum3	0.870	0.25	0.00	-1.65	25
145	*Sumey3*	0.251	0.59	0.00	15.65	25
182	Sgv-NB/MM-Sum3	1.111	1.33	0.63	-1.56	25
201	Sum3-81.60kKő	1.485	0.00	0.67	15.78	25
162	Sgv-NB/MM-Sum3	0.435	0.58	0.88	3.68	27
391	Ttj-81.F379	1.522	0.17	0.96	1.05	25
147	Sgv-NB/MM-Sum3	0.930	1.43	1.88	8.69	25
135	Bence	5.439	0.83	4.83	18.84	25
413	Praag8	0.215	1.34	5.04	3.84	31
164	Zu/Ré-NB	0.164	0.50	6.25	9.90	25
161	Sgv-NB/MM-Sum3	4.730	3.67	7.33	12.16	31
192	St902/Sgv-NB*MM-Sum3	1.619	0.10	7.38	12.23	25
169	FHB 143	8.404	0.00	9.37	5.28	27
95	Mérő	2.878	0.03	10.67	8.29	25
181	Sum2^2-81.60	2.680	1.42	10.88	22.10	25
193	Sgv-NB/MM-Sum3	3.266	0.67	11.38	4.40	25
31	Sum3-81.60*Kő	1.792	0.03	11.46	-2.69	25
420	Kő	10.802	24.17	11.67	36.05	25
36	Zu/Sgv-GT..	3.451	4.09	14.54	30.66	25
26	Bánság8	4.375	0.84	14.58	15.99	25
133	Sámson	3.603	1.08	16.00	30.90	25
83	Kende	3.488	5.75	18.17	26.27	25
177	Sgv-NB/MM-Sum3	4.330	3.43	18.17	26.87	25
89	Véka	2.975	0.63	18.25	22.23	25
80	Marcal	1.563	5.92	20.21	30.23	25
143	Zu/Ré-NB	2.498	3.50	20.91	32.18	25
107	64.96	5.913	3.83	21.83	23.68	25
13	Zugoly	10.803	22.10	31.93	40.12	25
411	SK 8090	13.051	24.66	36.41	21.14	27
129	Jutka	5.695	5.10	37.45	30.88	25
418	MM	18.180	31.92	42.75	50.41	25
	Mean	**4.28**	**5.00**	**13.75**	**18.45**	
	General mean	**8.23**	**11.32**	**16.76**	**22.62**	
	LSD 5 %	10.77	2.11	3.6	8.84	

Inoculation May 25-31

Trait	DON ppm	Kernel inf.%	FHB %	Yield loss %
Kernel inf.%	0.8867***			
FHB %	0.74897** *	0.7021***		
Yield loss %	0.6362***	0.6711***	0.7550***	
Inoculation day	-0.0059ns	-0.0164ns	-0.1326ns	-0.2598ns

*** P = 0.1 %, ns = non significant

Later investigations can clarify the causes. In some cases the resistance to kernel infection could be detected like in Jutka where 37 % head infection resulted only 5 % kernel infection compared with SK 8090 e 36 % FHB resulted in 24 % grain infection. A relative DON resistance was found in Forrás compared to Rába. Jubileynaya 50 seems to have also a relative DON resistance. We can identify also tolerance like in SK 8090, plot No 31 or Bánság where the yield loss is significantly less than the neighboring entries with similar FHB data.

Among winter wheat selections we should mention the Szeged lines Ringo Star and Ttj-81.F279, their performance is near to the better spring wheat resistance sources.

The lines are tested for other resistance traits like stem rust, leaf rust, powdery mildew and Septoria tritici. Also yielding ability and quality parameters are screened. Based on these data several of the highly FHB resistant lines will be suitable also for commercial production.

Among the Szeged commercial cultivars from regular breeding program GK Cipó, GK Forrás, GK Garaboly, GK Góbé, GK Jászság, GK Kende, GK Marcal, GK Mérő, GK Répce and GK Verecke have better resistance to FHB than leading more susceptible cultivars, they have appropriate yielding ability and baking quality. We can state now that a FHB resistant material can be bred without large scientific investment; even we have a number of open questions. The screening technology is present; sources of resistance are at hand. We see a greater problem to breed genotypes with FHB resistance accompanied with yielding ability, resistance to other important diseases and suitable quality parameters. However, this is also possible and we have a number of lines that represent a good genetic tool for this work.

There is a strong hope that is several years the most susceptible cultivars can be changed for more resistant and resistant ones and so the feed and food safety can be increased significantly.

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BREEDING FOR COMPLEX RESISTANCE TO LEAF DISEASES IN WHEAT

Mária Csősz
Cereal Research Non-profit Company, 6701 Szeged, POB. 391., Hungary

Leaf rust (*Puccinia triticina)*, powdery mildew (*Blumeria (Erysiphe) graminis* f.sp. *tritici*) and stem rust (*Puccinia graminis* f.sp. *tritici*) are the major wheat leaf diseases in Hungary.
During the last 11 years two severe leaf rust epidemics were recorded (1996, 1999) and in two years the plants were nearly symptomless (1995, 1998). The remaining years showed only moderate infection severities (Figure 1).
The severity of the powdery mildew epidemics varied heavily between 1990-2000. In the last 5 years the severity of epidemics was low (yearly means about 10 %), only the most susceptible genotypes showed considerable infection up to 20-40 % (Figure 2.).
Of the leaf rust epidemics the yield data of 1999 and 2000 are shown in Table 1. It caused significant yield reduction in 1999. The powdery mildew caused also significant yield decrease, but the damage was lower than that found for leaf rust. In 2000 the weather was very dry and warm, so the build up of the leaf rust epidemic was delayed and in spite of the considerable infection at the last scoring on susceptible cultivars the yield reduction was very moderate. The powdery mildew started more strongly, but its spreading was strongly inhibited by unsuitable ecologic conditions (Table 1.).

Table 1. Effect of the natural leaf rust and powdery mildew infection on yield of winter wheat genotypes at different stations, 1999-2000

Years	1999		2000	
Stations	Leaf rust	Powdery mildew	Leaf rust	Powdery mildew
Szeged	-0,5547***	-0,0846	-0,1852	-0,3074**
Zsombó	-0,7335***	-0,4662***	-	-
Gödöllő	-0,4969***	-0,4849***	-	-
Táplánszentkereszt Optimal sowing time	-0,0740	-0,1346	-	0,1325
Táplánszentkereszt Optimal sowing time+50kg N	-0,3945***	-0,3634**	-	0,0358
Táplánszentkereszt Late sowing time	-0,3593***	-0,3470**	-	0,0404
Fülöpszállás	-0,2631*	-0,2319*	-0,2298°	0,1682
Szarvas	-	0,0487	-	-
Öthalom	-	-0,4975***	-	-

***P = 0,1 %, **P = 1 %, *P = 5 %, ° = 10 %

The stem rust infections were rare in the last three decades: epidemics with losses were not recorded. Considerable infection and yield loss were found only in the artificially inoculated nurseries.

The identification of effective resistance genes is an important part of a breeding project.
Among the leaf rust near isogenic lines no symptoms were found on Lr9 and very low infection severity was present on the Lr19, Lr24, Lr25, Lr29, Lr35 and Lr38 (Csősz et al., 2000). On the other NILs the virulence of the present leaf rust population was medium to very high.
Among the powdery near isogenic lines the best line is Amigo, that includes the Pm 17. In the last three years the epidemic levels were very low, so we could not evaluate properly the effectiveness of the resistance genes.
Of the stem rust near isogenic lines (from 2000) no symptoms were found on Sr36 and very low infection severity could be identified on the Sr9e, Sr11, Sr24, Sr26, Sr27, Sr31, Sr32, Sr36.

The GK Kincső was registered in 1983. This cultivar derives from the Arthur 71/Sava cross. Arthur 71 includes the Sr36 gene being effective all over the world and additionally the Sr2, Sr5 and Sr8a genes. Sava contains Pm2 and Pm6 for powdery mildew. The resistance determined by Sr36 was effective for stem rust in the past 23 years. The Kincső had a good field resistance also to powdery mildew. It seems that beside Pm2 and Pm6 other genes might be present as the near isogenic line with Pm2 and Pm6 was always more severely infected.
The classical gene identification of the postulated Sr36 gene in Kincső was made. In this work we cooperated with Dr. Pavel Bartos and the seedling tests were made in Prague. For the adult plant tests in segregating populations the race 218 was used supplied by Dr. Klara Manninger. In the adult plant test no segregation was found between Kincső and NIL Sr36 that proves that the GK Kincső includes the Sr 36. In Prague also five other Kincső originated cultivars were tested. All contained the Sr36. For them, however, the segregation tests have

not been made. The GK Kincső was very widely used in our breeding program because of its good stem rust resistance. From this program many registered cultivars originated with similarly good stem rust and powdery mildew resistance (Csősz et al., 1997, Csősz et al., 1999, Manninger et al., 1998).

Of the cultivars listed GK Kincső, GK Garaboly, GK Góbé (Sr36) and GK Csörnöc (Sr31) were tested in twin plot design for stem rust resistance by artificial inoculation in 1992-1998. (The stem rust race mixture was supplied by Dr. Klara Manninger, Budapest). Beside the stem rust data also leaf rust and powdery mildew data from natural infection have been recorded.
The stem rust infection severity of the three highly resistant cultivars was never higher than 5 %. So the efficacy of their resistance was excellent. The infection severity on GK Csörnöc varied from very low to middle infection severity. The Sr 31 therefore is considered less effective than Sr 36 (Figure 3.). The cultivar's response to powdery mildew was low with slight differences between years, and it was significantly lower than that observed in susceptible cultivars (Figure 4.). Significant natural leaf rust infection was observed only in three years. The leaf rust infection severity of these cultivars was significantly lower than that experienced in the susceptible cultivars reaching 80-100 % infection (Figure 5.).
Therefore the resistance in these cultivars mediated by known or unknown Lr, Pm and Sr resistance genes should be considered for all four cultivars to be effective and long lasting.
The yield reaction of the four cultivars except for 1994 and 1997 remained stable, only low, but occasionally significant yield loss has been recorded without significant infection severity. However, the susceptible cultivars in the nursery suffered heavy losses. This means that under these conditions the energy consumption of the resistance plants caused the yield loss (Figure 6.).
The resistance genes for stem rust decreased very effectively the infection, for Sr36 it was nearly complete, for Sr31 rather moderate. For leaf rust and powdery mildew the resistance was medium and severe epidemics did not develop (Csősz and Mesterházy 2000).

In the last years the *Drechslera tritici-repentis* (DTR) was considered to have a high importance in Hungary. To investigate this, 1800 samples from 15 locations were collected in April, May and June 2000 (5-5 leaf/sample) from plants showing leaf spots. The leaves were placed in humid chamber. The Petri dishes were stored at 20 C° and 2-3 days later microscopic investigations followed. Of the data only the June records are presented.
Alternaria spp. and *Cladosporium* sp. were found in all samples. *Stemphylium* sp. (50 %), *Epicoccum nigrum* (34 %) and *Mucor* sp. (8 %) were much less represented. Some of them may cause leaf spots, but they are considered generally as saprofitic microorganisms (Table 2.).

Table 2. Incidence of pathogenes considered saprophitic on wheat leaves (June 2000, Szeged, Hungary)

Stations	Total	Saprofitic pathogenes				
		Alternaria spp.	*Cladosporium* sp.	*Epicoccum nigrum*	*Stemphylium* sp.	*Mucor* sp.
Fülöpszállás	72	72	72	47	15	17
Szeged	190	190	190	59	60	13
Táplánszentkereszt	141	141	141	47	105	7
Szombathely	66	66	66	4	62	8
Debrecen	66	66	66	28	38	1
Total	535	535	535	185	280	46
Occurence %		100,00	100,00	34,58	52,34	8,60

Among the known necrotrophic pathogens DTR (24 %), *Septoria tritici* (20 %) *Bipolaris sorokiniana* and *Septoria tritici* (6 %) were often found (Table 3.).

Table 3. Incidence of pathogenes considered necrotrophic on wheat leaves (June 2000, Szeged, Hungary)

Stations	Total	Necrotrophic pathogenes			
		Bipolaris sorokiniana	*Drechslera tritici-repentis*	*Septoria tritici*	*Septoria nodorum*
Fülöpszállás	72	8	14	17	3
Szeged	190	10	23	6	47
Táplánszentkereszt	141	1	38	-	7
Szombathely	66	2	32	-	1
Debrecen	66	2	25	9	51
Total	535	23	132	32	109
Occurence %		4,30	24,67	5,98	20,37

The conclusion is that most of the leaf spots can not be connected to DTR. So its decisive role could not be supported by the data. It seems that high majority of leaf spots judged as DTR infection were caused by other pathogens and sometimes saprophytic microorganisms. Is seems further that a differential diagnostic at initial leaf spot development based on symptoms is mostly not correct.

Summary

1. The Szeged breeding material has mostly MS, MR or R type of disease reaction for the yearly 1000 or more genotypes. Most of them have no known genetic background.
2. The efficacy of resistance genes for the three diseases is different. For stem and leaf rust we have highly effective genes even if they could not be identified in some cases, for powdery mildew the virulence was medium to high for all NILs tested. The Pm resistant materials therefore need a careful genetic analysis to identify gene combinations or unknown sources of resistance being useful in the future work.
3. The most successful stem rust resistance source is Artur 71, the Sr36 could be verified in its progenies with classical genetical methods.
4. Within the tested four cultivars the resistance to the leaf diseases seems to be durable and the same refers to the Kicső resistance, too.
5. DTR is less important as thought before. Leaf spots in many cases can not be identified only by symptoms.

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Figure 1. Leaf rust infection severity of winter wheat genotypes (1990-2000) Szeged, Hungary (n=1012-2472)

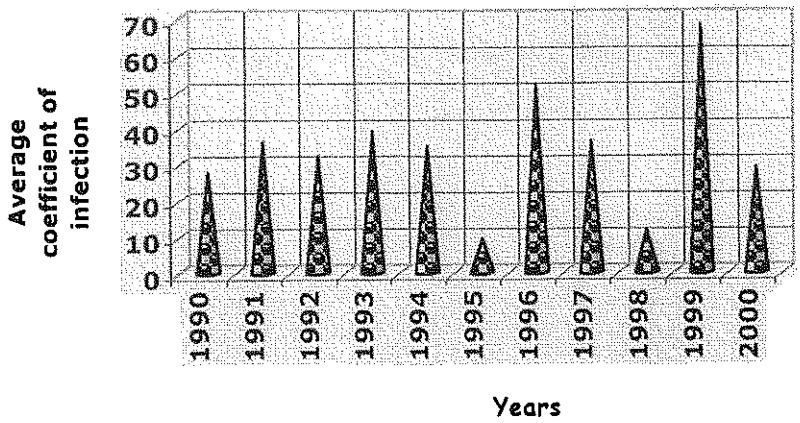


Figure 2. Powdery mildew infection severity of winter wheat genotypes (1999-2000) Szeged, Hungary (n=1012-2472)

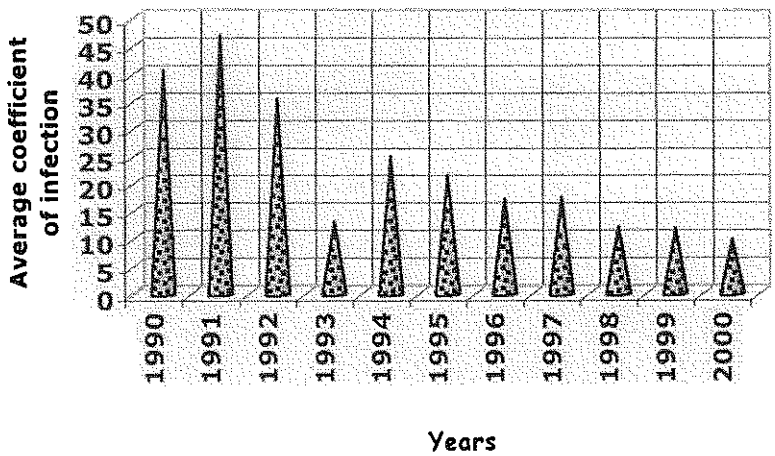


Figure 3. Stem rust infection (ACI) in GK Csörnöc (Sr31) and three other cultivars with Sr36 (Szeged, 1992-1998)

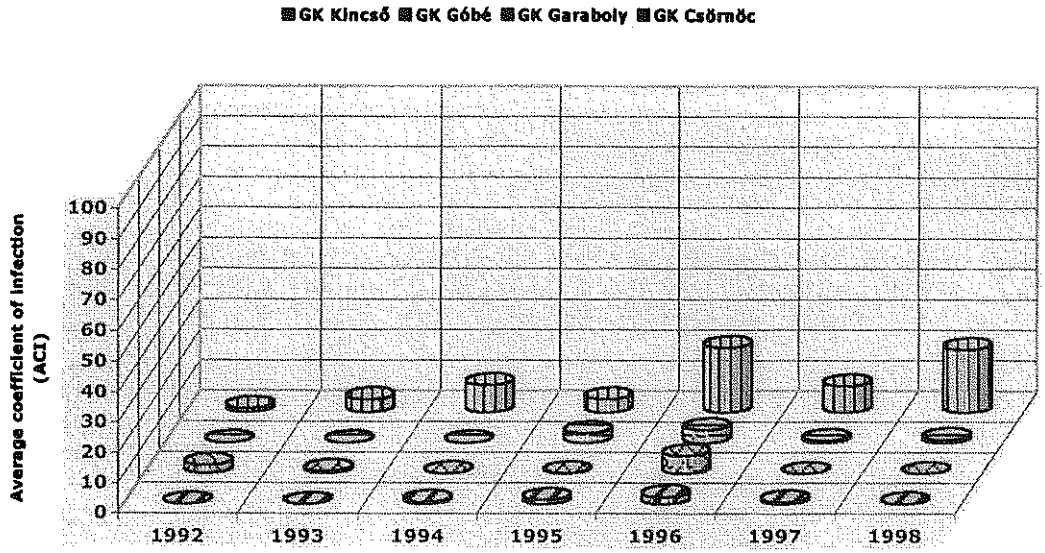


Figure 4. Powdery mildew infection (ACI) of four winter wheat varieties (Szeged, 1992-1998)

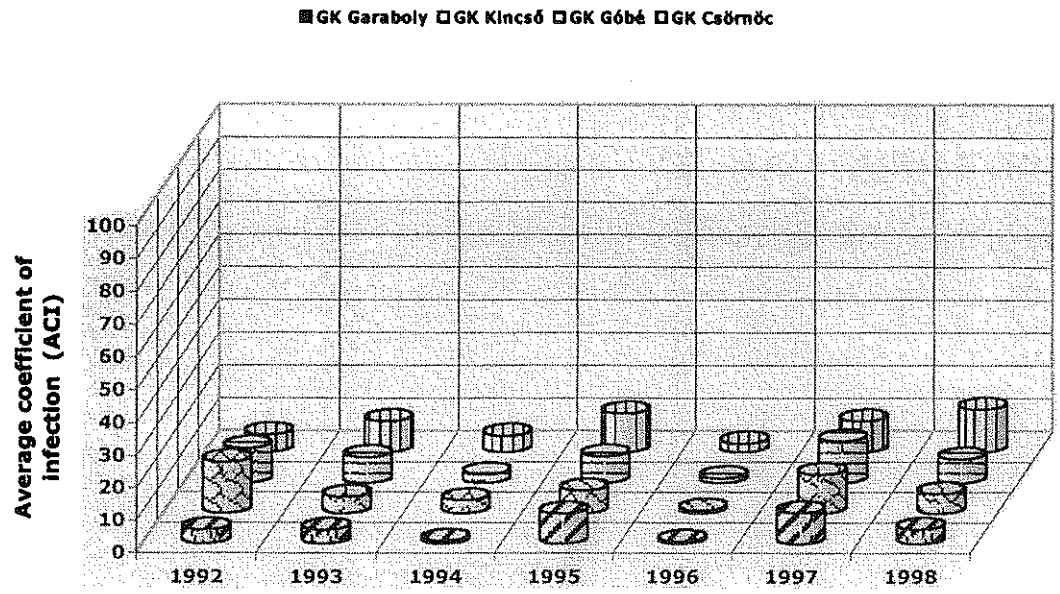


Figure 5. Leaf rust infection of four winter wheat varieteies (ACI) (Szeged, 1992-1998)

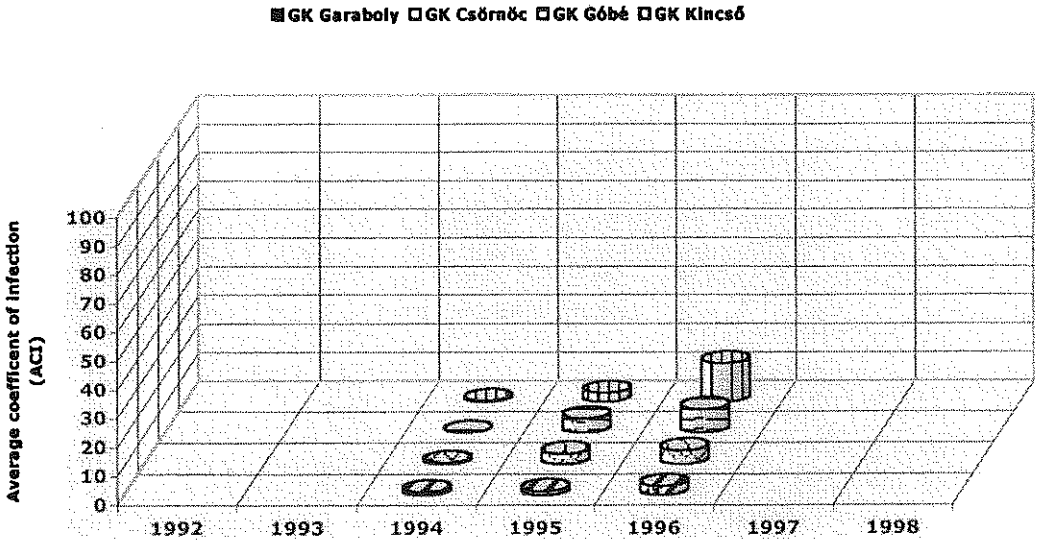
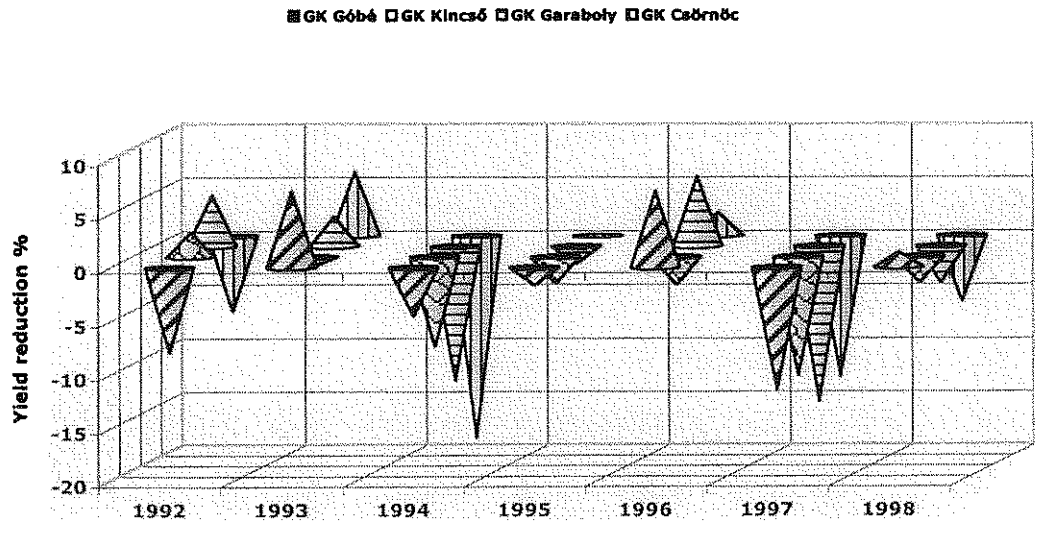


Figure 6. Yield reduction in four winter wheat varieties following stem rust, leaf rust and powdery mildew infection (Szeged, 1992-1998)



APPROACHES TO RESISTANCE BREEDING OF ANNUAL CARAWAY (*CARUM CARVI* L. VAR. *ANNUUM*) TO UMBEL BROWNING

Jutta Gabler

Federal Centre for Breeding Research on Cultivated Plants, Institute for Resistance Research and Pathogen Diagnostics, PO Box 1505, 06449 Aschersleben, Germany

Introduction

In recent years the interest in healthy diet and natural products has been increasing constantly on an international scale. This trend led not only to a growing demand for medicinal and aromatic plants but also to an increased need for research in this field. Reacting to this development, fungal diseases on different medicinal and aromatic plants have been investigated in the Institute for Resistance Research and Pathogen Diagnostics Aschersleben of the Federal Centre for Breeding Research on Cultivated Plants for several years. The main aim of these investigations is the development of the scientific and methodical basis for the breeding of resistant cultivars. In a first project, the umbel browning on annual caraway has been investigated. The results of these approaches to resistance breeding performed from 1997 to 1999 shall be reported here.

Caraway is originally a biennial plant under climatic conditions of Central Europe. As a consequence, producers can have yields only every two years. For improvement of the price-performance ratio, populations of annual caraway were selected. During the breeding process, an umbel browning of unknown cause with negative implications on yield and quality was observed repeatedly in the breeding material. For finding a solution to this problem, the cause of the umbel browning had to be identified first. There were three hypotheses: It is 1. a parasitic disease, 2. a physiological disturbance, 3. a genetic defect. Therefore, our research task was, above all, to analyse the causes of the disease.

Material and methods

Field experiment

A field experiment using 7 populations of annual caraway in 1997, and 12 in 1998 and 1999, respectively, was performed in order to take samples for the pathogen isolation, and to establish differences in the susceptibility to umbel browning within the host population. Every population was represented by 16 plants.

Taking samples

During the flowering stage, umbels with browning symptoms were taken weekly as samples for the pathogen isolation. The plant material was surface sterilised, than transferred on agar medium (SNA) and incubated at room temperature.

Pathogen isolation and pathogenicity tests

The fungal isolates were identified microscopically and tested for pathogenicity under greenhouse or lab conditions using intact caraway plants or detached umbels. For the inoculation, different methods were applied: Spraying with inoculum, dipping into inoculum or application of inoculum (5-10 µl) on the umbel ground. With the last mentioned method, the variants of "umbel ground wounded" and "not wounded" were compared additionally. For the preparation of the inoculum, mycelium from agar cultures was homogenised and suspended in destilled water (20 ml per petri dish with 6 cm in diameter).

Phomopsis diachenii Sacc.

Among the fungal pathogens, *P. diachenii* was studied particularly intensively. The temperature requirements were estimated. Furthermore, a polyclonal antiserum (IgG 59/1) raised from mycelial extracts was produced for pathogen detection. In addition, a PTA-ELISA was developed and tested for its suitability to assess resistance.

Visual scoring of the symptom severity

The following scale was developed for visual scoring of the symptom severity on intact plants and detached umbels infected artificially (especially with *P. diachenii*) under greenhouse conditions:

Symptoms	Symptom severity class
Umbel externally healthy	1
Browning of one flower in the umbel	3
Browning of several flowers in the umbel	5
Total browning of the umbel	7
Necrosis on umbel and stem	8
Pycnidia on necrotric parts of the plant	9

Plants infected under natural conditions were visually evaluated by counting all diseased and healthy umbels per plant. In addition, the following three developmental stages of the umbels were separately estimated: 'Before flowering', 'During flowering' and 'After flowering'. This time-consuming method was applied hoping to get more detailed information regarding the correlation between disease development and response of the populations to umbel browning.

Fungicide experiment

A fungicide experiment with four benomyl treatments within 8 weeks was performed using 3 high-susceptible populations for getting additional evidences regarding the causal agents of the disease.

Wild caraway populations

Wild caraway populations collected by Julia Forwick from the Institute for Agricultural Botany of the University Bonn were studied for umbel browning in a separate field experiment. The aim of these studies was to find out indirectly whether umbel browning is a specific genetic defect of annual caraway or not.

Results

Umbel browning was mainly caused by the fungal pathogens *Alternaria* spp., *Botrytis cinerea*, *Synchytrium aureum*, *Cladosporium* sp. and *P. diachenii* which mostly occurred as a complex. So, it could be proved that it is a parasitic disease. Some isolates among all fungal genera and species mentioned were also able to cause browning symptoms when they were inoculated separately. *P. diachenii* could be identified to be the most aggressive fungal pathogen, followed by *Alternaria*. The former was also able to kill whole plants within a short time by progressive necrosis. In pathogenicity tests with *P. diachenii*, the highest symptom severity was obtained by dipping the umbels into the inoculum suspension, compared with spraying or application of inoculum on the umbel ground. A distinct increase of the symptom severity was achieved by wounding the umbel ground with a needle. This procedure should simulate the stinging of the umbel ground by *Lygus* bugs under natural conditions. The result obtained confirms the hypothesis that *Lygus* bugs favour the spread of the disease. The detection of *P. diachenii* on caraway in 1998 was also the first in Germany (GABLER & EHRIG, 1999, GABLER & EHRIG, 2000[1]). Two years before, the fungus was detected for the first time in the Czech Republic, where it caused locally high yield losses (ONDREJ, unpubl.).

Morphological and physiological characteristics of *P. diachenii*

P. diachenii Sacc. is characterised by white mycelium, black pycnidia submersed in the substrate and two types of pycnospores: α and β. The β spores were not able to germinate and to infect the plants. The temperature optimum for the colony growth of *P. diachenii* (isolate Pdi 6) was between 25 °C and 30 °C; however, growth was also possible at 14 °C, 18 °C and 20 °C. No growth took place at 37 °C. A close correlation was found between the disease development in the field and some climatic data, above of all the mean and maximal air temperatures. This fact could probably explain the high disease level in hot summers observed by the breeder.

Bacteria

Occasionally, bacteria, especially *Pseudomonas* sp. and *Erwinia* sp. (ZIELKE, unpubl.), could be detected as causal agents of umbel browning symptoms, too, but they played a subordinate role compared to fungi. A visual differentiation between umbel browning caused by fungal or bacterial pathogens was not possible.

Wild caraway

Umbel browning occurred on wild caraway, too, indirectly showing that the disease is not a genetic defect of the annual caraway.

Fungicide experiment

Additionally, the predominant role of fungal pathogens was confirmed by the fungicide experiment. The disease level was reduced by the fungicide treatments by approximately 60% compared with the untreated controls.

Susceptibility of the populations under natural conditions

There were significant differences between the populations in their susceptibility to umbel browning, but none of them was resistant. The early flowering populations inclined partly to a higher susceptibility compared with later flowering ones (Fig. 1).

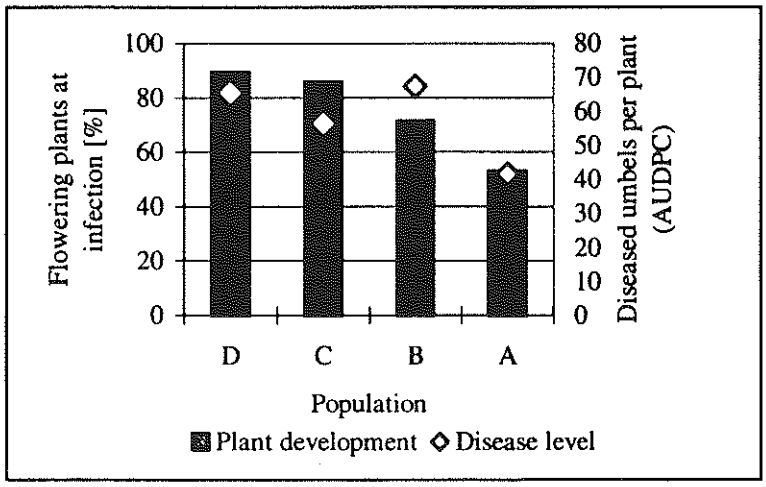


Figure 1: Correlation between the plant development of different caraway populations and the level of their susceptibility to umbel browning (Population D was significantly higher susceptible than B and A).

P. diachenii could be exactly detected in PTA-ELISA with the polyclonal antiserum IgG 59/1 (GABLER, 2000, GABLER & EHRIG,2000 [2]). A close positive correlation was found between the symptom severity evaluated visually and the corresponding ELISA values. A detection of latent infections was also possible by the PTA-

ELISA. The IgG showed in PTA-ELISA no cross reactions with mycelial extracts of *Alternaria spp., Botrytis cinerea, Synchytrium aureum, Fusarium* spp., *Septoria carvi, Mycocentrospora acerina* and *Erysiphe umbelliferarum* as well as with bacteria occurring on caraway, too. Therefore, it can be also used for a specific detection of *Phomopsis* in field material. The PTA-ELISA also proved to be suitable to assess differences in the susceptibility of populations infected artificially with *P. diachenii*. The susceptibility of the populations to *P. diachenii* evaluated by PTA-ELISA and the response of the same populations to umbel browning evaluated visually showed similar tendencies (Fig. 2).

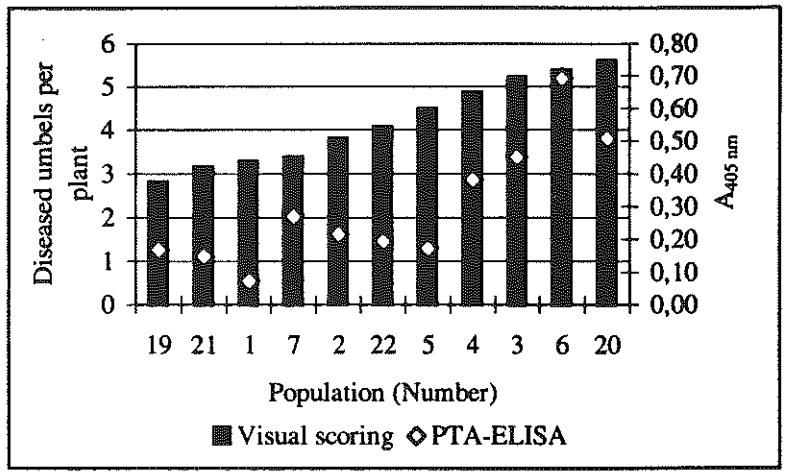


Figure 2: Comparison of the susceptibility of caraway populations to umbel browning under natural conditions (visual scoring) and to *P. diachenii* infected artificially in the greenhouse (PTA-ELISA).

Discussion

Umbel browning is neither a physiological disturbance nor a genetic defect but an infectious disease mostly caused by a complex of fungal pathogens. *P. diachenii* and *Alternaria* spp. could be identified as the most important causal agents. There are significant differences in the susceptibility to umbel browning between populations of annual caraway. PTA-ELISA for the detection of *P. diachenii* proved to be also suitable for the assessment of differences in the susceptibility. The results obtained could be used as a scientific and methodical basis for the breeding of caraway cultivars resistant to umbel browning.

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BREEDING APPROACHES FOR BUNT CONTROL IN WINTER BREAD WHEAT

ITTU M.*, N. N. SAULESCU & G. ITTU

Research Institute for Cereals and Industrial Crops (R.I.C.I.C.)
Fundulea, 8264 Calarasi
(*gittu@pcnet.pcnet.ro)
Romania

Introduction

Bunt disease has been effectively under control in Romania, for many years, by use of seed treatments on a routine basis. Hg chemicals were replaced after 1990 by very efficient organic chemicals. However residual infection occurs endemically in some regions in South-Romania characterised by dry climatic conditions. Increased levels of attacks were reported also for other regions of the country (Bobes and Florian 1986). More frequent epidemics of common bunt were particularly registered in small private farms formed after 1989, where the use of untreated seed still happens. In the last years higher prevalence of dwarf bunt was registered in some central and northern regions (Anonymous, 1996, 1997, 1998 and 1999). Higher yield losses were registered in several other countries: Canada (Laroche et al. 2000), Denmark (Nielsen and Jørgensen 1994), Hungary (Szunics and Szunics 1994), France (Champion and Raynal 1993) and USA (Mathre and Johnson 1976, Williams and Gough 1984, Ballinger and Gough 1989, Williams 1988, *cited by* Goates, 1996). The main factors generally incriminated for the re-emergence of bunt disease are: i) improperly use of fungicides, ii) monoculture, iii) change of climate, iiii) change of virulence in bunt population etc.
In many countries breeding for resistance is of major concern. Development of resistant cultivars seems to be an important alternative to chemical control, in order to produce healthy wheat seed with low inputs and reduced ecological risks. In this respect more than 15 specific resistance genes (*Bt*) which control the resistance to both common and dwarf bunt are available (Hoffman and Metzger 1976, Goates, 1996). In some areas several resistance genes proved to be highly durable, but the rapid evolution of new races is also possible. New races that observed in India (northwestern Himalayan region) defeated most of the identified common bunt resistance genes (Sood 1996). Partial resistance has also been described. High degrees of resistance to bunt were reported in wheat cultivars of different origins: Amigo, Franklin, Crest, Tjelvar, Stava etc (Jönsson and Svensson 1990, *cited by* Nielsen et al. 1998, Blazková and Bartos 1997, Nielsen et al. 1998).
New projects to pyramidate multiple resistance genes to common bunt in wheat are in progress. Recently a RAPD-based 590-bp marker linked to the very efficient *Bt*-10 gene was identified (Demeke et al 1996). Laroche et al. 2000 demonstrated the reliability of a PCR marker system using the FSD and RSA primer pair to select individual lines carrying *Bt*-10 gene of resistance to common bunt and to distinguish between homozygosity and heterozygosity.
The objective of this report is to present the breeding results regarding the improvement of resistance to bunt in winter bread wheat at R.I.C.I.C. Fundulea.

Material and Methods

Material included in trials for bunt resistance is the breeding germplasm obtained in the winter wheat bread breeding program from Fundulea and lines carrying different race-specific *Bt* resistance genes, obtained from the Oregon State University .
The efficacity of nine race-specific *Bt* genes following artificial inoculations with a large range of *Tilletia* spp. isolates of different geographic and host origins is permanently assessed.
Transfer of race-specific *Bt* resistance genes (*Bt* 5, *Bt* 8, *Bt* 9, *Bt* 10, *Bt* 11, *Bt* 12, *Bt* 13, *Bt* 14, *Bt* 15, *Bt u* and *Bt c)* into the cultivar Dropia was performed by backcross. Dropia (registered in 1993) has a high bread making quality and is now the most cultivated wheat bread cultivar in Romania. Association of resistance to bunt conferred by different *Bt* genes and bread making quality is the main of our current breeding focuses.
Artificial inoculations are performed by dressing the seed with teliospores (10 mg spores /1 g seeds). Inoculated seed are sown in the middle of October by hand in individual rows, 1 m long.
Inoculum consisted of single spore isolates or mixture of isolates. From each sample of pathogen (location, year, host-variety) 1-10 single spore isolates were selected, since 1992. Assessment of resistance (R) and segregation for resistance (R: S) following artificial inoculation was performed in the lines carrying different *Bt* genes.

Results

Efficacity of the race-specific Bt genes. In order to identify high virulent *Tilletia* spp. pathotypes, a large collection of isolates of different geographic and host origins was produced. Origin of 145 single spore isolates selected from 23 populations is presented in table 1.

Table 1. Origin of a collection of *Tilletia spp.* isolates utilised in the testing program from R.I.C.I.C. Fundulea.

Tilletia spp. Code	Origin		Year	No. of single spore isolates
	Geographic	Host		
1	Fundulea-RO	wheat	1992	8
2	Fundulea-RO	wheat	1992	8
3	Fundulea-RO	wheat	1997	5
4	Fundulea-RO	*Bt* 5	1998	6
5	Fundulea-RO	*Bt* 6	1998	1
6	Fundulea-RO	*Bt* 8	1998	4
7	Fundulea-RO	*Bt* 9	1998	1
8	Simnic-RO	wheat	1992	8
9	Simnic-RO	wheat	1993	8
10	Albota-RO	wheat	1993	8
11	Brasov-RO	wheat	1992	8
12	Brasov-RO	wheat-Apullum	1992	2
13	Brasov-RO	wheat-Ariesan	1998	2
14	Brasov-RO	wheat-Transilvania	1998	3
15	Brasov-RO	wheat	1999	10
16	Harghita-RO	wheat-Apullum	1998	7
17	Suceava-RO	wheat	1999	10
18	Suceava-RO	wheat-F 29	1999	10
19	Suceava-RO	wheat-Monopol	1999	10
20	Turda-RO	wheat	1999	14
21	Oradea-RO	wheat	1999	9
22	G.Toshevo-BG	wheat	1998	2
23	Colorado-USA	wheat	1999	1

Efficacity of the race-specific *Bt* genes was studied between 1994-2000 with different isolates of *Tilletia spp.* The most efficient race-specific gene in inoculations performed with 10 isolates was *Bt* 12, but a rather low frequency of virulences was also found on the genes *Bt 9* and *Bt 10*, at a rate of 20% and 10%, respectively (Table 2). Isolates from Simnic were the most virulent ones.

Table 2. Efficacity of race-specific *Bt* resistance genes against several virulent isolates found in Romania.

Bt gene	***Tilletia* spp.**									
	FUN	FUN	FUN	FUN	S	S	BV	BV	BV	SV
5	S:R	S:R	R	R	S:R	S:R	S:R	R	R	S:R
8	R	S:R	R	R	S:R	S:R	R	R	R	R
9	R	R	R:S	R	R	R	R	R	R	R
10	R	R	R:S	S	R	R	R	R	R	R
11	R:S	R:S	R	R	R:S	R:S	RS	R	R	R
12	R	R	R	R	R	R	R	R	R	R
13	R:S	R:S	R	R	R:S	R:S	R	R	R:S	R
u	R:S	R:S	R	R	R:S	R:S	R:S	R:S	R	R
e	R:S	R:S	R	R	VS	R	R	R	R	S:R

R=Resistant •S=Susceptible •VS=Very Susceptible

Diversification of Bt race-specific resistance genes. Initially in the bread wheat breeding program from Fundulea the line PI 178383 (*Bt* 8, *Bt* 9, *Bt* 10) was utilised as source of resistance. Selection was performed for resistance to bunt, as well as for other agronomic traits (yield capacity, height and resistance to leaf rust). As a result, advanced semidwarf lines with improved resistance to bunt and leaf rust and improved yield capacity was obtained.

After 1993, due to the wide cultivation of Dropia in many regions of Romania the concern to transfer resistance to bunt from different sources in this cultivar became prevalent. The approach used to improve the bunt resistance in this variety via introgression of the race-specific resistance *Bt* genes is synthesised in Fig. 1. After one cycle of backcross (F_1BC_1 and F_2BC_1), selection for resistance and important agronomic traits was

continued under artificial inoculation in F3-F6 generations. From F4 generation the first advanced breeding lines were included in preliminary trials in order to accelerate the breeding of a new cultivar.
At present 60 F6 winter bread wheat lines carrying different *Bt* race-specific resistant genes and an improved agronomic type are available (Table 3).

Table 3. F6 winter bread wheat lines derived from crosses Between cultivar Dropia and different *Bt* race-specific resistant genes

Cross DROPIA/*Bt* gene	Number of lines
Bt 5	5
Bt 8	15
Bt 10	5
Bt 11	10
Bt 12	5
Bt 13	5
Bt *T. urartu*	10

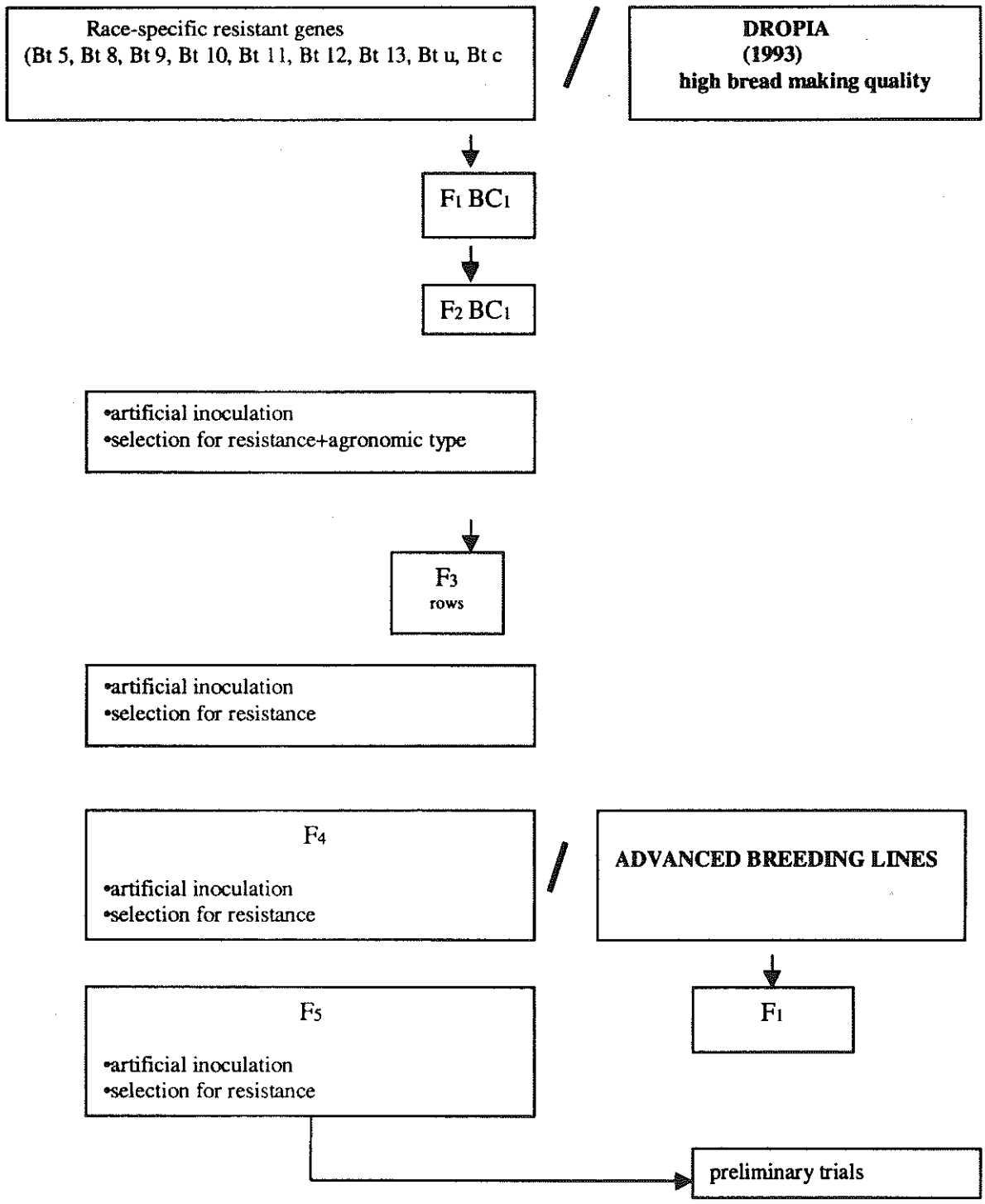
For the same purpose a new research project, funded by the national program ANSTI-RELANSIN (3435/2000) started in 2000. The studies are carried on in the breeding centers from Fundulea and Simnic (endemic occurrence of bunt disease). The main goals are. 1) evaluation of level and efficiency of resistance in nine advanced breeding lines to three *Tilletia* spp. isolates of different origins, 2) evaluation of other agronomic traits in advanced resistant lines and 3) identification of new sources of resistance in the breeding germplasms created in both centers.

Conclusions

-Evaluation of response to bunt in nine race-specific *Bt* resistance genes inoculated with 10 Romanian isolates of *Tilletia spp.* highlighted the total efficiency of *Bt 12* and a good level of resistance in *Bt 9* and *Bt 10.* However the recombination between genes having a lower effect, seems to be a realistic tool in the improvement of resistance to this pathogen in wheat.

-Following two cycles of selection introgression of several *Bt* genes (*Bt5, Bt 8, Bt 10, Bt 11, Bt 12, Bt 13 and Bt u)* in a commercial cultivar of winter bread wheat was possible. Production of advanced breeding germplasm adapted for wheat crop conditions from Romania combined with genetically resistance to bunt is now in progress at RICIC-Fundulea

Fig. 1. Diversification of resistance genes for bunt disease in winter bread wheat breeding program from R.I.C.I.C. Fundulea after 1993.



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EVALUATION OF PEA GENOTYPES FOR ASCOCHYTA BLIGHT RESISTANCE *IN VIVO* AND *IN VITRO*

R. Rodeva, G. Kosturkova, I. Georgieva and A. Mehandjiev

D. Kostoff Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

INTRODUCTION

Ascochyta blight of pea (*Pisum sativum* L.) is caused by three related phytopathogenic fungi, namely *Ascochyta pisi* Lib., *Ascochyta pinodes* Jones (teleomorph *Mycosphaerella pinodes* (Berk. & Blox.) Vestergr.) and *Phoma medicaginis* Marlbr. & Roum. var. *pinodella* (Jones) Boerema, formerly known as *Ascochyta pinodella* Jones. They are referred to as the "ascochyta complex" or "blackspot complex" and are identified as the major contributors to the pea yield decline syndrome (4). All three pathogens cause leaf and pod spot of pea. *P. medicaginis* var. *pinodella* and *M. pinodes* also cause the potentially much more damaging foot rot, the symptoms of which include lesions at the base of the stem and hypocotyl. Foot rots, if severe, can incite death of seedlings or lodging of a mature crop. Despite efforts to develop resistance to ascochyta blight pea crops throughout the world are still severily affected by the disease. Cultivars with improved resistance to this disease need to be developed.

Alternative approach to conventional methods for crop improvement is based on plant tissue- and cell-cultures. Using intact pathogen or its toxic metabolites as selective agents *in vitro*, offers the potential for *in vitro* studies of the plant-pathogen interaction (3, 13). It is important to determine whether the reactions *in vitro* bear relevance to the disease response of whole plants. A specific *in vitro* screening method is necessary for each host-pathogen system. Previously, culture filtrate of *A. pisi* was included in the media for *in vitro* development of pea immature embryos and lethal and sublethal concentrations were defined (9). In our further experiments this system for *in vitro* selection was improved and applied for two more genotypes (unpublished). This work describes results of experiments designed to investigate the applicability of *in vitro* inoculations for achievement a simulation of whole plant response.

MATERIALS AND METHODS

In vivo tests

Plant material. The commercial cultivar Sredetz and the advanced lines 333 and 383, all created at the Institute of Genetics, differing in many respects including growth, stem type, maturity date, yield and field reaction to ascochyta blight were involved in the study. The pea accessions were scored for ascochyta blight resistance in seedling and adult growth stage. To remove surface contamination all seeds were surface sterilized by shaking in 10 % sodium hypochlorite for 15 min, and washed in six changes of sterile distilled water. Seeds for the seedling test were left for germination in filter paper towels in termostate at 22 °C for 7 days. Seedlings were arranged on perforated polyethylene foil covering plastic pots filled with tap water, by inserting the roots through the holes. On day 17 plants were inoculated and placed in a humidity chamber for 48 h, then moved to a growth chamber with controlled conditions (20/13 °C, 12 h photoperiod) and kept there throughout the disease assessment period. Seeds for adult plant evaluation were sown 3 cm deep in metallic trays (50 by 50 by 15 cm) filled with sterilized soil with 12 plants in a tray. The experiment was arranged in 2 replicates giving 24

plants per genotype. Plants were grown in the glasshouse at 22 - 25 °C with natural light. Side shoots were removed to ensure plant uniformity. Five weeks after sowing plants were inoculated and put under polyethylene tunnel for 48 h and then left in the greenhouse to the end of experiment.

Inoculum production and inoculation procedure. A virulent single spore isolate Pmp 8-1 of *P. medicaginis* var. *pinodella*, obtained from naturally infected pea plant, was used throughout the study. Pycnidiospores were used in all experiments obtained by culturing the isolates on Coon's agar media (1) at 22°C with a 12-h photoperiod. Spore suspensions were prepared by flooding 15-day-old cultures with sterile deionized water containing 0.02 % Tween 20 and leaving for 10 min before rubbing the surface of each culture to dislodge spores. Mycelial fragments were removed by filtration through two layers of muslin. The concentration of spores was adjusted to 10^6 spores/ml using a haemocytometer. Plants were inoculated using a hand sprayer, until runoff.

Disease measurement. The incubation period was defined as the time in days from inoculation to the appearance of the first symptom. Disease scores were assessed separately for stems and leaves, following the 0-5 scales of Clulow et al. (2) 21 days after inoculation (stem reaction categories: 0 = symptomless, 1 = < 5 necrotic flecks, 2 = numerous necrotic flecks, 3 = coalescence of necrotic areas, 4 = lesions 1-2 cm long, 5 = girdling lesion > 2 cm long; foliar reaction categories: 0 = symptomless, 1 = < 5 necrotic flecks, 2 = numerous necrotic flecks, 3 = < 50 % of inoculated area necrotic, 4 = 50-100 % of inoculated area necrotic, 5 = necrosis spreading beyond inoculated area). Plants scored 0, 1, 2 were classified as resistant and those scored 3, 4, 5 as susceptible. At the end of greenhouse experiment the progress of infection up the stalk was recorded as number of nodes crossed.

***In vitro* test**

Initiation and maintenance of callus cultures. Callus cultures were initiated from immature embryos or shoots on medium containing MS inorganic salts (11) supplemented with 1.0 mg/l 2,4-D and 0.1 mg/l kinetin. The cultures were incubated in the dark at 24°C for 4 weeks, then transferred to fresh media in Petri dishes, each containing four calli and incubated until the calli were 15-20 mm in diameter.

Direct inoculation of callus. Each callus was inoculated centrally on its top with mycelium (c. 1 mm^2) taken from the edge of a 2-wk-old culture on potato dextrose agar in Petri dish. Mycelial inoculum was used, rather than suspension, to localize precisely the inoculation and to avoid drops of suspension reaching the agar. The cultures were incubated at 24°C in darkness. Eight replications, four calli each, were used for each genotype. Four of them were utilized for measurement of hyphal growth on the individual calli. Another four were used for histochemical examination made on 50 µm thick transverse frozen sections of the infected and control calli of each pea genotype. The phenolic compounds, polyphenols, lignin and callose were determined by relevant reactions. Initially, the diameter of each callus was measured, and then the diameter of the fungal colony (aerial mycelium) developing on the callus surface was recorded under a x 10 stereo dissecting microscope 3, 5 and 7 days after inoculation. Fungal growth was expressed as a percentage of callus diameter and scored using a 0-4 scale (0 = no visible mycelia, 1 = aerial mycelia on the upper 25 % of the callus, 2 = aerial mycelia on the upper 50 % of the callus, 3 = aerial mycelia on the upper 75 % of the callus, and 4 = mycelia mat on the entire callus. Non-inoculated calli were included in the experiment as controls, but were excluded from the analysis of variance.

Statistics: All experiments were repeated two times. Mean values were calculated for all variables. The data were subjected to analysis of variance and least significant differences (LSDs) were calculated if F-tests indicated statistical significance (10). Significance of differences was given by the Student's test at $P \leq 5$ %, 1% and 0.1 %.

RESULTS

In vivo evaluation

Seedling evaluation. The first symptoms of disease appeared from 5 to 11 days after inoculation and were expressed as a number of small blue-black spots of aerial organs. Line 333 had the shortest incubation period and line 383 the longest (Table 1). The spots enlarged quickly, coaleshing to form necrotic lesions. Significant differences in stem and lear reaction were established. Those plants with disease score of less than 2 were considered as resistant.

Table 1. Disease response parameters following artificial inoculation with isolate Pmp 8-1 in seedling test

Cultivar/line	Incubation period (days)	Stem reaction	Foliar reaction
line 333	5.8	3.33	3.58
line 383	9.6	1.58	1.63
cv. Sredetz	7.0	1.82	1.94
LSDs $P \leq 5$ %, 1%, 0.1 %	1.3, 1.8, 2.6	0.58, 0.87, 1.40	0.90, 1.37, 2.20

Adult plant evaluation. A similarity was found between seedling response and the reaction of mature plant in the greenhouse test both on stems and leaves. The incubation period recorded in adult plant test showed the same tendency as in seedling test. Line 333 stems were girdled well above the tenth node at a time when girdling of line 383 and cv. Sredetz was restricted to the lower nodes (6 and 7, respectively) (Table 2).

Table 2. Disease response parameters following artificial inoculation with isolate Pmp 8-1 in adult plant test

Cultivar/line	Incubation period (days)	Stem reaction	Foliar reaction	No. of infected stem node
line 333	8.58	2.76	3.36	9.3
line 383	11.74	1.26	1.05	5.7
cv. Sredetz	11.50	1.70	1.45	6.8
LSDs $P \leq 5$ %, 1%, 0.1 %	1.43, 1.99, 2.77	0.59, 0.90, 1.44	0.61, 0.92, 1.49	2.9, 4.3, 7.0

In vitro evaluation

The three pea genotypes under study expressed good capacity for cultivation *in vitro* before inoculation. New growth of aerial hyphae on directly inoculated calli was noted 24 h after inoculation. Significant effects were apparent at both 3 and 5 days after ioculation. By 7 days all inoculated calli were completely overgrown of fungus onto the tissue culture media. Callus of line 383 and cv. Sredetz supported significantly less fungal growth on day 3 and 5 ($P < 0.1$ % for both accessions), compared with callus of line 333 (Table 3).

Table 3. Fungal growth on the callus surface scored using a 0-4 scale 3, 5 and 7 days after inoculation

Scoring day	Pea line/cultivar			LSD		
	line 333	line 383	cv. Sredetz	P ≤ 5 %	P ≤ 1%	P ≤ 0.1 %
3	3.25	2.12	2.25	0.44	0.59	0.77
5	3.50	2.50	2.38			
7	4.00	3.62	3.75			

The histochemical reaction for phenolic compounds after staining with toluidine Blue 0 (5) revealed in non-infected calli presence of total phenolics in the meristema cells stained in blue-greenish to green and pectins in the parenchyma metachromatically stained in rose. The fungal infection of the three genotypes under study was connected with the appearance of phenolic compounds in the callus cells near by the fungal mycelium, which also showed a positive reaction for total phenolics. The nitrose reaction (14) is associated with staining of the different polyphenolic compounds in different colors. In the control calli some superficial and some inner cells showed yellow staining. In the infected calli of line 333 all cells of the superficial layer were stained in yellow. Besides, some xylem vessels were dark orange. In infected calli of line 383 groups of dark orange or yellow cells on the callus surface were observed. A large area of cells was observed on the callus and bud surface after infection of cv. Sredetz. Phloroglucinol-HCl reaction for lignin detection (6), expressed as reddish violet to cherry red color resulted in positive reaction only in the xylem of control calli. The fungal invasion provoked positive reaction in the cell walls of groups of parenchyma cells close to the penetrating hyphae. In calli of line 383 and cv. Sredetz these groups of cells were larger in size and deeply stained compared with those of line 333. Yellow fluorescence characteristics for callose depositions (8) was registrated only in single cells of non-infected callus. The inoculation led to the fluorescence in group of callus cells.

DISCUSSION

Three pea accessions were chosen on the basis of their amenability to *in vitro* culture techniques. Line 333 expressed a higher degree of susceptibility to ascochyta blight in both seedling and mature plant tests, which made it suitable for comparison with the more resistant line 383 and cv. Sredetz. Isolate Pmp 8-1 was chosen for experiments because of its consistently moderate virulence. An agreement was found in the present study between seedling response and those of mature plants, indicated that although the conditions imposed by the greenhouse and the growth chamber were not ideal, they gave meaningful data with which to compare the *in vitro* responses.

The relatively resistant line 383 and cv. Sredetz supported significantly less mycelial growth than relatively susceptible line 333 by direct inoculations of callus with fungal mycelium. Lignin deposition and accumulation of polyphenolic compounds in infected calli were greater in resistant genotypes. The production of phenolic compounds is assumed to play a multilateral role in resisting of plants to pathogen attack or in limiting damage to the host by inhibition or toxicity against pathogen and mechanical barrier formation (12).

In vitro methods offer several advantages in the study of disease resistance compared to conventional glasshouse and field tests, although results must always be confirmed in field trials. The development of symptoms in field grown plants can be slow, being subjected to unpredicable and uncontrolled changes in weather. In contrast, *in vitro* techniques provide highly controlled environment eliminating the fluctuations in temperature and humidity. Jayasankar et al. (7) have demonstrated that treatment of grapevine embryogenic

cultures with culture filtrate from *Elsinoe ampelina* resulted in resistance at cellular level translating to the whole plant. They supposed that recurrent *in vitro* selection with the fungal culture filtrate have probably induced systemic aquired resistance or could have encouraged a preexisting population of resistant cells.

CONCLUSION

Culture of pea callus with causal agents of ascochyta blight could be a useful approach in selection of host resistance and in studies of host-pathogen interactions. A combination of *in vitro* cultured tissues and the application of fungal culture filtrates may further intensify the utility of such systems in studies of resistance. Direct inoculations and dual cultures would provide rapid *in vitro* assays for host resistance.

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RESISTANCE OF TOMATO TO FUNGAL DISEASES

Bagirova S.F. [1], Ignatova S.I. [2], Tereshonkova T.A. [2], Gorshkova N.S. [2], Zlotnikov A.K. [3]
[1] Moscow State University, [2] All-Russian Institute for Vegetable Crops,
[3] Center for Biocontrol and Stimulation of Plant Growth, NPF BIO-Biz, Russia

Production of tomato is a matter of great importance in Russian agriculture. Tomato, which is exceptionally rich source of vitamins, minerals and antioxidants such as lycopene, provides essential nutrients and plays a significant role in Russian diet. Over the past few years consumption of tomato in Russia has been constantly growing. Total tomato production of Russia is 1676000 tonnes that means 1,76 % of the world tomato production and 7,61 % of Europe one. Tomato growing area in Russia is about 140000 ha. In the Moscow Region the majority of tomato grows in sheltered tunnels or greenhouses, which cover 470 ha. Tomato production makes up to 30% of total sheltered crops production in the Moscow Region. Annually the Moscow Region produces about 30000 tonnes of tomato, average harvest is 19 kg/m^2, and maximal- 29,3 kg/m^2. In 1980 there was only one state company produced tomato in the Moscow Region The company possessed about 40 ha of sheltered area. Nowadays there are 27 big companies that deal with greenhouse tomato production in the Moscow Region. Additionally there are 3 private companies specialized in tomato breeding and home seed production, and also numerous farmers, which grow tomato mainly for themselves, rather than for sell. Some of the companies use modern techniques to grow tomato crops (such as hydroponics, drip irrigation/fertigation), new approaches to protect plants, apply enough chemicals and fertilizers, but the majority does not. Large and ever increasing number of pathogens is distributed, mainly in those greenhouses, that belong to old-fashioned companies with low incomes. There is risky agriculture in the Moscow Region due to the continental north climate (morning frost in June, cold and wet August; period for tomato growth is very short) and economic factors (instability, low-invested agriculture, low price for tomato fruits (from 15 rubles =0,5$ to 45 rubles=1,5 $, expensive heating). Current harsh economic conditions make fungicides unaffordable for the small farm holdings, that can't apply expensive imported fungicides to battle successfully the diseases. Hall Russian tomato industry increasingly seeks to reduce production costs avoiding chemical intervention for crop protection. The main reason for tomato yield losses which stopped many producers from tomato business in Russia is spectacular infectious diseases. The most important tomato pathogens and corresponding tomato resistant genes are listed in the table below:

Pathogens	Tomato resistant genes
Phytophthora infestans	Ph-1, Ph-2
Phytophthora spp *(P.capsici, cryptogea, nicotianae, cactorum)*	Ph oligogenic resistance
Oidium lycopersicum	Ol-1, Ol-2 plus set of extra genes
Verticillium dahlia	Ve
Fusarium oxysporum f.sp. licopersic	I (pathotype 0), I-2 (pathotype 1)
Fulvia fulva (=Cladosporium fulvum)	Genes of the Cf series
Fusarium oxysporum f.sp.radicis lycopersici(=FORL)	Frl
Pyrenochaeta lycopersici	Pyl

Relatively new tomato diseases are white rust (*Albugo=Cystopus*), south phytophthorosis (soilborn *Phytophthora* species) and powdery mildew (*Oidium lycopersicum*). Russian tomato varieties and F1 hybrids usually cumulate 3-4 resistances. The most frequent ones are resistances to *Fusarium oxysporum, Fulvia fulva* ,viruses (TMV), and nematodes *(Meloidogyne spp).*

Consequence of described above complicated situation with tomato production in Russia is an attempt to find nonstandard, and simultaneously cheap, effective and environmentally friendly protective means. The most perspective ones may influence directly upon plant resistance, not pathogen activity. Among such means is preparation called Narciss. This preparation is proved to be very effective on indoors tomato against several pathogens including causal agents of phytophthorosis, powdery mildew, root rots. It consists of two organic acids (succinic and oxalic), chitosan and micro supplements, and is characterized by plant growth stimulation and immune modulating protective features. Another approach used in practice in Russia is supplementation of substrate for plant growth with sodium humate. It becomes an important element of greenhouse vegetable growing technology. Growth-stimulating and adaptation-creating properties of sodium humate is well-known, while its immune- modulating effect was found recently. Treatments with sodium humate reduce injury of plants by phytophthorosis, powdery

mildew, grey mold , root rots in average 1.5-2 times (Trusevich, 2000). Supplementation of substrate for plant growth with humus, microelements (Mn, Zn, Cu, B), active carbon also shows capacity to decrease plant injury. Additionally to using advanced substrates for plant growing improvement of greenhouses constructions regarding to covering material affected light spectrum transmission can play a definite role in tomato crop protection. Coverings of different types influence through light spectrum upon disease development, in some cases notably decreasing disease plant severity. Crop rotation, irrigation management, sanitation, clean seeds and transplants, delayed planting, cultivation, and hand weeding are widely used methods of avoiding or preventing chemical intervention. Risk assessment models or phenology models that available for powdery mildew, and being developed for late blight can be very important in a tomato protection system. New hopes to control tomato diseases appear with biotechnology. In Moscow transgenic tomato was created by introducing Rs-AFP2 gene from *Brassica napus*. Introducing of this gene was found to give resistance to several pathogens, most importantly to *Phytophthora infestans* (Parashina et al, 1999).

We will stress here on our recent results on tomato resistance to fungal diseases. Little known form of induced plant resistance is resistance triggered by root-colonizing non-pathogenic bacteria that extended to the above-ground plant parts and effective against foliage pathogens. Our study was aimed at researching action of *Bacteria Klebsiella terrigena* E6 and *Bacillus firmus* E3 on growth and resistance of tomato plants to foliage pathogens: *P. infestans, Botrytis cinerea* and *Oidium lycopersicum*. Those selected bacteria strains were isolated from rhizosphere of *Dactylus glomerata*, and characterized by high nitrogenase and plant growth promoting activity. The bacteria were appeared to suppress growth of some fungi including plant pathogens in our tests on cultural media. In further experiments tomato seeds, young and adult tomato plants were treated with bacteria suspension (10^6 CFU per ml). Pure cultures, cultural liquid and consortia of the bacteria were tested. It was revealed that the bacteria not only stimulate plant growth, but also suppress the pathogenic fungi development through plant mediated mechanisms. In our study the consortia was more effective than pure cultures or cultural liquid. Obtained date indicate the high activity of the rhizosphere bacterial strains to suppress the foliage pathogens in the tested plant-pathogen system. To improve biocontrol of tomato diseases we suggest to combine the bacterial strains.

Plant breeding towards genetic control of pathogens remains in practice the most important component of integrated control. Our breeding programs are focused on tomato resistance to *O. lycopersicum, B. cinerea, P. infestans*. Powdery mildew of tomato caused by *O. lycopersicum* is relatively new disease, that was not previously occurring on this crop. Recently the fungus was found on tomato in the USA, Canada, Asia, and Europe. The disease becomes one of the most serious on indoor and outdoor tomatoes in the Central and the North-Western regions of the Russian Federation. This tomato disease is distributed mainly in plastic tunnels, less in greenhouses. Tomato resistance to powdery mildew is oligogenic with low level of expression Resistant genes are recessive or partially dominant (Latterot 2000; Cirulli, unpublished data). The causal agent is an obligatory pathogen. Tests for resistance are very delicate. Screening for resistance of a set of wild tomato species and a row of the simple and complex interspecies hybrids between the selected resistant forms and *Lycopersicon esculentum* was carried out. Original system of methods for estimation of resistance to *O.lycopersicum* (Tereshonkova et al., 1999) was created. It includes inoculation of detached leaves by direct contact, inoculation of tomato seedlings in laboratory and adult plants in greenhouses by spraying with spore suspension. Disease evaluation was based on definition of percentage of visible foliar area affected. Estimation scale 0-4 (0-1-resistant plant, 2-4-susceptible plant) was involved. Using of those methods allowed to select very promissing tomato forms of F7-F9 generations with high level of resistance. Several wild species were found to be a source of resistance to the disease.The best results were obtained for hybrids derived from *L.cheesmanii and L.pimpinellifolium*. Some of the hybrids have fruits of very popular cherry type, the others have large fruits. Obtained data are present in the table.

Table 1. Resistance of studied tomato hybrids to powdery mildew

Combination	Disease severity, scale 0-4	Portion resistant patterns out of investigated ones, %
L.esculentum x L.pimpinelifolium	1-2	55
L.esculentum x L.hirsutum glabratum	0-1	71
L.esculentum x L.humboldtii	1-2	66
L.esculentum x L.chesmanii	0-1	41
L.esculentum x	1-2	1

(L.chesmaniixL.esculentum v.cerasiforme)		
(L.esculentum x L.humbotii) x (L.esculentum x (L.chesmanii x L.esculentum v. Cerasiforme)	1-2	11

Tomato grey mold is a common disease worldwide, and often causes serious production losses by infecting leaves, stems, flowers and fruits. Presently, no resistant cultivars are available. Grey mold is not controlled by genetic resistance in commercial varieties. Estimation of tomato stem damage in F1 hybrids grown in two rotations caused by *B.cinerea* was carried out. The estimation was made in a period of mass fruitage. As a result it was shown, that the hybrids with determinate type of growth (sp) are damaged more strongly that then indeterminate ones (sp+). An average disease severity in the first group was 2.8 against 2.3 in the second one using a scale 0-4. Number strongly injured (2.5-4) plants (SIP) in each group were 44.1% and 30.7% and number of moderate resistant (0-1.5 numbers) plants were 10% and 8.5 % accordingly. The analysis of resistance of F1 hybrids showed significant effect of parental lines on a degree of plant stem damage caused by B.cenerea. The amount of SIP was 32.3% when the most susceptible hybrids showed 72-75% SIP. The good resistance was shown by commercial hybrids F1 Pilgrim, F1 Vlad, F1 Blues and F1 of Marquises derived from crosses of lines T850 and K 512. On the average for 2 years they had 11.1-17.6% of SIP when the most susceptible hybrids showed 53-4-65.9% SIP. Received data allow correcting the practical breeding programs in the direction of creating hybrids with the increased resistance to the disease. Late blight of tomato is very difficult to control. Now new *Phytophthora* populations are distributed in the greenhouses. This new virulent type is more aggressive than that one which caused well-known Irish potato disease last century. Abundant resting sexual spores of *Phytophthora* (oospores) are formed within tomato tissue. Oospores can survive in the soil without any potato tubers being present. Both resistant genes Ph-1 and Ph-2 are broken now. Ph-2 has been more stable, but recently Ph-2 tomato hybrids were found to be destroyed within two weeks. Our study was aimed at re-evaluating tomato breeding material and searching for sources of tomato durable resistance against late blight. During two late blight epidemics of years 1998 and 1999 selected tomato breeding material was evaluated under natural conditions of severe epiphytoty in greenhouses in the Moscow Region. More than 1500 tomato lines or hybrids were screened for the resistance against new more aggressive population of tomato late blight pathogen. Eighteen lines (F3-F8) that created with involving different wild tomato species as resistant sources were found to show the greatest resistance to the late blight. Simultaneously, *Phytophthora* strains were collected from diseased plants and studied. High polymorphism of the new sexual population of the late blight pathogen that similar to polymorphism of the Mexican populations was revealed. Our data concerning population diversity suggest that the monitoring of *P.infestans* in the Moscow Region is a good model for study of different aspects of population biology of *P.infestans* (the spread of new pathotypes, role of oospores in disease development, interrelationships between the tomato and potato populations) and for reevaluation of plant breeding material.

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EFFECTS OF SEPTORIA AND PYRENOPHORA ON REDUCTION OF WINTER WHEAT YIELD

HORČIČKA P., MARKOVÁ O.*, HANIŠOVÁ A.
SELGEN a.s., Plant Breeding Station Stupice, 250 84 Sibřina Czech Republic,
*Plant Breeding Station Úhřetice

Introduction

Foliar fungal diseases of wheat caused by *Septoria, Stagonospora* and *Pyrenophora* species have become economically significant for the wheat growing in Czech republic. Septoria tritici and Pyrenphora have occured more widely in last 5 years. *Septoria, Stagonospora* and *Pyrenophora spp.* induce a decrease in assimilation, developmental retardation, premature leaf disiccation and reduction TKW. In epidemic year, yield losses may reach 30-50% (Kolomiets, 1999), up to 100% according to Mentha (1998). Total yield losses caused by these pathogens all over the world are estimated at 9 million tons of wheat.

The most economical way to control these diseases is to use resistant or tolerant varieties in practice. The success of practical resistance breeding depends on combining resistance to economical important diseases with yield, quality and further traits in an optimal way (new variety).The screening on resistance of this diseases in breeding process is very difficult and labour. In early and advance generations it is possible only to evaluate the field symptoms by scoring. Scoring of *Septoria, Stagonospora* and *Pyrenophora* is in weak correlation to classifications by yield components .

Evaluation of gene resources and their introduction in breeding programmes is basic strategy of wheat breeders. Our objectives were to compare of yield reduction caused by *Stagonospora nodorum* (SN), *Septoria tritci* (ST) and *Pyrenphora tritici- repentis* (PT) at 33 varieties and advanced lines at two locations. We compared diseases score to yield parameters thousands kernel weight (TKW), number of kernel per ear (NKE) and weight of kernels per ear (WKE).

Materials and Methods

33 varieties of winter wheat (tab.1) were planted at two locations (Stupice, Uhretice) in hill plots in two replications. Inter-plot spacing were 30 cm. Every disease and control had own nursery in 1998,1999. The initial inoculom sources were the same at both sites.

Stagonospora nodorum (SN)

For inoculations, a spore suspension was prepared through washing of dried inoculated wheat grains. It contained a mixture of pathogen isolates originated from whole country, supplied by Dr. E. Sychrova VURV Ruzyně. The suspension ($1*10^6$ spores/ml) was sprayed in the evening. After inoculation supplementary irrigation was applied to obtain uniform disease development for 4 days. Two inoculations were applied before heading and after heading for successful infection

Pyrenophora tritici-repentis (PT)

For inoculations, a spore suspension was prepared through washing of dried inoculated oat grains. It contained a mixture of pathogen isolates originated from whole country, collected by SELGEN. The suspension ($1*10^6$ spores/ml) was sprayed by hand sprayer in stage (EC33 and EC39). Two inoculations were applied for successful infection.

Septoria tritici (ST)

For inoculations, a spore suspension was , supplied by Dr. E. Sychrova VURV Ruzyně. The suspension ($1*10^6$ spores/ml) was sprayed in the evening. Two inoculations were applied in stage (EC33 and EC39) for successful infection. After inoculation supplementary irrigation was applied to obtain uniform disease development for 4 days.

Assessments of attack were made on the three upper leaves and on ears (for SN) two times at dates depending on development of the disease. Used scores from 9-1 indicate no attack (score 9) resp. very high attack (score 1). Estimation of degree of severity for tested diseases was done on plot basis. 15 ears from every replication were harvested and evaluated for yield (WKE), for weight of thousands grains (TKW) and number of grains per ear (NKE). Data analyses were performed by STATGRAPHICS statistical packages.

Results and Discussion:

The trials have shown negative effects of *Septoria tritici* (ST), *Stagonospora nodorum* (SN) and *Pyrenphora tritici- repentis* (PT) on the relative yield/ear of winter wheat (Fig.1). There were significant difference between diseases. The significantly highest reduction was caused by SN, relative yield was 69 % of control. For PT and ST relative yield was 81 and 82 % resp. Gonzales et al. (1999) determined relative yield losses of 10-32%.

ST, SN and PT were effected differently yield components.. Reduction of numbers of kernels per ear was similar for all three diseases for 14-11% (Tab.2).. In case of TKW the significantly highest reduction was caused by SN (25%)(with highest reduction up to 38%). For PT and ST reduction of TKW was similar to reduction of

NKE 13,7 and 12. 3% resp. The highest reduction grain weight is effected by duration of SN and by attack of ears .

WKE for all varieties was 83,8 % (table 2), susceptible varieties suffered 15-21% loss in yield, medium susceptible varieties lowered WKE about 14-15%, while moderately resistant varieties lowered WKE about 8-13 % depending on year and location of testing (table 1). SASKIA, ALANA and MONA were the best in tested set of varieties. For all three diseases reduction was between 8-10%. The most susceptible cultivars were. ŠÁRKA, VERSAILLES and BLAVA, mean yield/ear reduction was 21 % for all three pathogens. For single pathogens differences between resistant and susceptible varieties were bigger .

Fig. 1. Weight of kernels per ear (%) in disease nurseries and 95 % Tukey HSD Intervals (Stupice, Uhretice, 1998,1999)

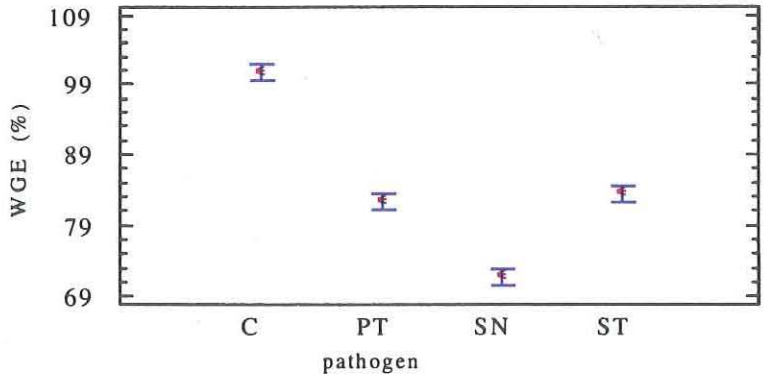


Fig. 2: Average score of leaf diseases of winter wheat in disease nurseries (Stupice, Uhretice, 1998, 1999)

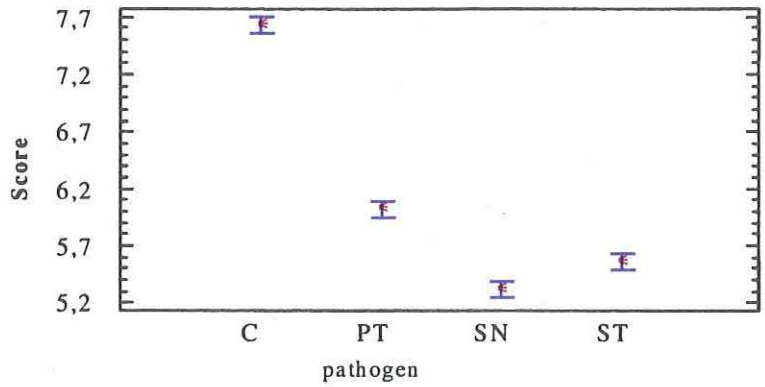


Table 1. Least Squares Means for weight of kernels per ear (WKE) relative yield with 95,0 Percent Confidence Intervals

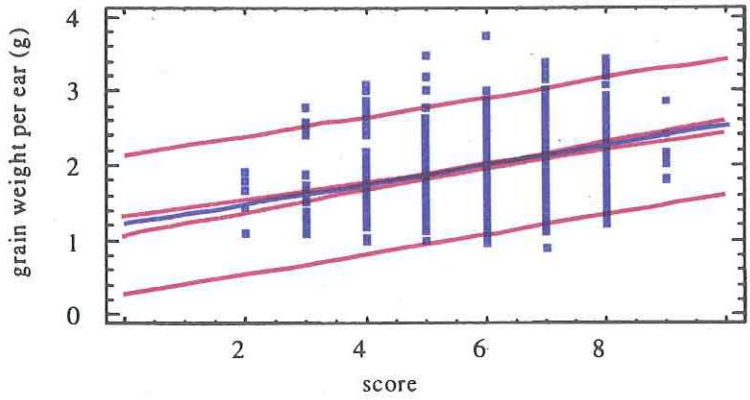
Level	Count	Stnd. Mean	Error	Lower Limit	Upper Limit
GRAND MEAN	1056	**83,8**			
location					
Stupice	528	**81,1**	0,44	80,3	82,0
Uhretice	528	**86,5**	0,44	85,6	87,3
year					
98	528	**86,1**	0,44	85,3	87,0
99	528	**81,5**	0,44	80,6	82,4
pathogen					
Control	264	**100,0**	0,62	98,7	101,2
PT	264	**81,6**	0,62	80,3	82,8
SN	264	**70,8**	0,62	69,6	72,0
ST	264	**82,9**	0,62	81,7	84,1

Table 2. Effect of diseases on varieties of winter wheat (Stupice, Uhřetice, 1997-99)

variety	number of kernels per ear				weight of thousand kernels				weight of kernels per ear for all three diseases (% to control)		
	control (number)	PT %of control	SN %of control	ST %of control	control (g)	PT %of control	SN %of control	ST %of control			
ALANA	**46,8**	91	96	101	**51,1**	99	82	90	**91,5**	a	MR
SASKIA	**46,1**	99	99	99	**50,0**	94	86	85	**91,3**	a	MR
MONA	**49,9**	89	84	88	**51,5**	98	95	96	**89,6**	a	MR
SG-S1108	**55,2**	95	92	93	**46,8**	92	81	92	**88,1**	a	MR
ESTICA	**48,7**	86	101	96	**46,4**	94	75	90	**86,9**	a	MR
VEGA	**51,2**	91	87	89	**46,7**	94	77	95	**86,6**	a	MR
BRUTA	**47,0**	93	97	94	**49,4**	95	77	91	**86,1**	ab	MS
ALKA	**46,4**	86	90	91	**49,3**	92	83	92	**86,0**	ab	MS
TORYSA	**57,1**	83	90	87	**49,6**	94	89	91	**85,9**	ab	MS
SG-S148	**51,2**	97	95	94	**47,1**	96	78	90	**85,8**	ab	MS
SIDA	**55,4**	84	89	90	**47,2**	94	85	91	**85,3**	ab	MS
REXIA	**49,7**	85	90	94	**44,9**	97	86	96	**85,1**	b	S
INA	**51,3**	100	98	101	**45,5**	85	68	82	**84,8**	b	S
BREA	**47,7**	83	81	101	**48,7**	90	72	92	**84,7**	b	S
SAMANTA	**52,3**	84	94	84	**48,5**	99	76	92	**84,0**	b	S
SULAMIT	**53,3**	99	92	94	**48,5**	86	72	86	**83,4**	b	S
ATHLET	**60,5**	94	95	89	**45,3**	85	74	79	**83,3**	b	S
EBI	**51,0**	93	89	93	**47,1**	82	75	90	**83,0**	b	S
HANA	**51,6**	83	85	91	**47,1**	92	84	93	**83,0**	b	S
RU-488	**60,8**	96	92	101	**49,1**	81	75	77	**82,6**	b	S
TRANE	**52,7**	87	89	89	**47,4**	86	83	86	**82,6**	b	S
BOKA	**44,4**	67	98	90	**46,8**	73	79	95	**82,1**	b	S
ARINA	**45,1**	90	92	97	**45,8**	93	72	89	**82,0**	b	S
SIRIA	**58,2**	87	93	86	**45,9**	89	77	90	**81,2**	b	S
RITMO	**50,9**	90	95	93	**40,5**	88	79	86	**80,9**	b	S
SG-S1365	**61,7**	87	80	91	**43,8**	90	84	90	**80,9**	b	S
ASTELLA	**57,0**	92	89	83	**47,7**	88	70	85	**80,6**	b	S
SAMARA	**51,4**	84	89	84	**48,6**	88	82	91	**80,6**	b	S
ILONA	**59,5**	87	91	92	**44,4**	91	73	89	**80,4**	b	S
SVITAVA	**54,7**	89	82	92	**48,6**	88	71	78	**80,4**	b	S
VERSAILLES	**55,4**	93	88	86	**43,1**	85	69	87	**79,9**	b	S
BLAVA	**53,3**	87	81	86	**46,1**	92	73	89	**79,8**	b	S
SARKA	**50,1**	90	85	90	**47,8**	90	72	88	**79,3**	b	S
average	**50,81**	**86,5**	87,88	89,09	**45,77**	87,65	75,71	86,26	**83,8**		

Number with different lower case letters are significantly different at the 0.05 level probability according to Tukey.

Fig. 3. Disease score for SN and grain weight per ear (linear model GWE= 1,22339 + 0,130234*score, r^2=15,1%)



Average score of leaf diseases of winter wheat in Fig.2 showed the significant differences in scoring of all pathogens. Score for SN was the lowest at 5.2, for this pathogen was measured lowest relative yield as well. The correlation coefficient equals 0,3636, indicating a relatively weak relationship (Fig.3)between the scoring and WKE. Loughman (1994) described similar relationship between yield components and field scoring. Jlibene et. all (1992) not recommended scoring of wheat plant reaction on plot basis, because of pronounced effect of height and environment on S. tritici.

For practical purposes in breeding process still visual evaluation of artificial or natural infection have played dominant role. All tests need several years and sites. In the future the use of the molecular markers would help for identification plants or breeding lines with higher resistance (Murphy et. all, 1999)

Between current commercial varieties have been identified varieties with moderate resistance to different leaf spot diseases some of them combine all of them. Because S.nodorum, S.tritici and P.tritici-repentis frequently occur as disease complex in Czech Republic combinations of resistance are important for effective disease management.

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Colletotrichum infection in maize

Holger B. Deising and Stefan Werner

Martin-Luther-University Halle-Wittenberg, Faculty of Agriculture, Phytopathology and Plant Protection, Ludwig-Wucherer-Str. 2, D-06099 Halle (Saale), Germany.
E-mail: Deising@landw.uni-halle.de

Colletotrichum graminicola (Cesati) Wilson [teleomorph *Glomerella graminicola* (Politis)] is the causal agent of anthracnose of different cereals and grasses, and especially epidemics on maize in the early 1970s demonstrated the destructive power of this fungus (3, 10). However, not only *C. graminicola*, but also other *Colletotrichum* species pathogenic on an array of host plants cause significant losses each year (1). Several features, e.g. easy cultivation, spore production in large amounts, infection-related morphogenesis, and accessability to all modern molecular genetic techniques, make this group of fungi, and particularly *C. graminicola* an excellent model system in plant pathology (5, 11).

After getting spread onto maize leaves, conidia germinate and undergo infection-related morphogenesis. A highly specialized infection cell, called an appressorium, is differentiated at the tip of a short germ tube, and during maturation this cell incorporates melanin into the inner appressorial wall (5). Thus, infection structures of *C. graminicola* are reminiscent of those formed by the rice blast fungus *Magnaporthe grisea* (6). Both fungi generate extremely high turgor pressure in order to breach the plant cuticle and cell wall. To accomplish this, osmotically active solutes are synthesized in the appressorium; Talbot and co-workers reported of glycerol reaching concentrations of more than 3 M in appressoria of *M. grisea* (4). The corresponding turgor pressure is between 5 and 8 MPa (50 to 80 bar) (7, 9), as determined by indirect measurement. This turgor is then translated into force, which is directed to the penetration pore at the appressorial base. To precisely measure the force applied by single appressoria of *C. graminicola*, Bechinger et al. (2) used an optical waveguide technique and found that these cells can exert, on average, some 17 μN. If a force of 17 $\mu N\ \mu m^{-2}$ were exerted over the palm of a hand, a human could lift an 8,000-kg school bus or a killer whale (8). These figures clearly illustrate that significant turgor-based forces occur, and, at the same time, that rigid cell walls are needed in functional appressoria.

In order to study the role of chitin, one of the major structural carbohydrates in cell walls (12), four chitin synthase gene fragments of *C. graminicola* were cloned by a PCR-based strategy, and the corresponding genes were isolated from a cosmid library. These genes were designated *CgCHSA* to *CgCHSD* (Werner and Deising, unpublished result). As *C. graminicola* is fully accessable to techniques of molecular genetics, the function of genes of interest can be studied by gene inactivation experiments. While mutants defective in *CgCHSA* and *CgCHSB* did not show a phenotype differing from that of the wild type, *CgCHSC* mutants were unable to grow on oat meal agar without osmotic stabilization. This mutant was unable to infect intact maize leaves. Interestingly, acervulus formation was initiated, as indicated by setae formation, but normal conidia were not formed (Werner and Deising, unpublished result). These results clearly indicate that *CgCHSC* is a gene essential for growth, conidiogenesis, vitality and pathogenicity in *C. graminicola*. The results presented may also define a new target for efficient fungicides.

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List of Participants

Surname	Firstname	Adress	e-mail
Afanasenko	Olga	All-Russian Institute of Plant Protection Laboratory of Mycology and Phytopathology Podbelski str. 3, 196608 St. Petersburg-Pushkin Russia	
Bagirova	Svetlana	Department of Mycology & Algology Moscow University 119899 Moscow, Russia	slana@sbagirova.home.bio.msu.ru
Barchend	Gudrun	Federal Centre for Breeding Research on Cultivated Plants Institute for Resistance Research & Pathogen Diagnostics P.O. Box 1505, D – 06435 Aschersleben, Germany	g.barchend@bafz.de
Berestetski	Alexander	Federal Centre for Breeding Research on Cultivated Plants Institute for Resistance Research & Pathogen Diagnostics P.O. Box 1505, D – 06435 Aschersleben, Germany	a.berestetski@bafz.de
Csösz	Maria	Cereal Research Institute P.O.Box 391 H - 6701 Szeged, Hungary	laszlone.csosz@gk-szeged.hu
Deising	Holger	MLU Halle Landwirtschaftliche Fakultät Institut für Pflanzenzüchtung und Pflanzenzucht Ludwig-Wucherer-Str. 2, D - 06099 Halle, Germany	deising@landw.uni-halle.de
Dragavtseva	Elena	Federal Centre for Breeding Research on Cultivated Plants Institute for Epidemiology & Resistance P.O. Box 1505, D – 06435 Aschersleben, Germany	e.dragavtseva@bafz.de
Drescher	Anke	Institute for Plant Genetics and Crop Plant Research Corrensstr. 3, D - 06466 Gatersleben, Germany	drescher@ipk-gatersleben.de
Gabler	Jutta	Federal Centre for Breeding Research on Cultivated Plants Institute for Resistance Research & Pathogen Diagnostics P.O. Box 1505, D – 06435 Aschersleben, Germany	j.gabler@bafz.de
Gagkaeva	Tatiana	All-Russian Institute of Plant Protection Laboratory of Mycology and Phytopathology Podbelski str. 3, 196608 St. Petersburg-Pushkin Russia	tug@MN1780.spb.edu
Greif	Peter	Saatzuchtgesellschaft Streng`s Erben Aspachhof 1, D - 97215 Uffenheim, Germany	p.greif@aspachhof.de
Griesbach	Erika	Federal Centre for Breeding Research on Cultivated Plants Institute for Epidemiology & Resistance P.O. Box 1505, D – 06435 Aschersleben, Germany	e.griesbach@bafz.de
Gultiaeva	Elena	Federal Centre for Breeding Research on Cultivated Plants Institute for Epidemiology & Resistance P.O. Box 1505, D – 06435 Aschersleben, Germany	e.gultiaeva@bafz.de

Habekuß	Antje	Federal Centre for Breeding Research on Cultivated Plants Institute for Epidemiology & Resistance P.O. Box 1505, D – 06435 Aschersleben, Germany	a.habekuss@bafz.de
Hammann	Thilo	Saatzucht Hadmersleben GmbH Kroppenstedter Str. 4, D - 39398 Hadmersleben, Germany	thilo.hammann@swseed.se
Hanicova	Ellena	Wheat Breeding Department SELGEN Plant Breeding ST. Stupice 25084 Sibrina, Czech Republic	
Hartl	Lorenz	Bayerische Landesanstalt für Bodenkultur und Pflanzenbau, Postfach 1641, D - 85316 Freising, Germany	
Henning	Frank	Institut für Gemüse- und Zierpflanzenbau Großbeeren / Erfurt e. V. Abteilung Zierpflanzen Mittelhäuser Str., D - 99189 Kühnhausen, Germany	
Horcicka	Pavel	Wheat Breeding Department SELGEN Plant Breeding ST. Stupice 25084 Sibrina, Czech Republic	horcicka@zero.cz
Ittu	Mariana	Research Institute for Cereals and Industrial Crops (ICCPT) 8264 Fundulea, Romania	gittu@pcnet.pcnet.ro
Jahoor	Ahmed	Risø Nat. Lab., Pl. Biol. and Biochem. Dept., Roskilde PBK-301, P.O.Box 49 4000 Risø , Dänemark	a.jahoor@risoe.de
Jaimes	Oskar	MLU Halle Landwirtschaftliche Fakultät Institut für Pflanzenzüchtung und Pflanzenzucht Ludwig-Wucherer-Str. 2, D - 06099 Halle, Germany	jaimes@landw.uni-halle.de
Kastirr	Ute	Federal Centre for Breeding Research on Cultivated Plants Institute for Resistance Research & Pathogen Diagnostics P.O. Box 1505, D – 06435 Aschersleben, Germany	u.kastirr@bafz.de
Kema	Gert H.J.	DLO-Research Institute for Plant Protection (IPO-DLO) P.O.Box 9060 N-6700 GW Wageningen, Netherlands	G.H.J.Kema@plant.wag-ur.nl
Kempf	Hubert	Saatzucht H. Schweiger & Co. Feldkirchen 3, D - 85368 Moosburg, Germany	
Klatt	Elke	Nordsaat Saatzuchtgesellschaft mbH Saatzucht Langenstein Hauptstr. 1, D - 38895 Böhnshausen, Germany	
Kofoet	A.	Institut für Gemüse- und Zierpflanzenbau Theodor-Echtermeyer-Weg 1, D - 14979 Großbeeren, Germany	kofoet@igzer.de
Kopahnke	Doris	Federal Centre for Breeding Research on Cultivated Plants Institute for Epidemiology & Resistance P.O. Box 1505, D – 06435 Aschersleben, Germany	d.kopahnke@bafz.de
Krämer	Ilona	Federal Centre for Breeding Research on Cultivated Plants Institute for Epidemiology & Resistance P.O. Box 1505, D – 06435 Aschersleben, Germany	i.kraemer@bafz.de

Krämer	Reiner	Federal Centre for Breeding Research on Cultivated Plants Institute for Horticultural Crops Neuer Weg 22/23 D – 06484 Quedlinburg, Germany	r.kraemer@bafz.de
Kühne	Thomas	Federal Centre for Breeding Research on Cultivated Plants Institute for Resistance Research & Pathogen Diagnostics P.O. Box 1505, D – 06435 Aschersleben, Germany	t.kuehne@bafz.de
Kusterer	Anette	Federal Centre for Breeding Research on Cultivated Plants Institute for Resistance Research & Pathogen Diagnostics P.O. Box 1505, D – 06435 Aschersleben, Germany	a.kusterer@bafz.de
Levitin	Mark	All-Russian Institute of Plant Protection Laboratory of Mycology and Phytopathology Podbelski str. 3, 196608 St. Petersburg-Pushkin Russia	levitin@IF3960.spb.edu
Lind	Volker	Institute for Resistance Genetics Graf-Seinsheim-Str. 23 D - 85461 Grünbach, Germany	
Mackinaite	Rimute`	Institute of Botany Zaliuju ezeru 49 LT-2021 Vilnius, Lithuania	rimute@server.botanika.lt
Manninger	Klara	Plant Protection Institute Hungarian Academy of Sciences P.O. Box 102 H - 1525 Budapest, Hungary	sman@nki.hu
Mironenko	Nina	All-Russian Institute of Plant Protection Laboratory of Mycology and Phytopathology Podbelski str. 3, 196608 St. Petersburg-Pushkin Russia	
Nachtigall	Marion	Federal Centre for Breeding Research on Cultivated Plants Institute for Resistance Research & Pathogen Diagnostics P.O. Box 1505, D – 06435 Aschersleben, Germany	m.nachtigall@bafz.de
Naumann	Klaus	Auf der Alten Burg 15, D - 06449 Aschersleben, Germany	
Neumann	Manfred	Federal Centre for Breeding Research on Cultivated Plants Neuer Weg 22/23, D - 06484 Quedlinburg, Germany	m.neumann@bafz.de
Proeseler	Gerhard	Federal Centre for Breeding Research on Cultivated Plants Institute for Epidemiology & Resistance P.O. Box 1505, D – 06435 Aschersleben, Germany	g.proeseler@bafz.de
Rabenstein	Frank	Federal Centre for Breeding Research on Cultivated Plants Institute for Resistance Research & Pathogen Diagnostics P.O. Box 1505, D – 06435 Aschersleben, Germany	f.rabenstein@bafz.de
Richter	Klaus	Federal Centre for Breeding Research on Cultivated Plants Institute for Epidemiology & Resistance P.O. Box 1505, D – 06435 Aschersleben, Germany	k.richter@bafz.de
Richter	Klaus	Saatzucht Hadmersleben GmbH Kroppenstedter Str. 4, D - 39398 Hadmersleben, Germany	klaus.richter@swseed.se

Rodeva	Rossitza	Bulgarian Academy of Sciences Institute of Genetics „Academy D. Kostoff" Mladost 479-1-34, 1113 Sofia, Bulgaria	dyakov@1.mycol.bio.msu.ru
Scholze	Paul	Federal Centre for Breeding Research on Cultivated Plants, Institute for Horticultural Crops Neuer Weg 22/33, D - 06484 Quedlinburg,Germany	p.scholze@bafz.de
Sperling	Ursel	Landespflanzenschutzamt Sachsen-Anhalt Silberweg 5, D - 39128 Magdeburg, Germany	Sperling@LPSA.ML.LSA-net.de
Streng	Stefan	Saatzuchtgesellschaft Streng`s Erben Aspachhof 1, D - 97215 Uffenheim, Germany	S.Streng@aspachhof.de
Sugni	Janyce	MLU Halle Landwirtschaftliche Fakultät Institut für Pflanzenzüchtung und Pflanzenzucht Ludwig-Wucherer-Str. 2, D - 06099 Halle, Germany	sugni@landw.uni-halle.de
Taubenrauch	Kerstin	Federal Centre for Breeding Research on Cultivated Plants Institute for Resistance Research & Pathogen Diagnostics P.O. Box 1505, D – 06435 Aschersleben, Germany	k.taubenrauch@bafz.de
Unger	Otto	Nordsaat Saatzuchtgesellschaft mbH Saatzucht Langenstein Hauptstr. 1, D - 38895 Böhnshausen, Germany	nord.boehnshausen@t-online.de
Walther	Ursula	Planstr. 38, D - 39398 Hadmersleben, Germany	
Weber	Inge	Seminis Vegetable Seeds Europe P.O. Box 22, N - 1600 AA Enhuizen, Netherlands	iweber@svseeds.nl
Weyen	Jens	Saaten-Union Resistenzlabor GmbH Hovedisser Str. 92, D - 33818 Leopoldshöhe, Germany	weyen@sulab.kunden.de

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