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PATHOGENICITY AND RESISTANCE IN *FUSARIUM* SPP. - WHEAT, TRITICALE AND RYE PATHOSYSTEMS AT THE SEEDLING STAGE.

ABSTRACT

In our previous study on pathogenicity of 20 isolates of 11 *Fusarium* species towards seedlings of 14 cultivars of wheat, triticale and rye no significant interaction isolates by cultivars was found. The finding suggested that there was non-specificity in those pathosystems and stimulated further study on the subject, which was continued with the same set of isolates and cultivars but on a different substrate. Instead of planting to soil *Fusarium* inoculated. in oculated kernels were plated on water agar and incubated under controlled environment conditions. The aim of the substrate change was to increase sensitivity and precision of the test to detect smaller differences in agressiveness and seedling reaction. Disease severity was estimated on the extent of coleoptile necrosis. Average percentage of disease was calculated from disease scores on ten individual seedlings.

With one exception, all *Fusarium* spp. isolates were pathogenic towards seedlings of all cereal cultivars, Mean disease severity was 23.7% of coleoptile necrosis. Significant differences in aggressiveness among isolates and in resistance among cultivars were observed.

Statistically significant correlation was found for the aggressiveness of isolates in this experiment and the previous one, where the soil was used as a substrate. However, the resistance of cultivars in both tests did not correlate. The latter finding could be explained by the change of experimental conditions that influenced the reaction of seedlings.

The main effects of cultivars and isolates were highly significant but the effect of interaction of isolates by cultivars was not significant, again. The findings correspond with results obtained in the previous experiment. The analysis of variance of the data combined from both experiments produced results similar to ones for each experiment separately. It seems that there is no specificity in the pathosystems neither at the genotype nor at the species level.

The aggressiveness of isolates significantly correlated with the variance in disease reaction to the respective isolates among cultivars. In both experiments the mixture of isolates produced the widest variance among cultivars. When aggressiveness score was close to 25% or more the variance among cultivars tended to decrease. Correlation of the resistance of cultivars with the variance in disease induction on seedlings of the respective cultivars among isolates showed that isolates were differentiated the best at the disease level about 25-30%. The variance among isolates decreased at the higher disease level.

Key words: cereals, *Fusarium*, resistance, rye, *Secale cereale*, seedling blight, triticale, *Triticum aestivum*, X *Triticosecale*, wheat.

INTRODUCTION

Cereals can be heavily damaged by seedling blight caused by *Fusarium* fungi which infects heads and are transmitted with kernels (Mańka 1989, Arseniuk *et al.* 1991a, Daamen *et al.* 1991). Pathogenic *Fusarium* fungi are also widely present in the soil, where they can survive as saprophytes (Parry *et al.* 1995). Therefore resistance of seed-

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lings to this disease is one of goals pursued in breeding programs (Mesterhazy 1987). The resistance of cereals to the seedling blight can be tested under field conditions or under controlled environments (Chełkowski and Mańka 1983, Grey and Mathre 1988, Mańka 1989, Arseniuk *et al.* 1991a). Repeatability of results of tests carried out in different environments and/or research centers seems to be insufficient. In addition to environmental conditions results are affected by composition of inoculum of *Fusarium* spp. Resistance can be tested with a use of single isolates or with a mixture of isolates of one or more *Fusarium* species causing seedling blight. The fact that *Fusarium* diseases of cereals (seedling blight, crown rot, head blight) are caused by several species generates the need to determine whether resistance to various *Fusarium* species has the same genetic background. Published results suggest lack of interaction between host genotypes and *Fusarium* species/isolates (Mesterhazy 1987, Miedaner *et al.* 1993, Meadway 1994, Snijders 1994, Van Eeuwijk *et al.* 1995, Miedaner 1997).

In our previous study on pathogenicity of *Fusarium* species towards seedlings of winter cereals no significant interaction of isolate by cultivar was detected (Arseniuk *et al.* 1993). The finding suggested that there was nonspecificity in those pathosystems. The above findings stimulated further study with the same set of isolates and cultivars but on a different substrate. The aim of the substrate change was to increase sensitivity and precision of the test in order to detect smaller differences in aggressiveness and seedling reactions. In this study reaction of winter wheat, winter triticale and winter rye seedlings to artificial inoculation with single *Fusarium* spp. isolates or their mixture was studied in order to determine the magnitude of host - pathogen interaction and to evaluate the effect of the testing method (substrate) on aggressiveness and resistance patterns.

MATERIALS AND METHODS

Experimental protocol

Isolates of 11 *Fusarium* species studied in this experiment were recovered from triticale seed (Arseniuk *et al.* 1991b). Tests were made with two isolates of each *Fusarium* species, except that one isolate was used for *F. sporotrichioides* and *F. tricinctum*. Inoculum was produced on potato-dextrose agar (PDA) in plastic petri plates. Isolates were grown at 20°C under near ultraviolet lamps with photoperiod 12/12h to stimulate sporulation. After 2 weeks of incubation plates were washed out with distilled water. Obtained suspension was filtered through gauze to remove mycelial fragments. The spore density of all isolates was adjusted to 5×10^6 spores/ml with a haemocytometer. Mixture of isolates was made by mixing proportional volumes of spore suspension of all isolates.

The pathogenicity test was conducted with 4 winter triticale cultivars, 6 winter wheat cultivars and 3 winter rye cultivars. Seeds of tested cultivars were surface sterilised for 3 min. in a mixture of sodium hypochloride (10%), 95% ethanol (25%) and sterilised water (65%). Then seeds were dipped into 0.5% water solution of carboxymethyl cellulose for 5 min. After drying, seeds were soaked in the spore suspension for 1h and dried overnight at room temperature. The control set of seeds was soaked in distilled water.

Inoculated seeds were plated on glass plates (Ø 7cm) filled with water agar. Ten seeds per plate were applied. Plates were placed in phytotrone chamber. After removing the caps, plates were covered with 1-litre Weck's jars. Environmental conditions were set as follows: day length 16h, temperature 22/16°C day/night, light intensity 350 $\mu E \times m^{-2} \times s^{-1}$ PAR (3000 lux). The extent of coleoptile necrosis was scored on individual seedlings 10 days after inoculation. Seedlings were at the growth stage 12 according to the decimal scale of Zadoks *et al.* (1974). The necrosis was rated on a four-degree scale (Grey and Mathre 1988):

0 - no discoloration,
1 - pinpoint lesions,
2 - extended linear lesions,
4 - discoloration of at least 50% and/or blighted seedling.

For each isolate-cultivar combination a percent disease rating (DR) was calculated as follows:

$$Intensity(DR)\% = \sum_{1,2,4}^{i=0} \frac{n_i D_i}{N D_{\max}}$$

where;

n_i is the number of seedlings of the *i*th category,
D_i is the numerical value of the *i*th category,
N is the total number of seedlings in the sample, and
$D_{\max}$ is the maximum category value.

To reduce the effect of natural seed infection and differences in germination ability, the disease ratings of control seedlings were subtracted from the DR's of artificially inoculated ones. Three consecutive tests without replications were carried out.

Statistical analysis

There was performed *n* analysis of variance using randomized complete block design with a single test as a block. For the analysis of variance of the data combined from the present experiment and the previous one (Arseniuk *et al.* 1993) split-plot design was applied with experiment as a main plot and isolates and cultivars as subplots.

Results from both experiments separately were analyzed by a method proposed by Carson (1987). Mean isolate aggressiveness was regressed against variance in resistance among cultivars and mean cultivar resistance was regressed against variance in aggressiveness among isolates. Linear regression model $\left(y = a + bx \right)$ and non-linear quadratic model $\left(y = a + b_1 x + b_2 x^2 \right)$ were applied.

RESULTS

All *Fusarium* spp. isolates were pathogenic towards seedlings of all cereal cultivars tested, with exception of isolate 'Fan-2' on wheat 'Parada', with the mean disease severity of 23.7% coleoptile necrosis (Table. 1). Significant differences in aggressiveness among isolates and in resistance among cultivars were observed (Fig. 1).

Statistically significant Pearson and Spearman rank correlations were found for the aggressiveness of isolates in this experiment and the previous one (r = 0.61,

$p<0.01$ and $r_s = 0.65$, $p<0.01$), where the soil was used as a substrate (Arseniuk *et al.* 1993). However, the resistance of cultivars in both tests did not correlate as calculated by Pearson correlation ($r = 0.39$ n.s.) or Spearman rank correlation ($r_s = 0.48$ n.s.). When correlating the resistance of cultivars to the mixture of isolates alone, Pearson and Spearman correlation coefficients proved higher ($r = 0.53$ n.s.) or significant ($r_s = 0.59$, $p<0.05$), respectively.

Table 1

Disease (percentage of coleoptile necrosis[a]) produced by 20 isolates of *Fusarium* spp. and their mixture on seedlings of 13 winter cereal cultivars

Cultivar/ Isolate[b]	Moniko (t)[c]	Motto (r)	Madar (r)	Malno (t)	D.Nowe (r)	Kamila (w)	Lasko (t)	Liwilla (w)	Almari (w)	Alba (w)	Almo (t)	Begra (w)	Parada (w)	Meane
Fc-1[d]	55.3	49.3	44.3	43.3	37.7	41.7	32.7	39.3	34.3	38.3	31.0	39.3	41.0	40.6
Fsp-2	52.7	49.3	46.7	38.3	34.3	40.0	38.7	29.3	18.7	30.0	23.7	26.7	11.0	33.8
Fa-2	41.0	30.0	32.7	48.3	23.3	28.3	44.3	39.3	32.7	12.7	12.0	33.3	25.3	31.0
Fc-2	41.3	41.0	32.7	42.7	30.0	38.3	36.7	34.3	22.7	18.3	21.0	5.0	31.0	30.4
Fsc-1	47.0	34.3	40.0	51.3	30.0	33.3	19.7	30.3	36.0	13.3	22.7	19.3	15.0	30.2
Fg-2	46.0	44.3	38.3	22.0	34.3	26.7	21.0	19.7	27.7	15.0	24.3	16.0	21.7	27.5
Feq-2	37.0	36.0	37.7	37.0	35.0	21.0	27.0	21.0	16.0	15.0	14.3	23.3	17.7	26.0
Feq-1	42.0	26.0	28.3	45.0	25.0	23.3	30.0	24.3	13.3	10.0	11.0	15.0	11.0	23.4
Mixture	39.3	39.3	43.3	29.3	26.0	25.0	20.0	13.3	17.7	18.3	12.7	4.3	15.0	23.3
Fg-1	41.0	31.0	33.3	20.0	23.3	29.3	27.7	14.3	18.3	25.0	19.3	1.0	14.3	22.9
Ft-1	29.3	27.7	26.7	26.7	27.7	33.3	15.0	16.7	18.3	18.3	6.0	23.3	24.3	22.6
Fsc-2	33.7	39.3	30.0	35.0	23.3	30.0	20.0	12.7	3.3	14.3	12.0	12.0	5.0	20.8
Fa-1	32.7	41.0	30.0	21.0	20.0	17.7	11.0	13.3	22.7	12.7	31.0	5.0	10.0	20.6
Fsu-2	56.0	33.3	27.7	24.3	16.7	20.0	15.0	15.0	8.3	9.3	21.0	11.0	10.0	20.6
Fp-1	33.3	32.7	30.0	28.3	20.0	23.3	15.0	6.7	15.0	33.3	7.7	13.3	7.7	20.5
Fp-2	21.7	26.7	26.7	34.3	21.7	27.7	16.0	14.3	32.7	11.7	9.3	10.0	8.3	20.1
Fan-1	42.7	36.0	15.0	34.3	23.3	26.7	21.7	10.0	11.7	15.0	13.7	6.7	1.0	19.8
Fcr-1	36.7	32.7	27.7	18.3	31.0	25.0	11.7	6.7	12.7	13.3	11.0	6.7	9.3	18.7
Fan-2	21.0	29.3	18.3	26.7	21.7	11.0	11.7	23.3	15.0	11.7	4.3	11.7	0.0	15.8
Fcr-2	8.3	27.7	41.7	10.0	22.7	16.7	16.7	6.7	11.7	9.3	4.3	11.7	3.3	14.7
Fsu-1	31.7	31.0	16.7	11.7	16.7	4.3	14.3	14.3	13.3	14.3	4.3	12.7	3.3	14.5
Mean[f]	37.6	35.1	31.8	30.8	25.9	25.8	22.2	19.3	19.1	17.1	15.1	14.6	13.6	23.7

[a]Average percentage of disease calculated from disease scores on ten individual seedlings; [b]Cultivars and isolates arranged according to decreasing resistance and aggressiveness; [c]r - rye, t - triticale, w - wheat; [d]Fa - *F. avenaceum*, Fan - *F. anthophilum*, Fc - *F. culmorum*, Fcr - *F. crookwellense*, Feq - *F. equiseti*, Fg - *F. graminearum*, Fp - *F. poae*, Fsc - *F. sambucinum* var. *coeruleum*, Fsp - *F. sporotrichioides*, Fsu – *F. subglutinans*, Ft - *F. tricinctum*; [e]Isolate means, $LSD_{0.01} = 9.0$; [f]Cultivar means, $LSD_{0.01} = 7.1$

The main effects of cultivar and isolate were highly significant but the effect of interaction of isolate by cultivar was not significant (Table 2). The analysis of variance of the data combined from both experiments produced results similar to ones for each experiment separately (Table 3). Isolate and cultivar effects remained sig-

nificant and interaction of isolate by cultivar remained insignificant. Moreover, significant interactions of experiment by isolate and by cultivar were found.

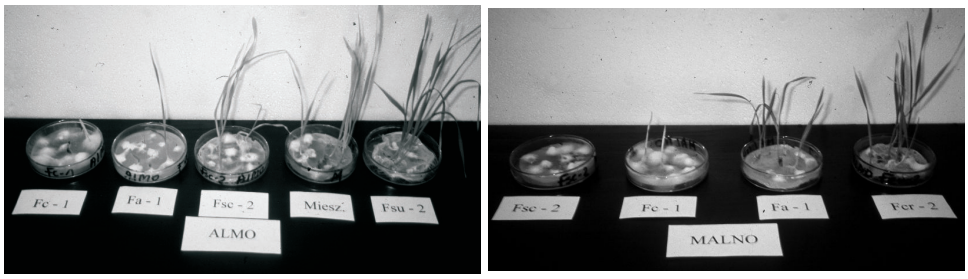

Fig. 1. Reaction of seedlings of winter triticale cultivars 'Almo' and 'Malno' to different *Fusarium* spp. isolates following seed inoculation. Fa - *F. avenaceum*, Fc - *F. culmorum*, Fcr - *F. crookwellense*, Fsc - *F. sambucinum* var. *coeruleum*, Fsu - *F. subglutinans*, Miesz. - mixture of isolates

Table 2

Analysis of variance for the disease (percentage of coleoptile necrosis[a]) produced on seedlings of 13 winter cereal cultivars by 21 isolates of *Fusarium* spp.

Source of variation	df	Mean square
Block/experiment	2	14152.4*
Isolate	20	1693.5*
Cultivar	12	4174.2*
Isolate × cultivar	240	141.3
Error	544	238.4
Total	818	...

[a]Average percentage of disease calculated from disease scores on ten individual seedlings;
**F*-value highly significant (P < 0.01).

Table 3

Analysis of variance for the disease (percentage of coleoptile necrosis[a]) produced on seedlings of 13 winter cereal cultivars by 21 isolates of *Fusarium* spp. [data combined from experiment described by Arseniuk *et. al.* (1993) and the present experiment]

Source of variation	df	Mean Square
Experiments	2	16944.8
Error 1	6	4683.3
Isolate	20	2771.2*
Experiments × isolate	40	902.0*
Cultivar	12	6734.0*
Experiment × cultivar	24	1912.1*
Isolate × cultivar	240	181.6
Experiment × isolate × cultivar	480	168.5
Error 2	1632	169.5
Total	2465	...

[a]Average percentage of disease calculated from disease scores on ten individual seedlings;
**F*-value highly significant (P < 0.01).

Isolates of *F. culmorum*, *F. sporotrichioides*, *F. avenaceum* and *F. sambucinum* var. *coeruleum* were the strongest pathogens (Table 1). Mixture of isolates was medium aggressive, significantly different from the strongest aggressive isolates. Winter wheat cultivars 'Parada' and 'Begra' and winter triticale cultivar 'Almo' were the most resistant to seedling blight. Winter triticale cultivars 'Moniko' and 'Malno' and winter rye cultivars 'Madar' and 'Motto' proved to be the most susceptible. Among cereal species winter wheat was the most resistant to the disease at the seedling stage while winter rye was the most susceptible. Winter triticale reaction was intermediate. A broad range of resistance to seedling blight was observed among triticale cultivars. 'Moniko' was the most susceptible and 'Almo' the most resistant (Table 1).

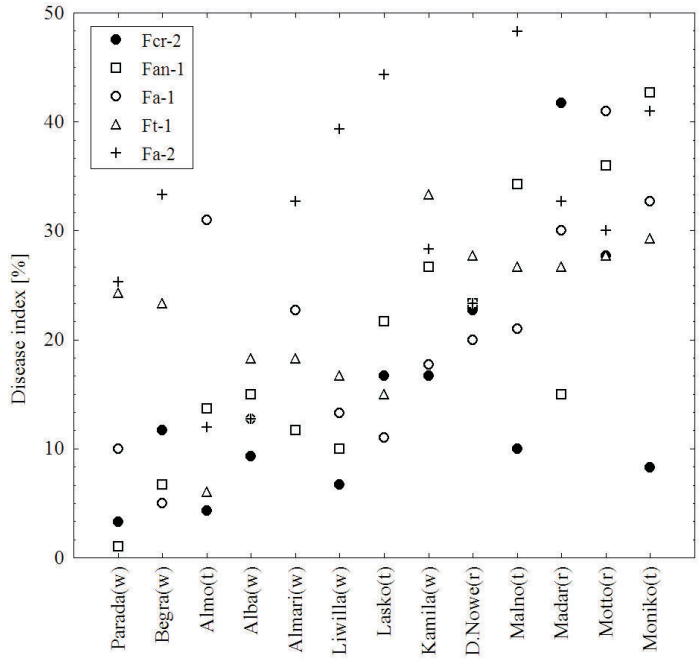

Fig. 2. Disease produced by 5 *Fusarium* spp. isolates showing differences in aggressiveness patterns on seedlings of 13 winter wheat (w), triticale (t) and rye cultivars

Closer analysis of aggressiveness/resistance patterns revealed ranking differences for some *Fusarium* isolates, e.g. 'Fcr-2' and 'Fan-1' on 'Malno' and 'Madar', 'Fa-2' and 'Fan-1' on 'Almo' and 'Begra' (Fig. 2). These differences were the most clearly pronounced when comparing isolate aggressiveness on cultivars of different cereal species.

Linear regression of mean isolate aggressiveness against variance in resistance among cultivars was highly significant in previous experiment but proved not significant in this one (Fig. 3a, b). The non-linear regression model applied for the present experiment data appeared to be highly significant (Fig. 3b). It was observed that if aggressiveness score was close to 25% or more the variance among cultivars

tended to decrease. It is to add that non-linear regression did not fit the relationship between analysed variables in previous experiment.

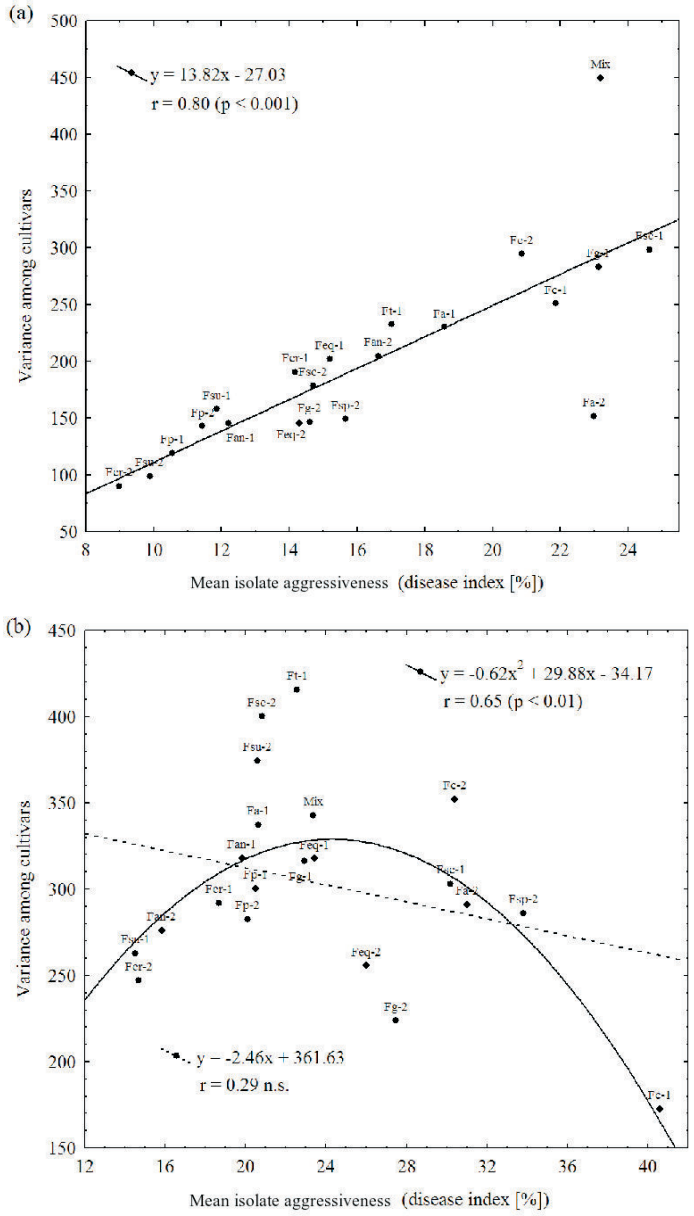

Fig. 3. Relationship of the mean aggressiveness of 21 isolates of *Fusarium* spp. and the variance in resistance among 13 winter cereal cultivars. a) experiment described by Arseniuk *et al.* (1993), b) present experiment; r - rye, t - triticale, w - wheat

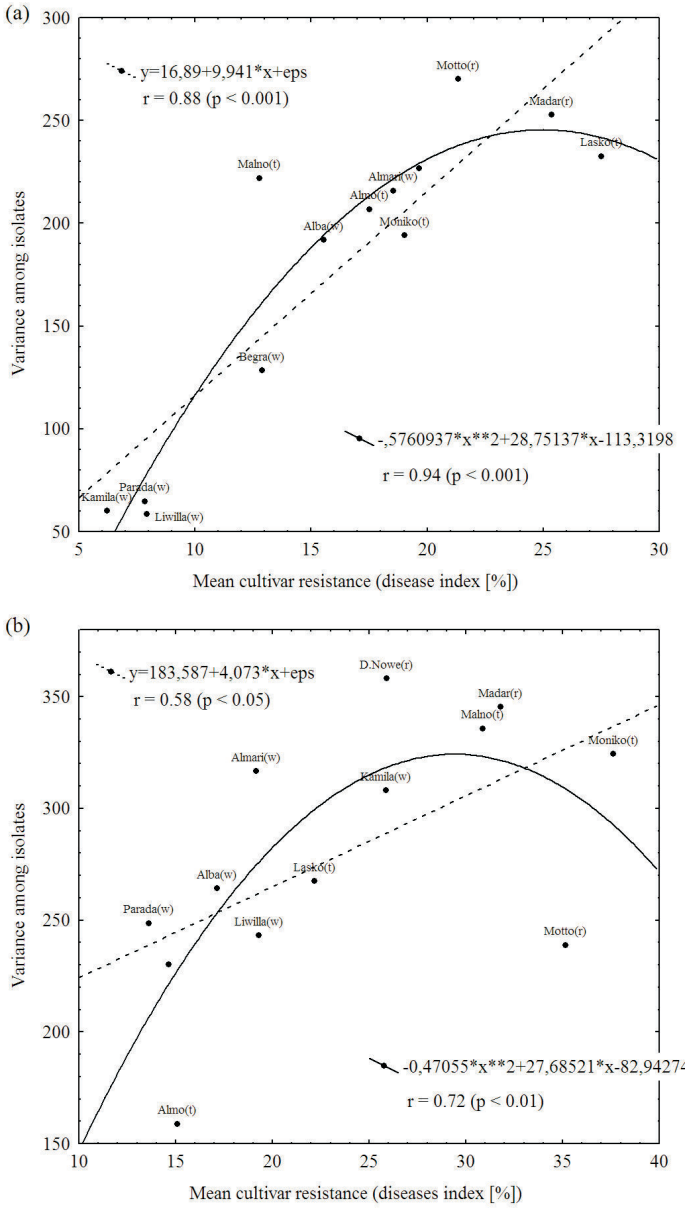

Fig. 4. Relationship of the mean resistance of 13 winter cereal cultivars and the variance in aggressiveness among 21 isolates of *Fusarium* spp. a) experiment described by Arseniuk *et al.* (1993), b) present experiment; r - rye, t - triticale, w - wheat

Linear regression of mean cultivar resistance against variance in aggressiveness among isolates was highly significant in both experiments (Fig. 4a, b), but it appeared that non-linear regression was significant too and explained the relationship between analysed variables more precisely. Isolates were differentiated the best at

the disease level about 25-30%. The variance among isolates decreased above the indicated range of values.

DISCUSSION

According to the results the following species were found as the most aggressive to cereal seedlings: *F. culmorum*, *F. avenaceum*, *F. sambucinum* var. *coeruleum* and *F. sporotrichioides* (Table 1). In the previous experiment (Arseniuk *et al.* 1993) the first three species were found also highly aggressive. *F. sporotrichioides* possessed medium aggressiveness. The results are in agreement, with some exceptions, to other published data (Chełkowski *et al.* 1985, Mańka 1988, Chełkowski *et al.* 1989, Mańka *et al.* 1989, Wiśniewska and Buśko 2005). Mańka *et al.* (1985) found that *F. avenaceum* was low to medium aggressive to cereals. *F. tricinctum*, medium aggressive in this study, was not pathogenic to cereal seedlings according to other authors (Chełkowski *et al.* 1989, Mańka 1989). *F. crookwellense*, in contrast to the results reported by Chełkowski *et al.* (1988) and Mańka (1989), was a weak pathogen of cereal seedlings. Mixture of isolates possessed medium aggressiveness, what contrasted with results of the previous experiment (Arseniuk *et al.* 1993).

All *Fusarium* species used in this experiment are known as capable of mycotoxin production such as trichothecenes (e.g. deoxynivalenol, T-2 and HT-2 toxins, nivalenol), monilformin, zearalenone (Marasas *et al.* 1984). Most of this toxins have phytotoxic effect (Menke-Milczarek and Zimny 1991, Bruins *et al.* 1993, Wiśniewska and Chełkowski 1994) Four isolates of *F. culmorum* and *F. graminearum* were tested for toxins production *in vitro* (Góral *et al.* 2002, Ochodzki and Góral 2006). They produced mainly deoxynivalenol and zearalenone. Other isolates were not tested for their toxinogenicity. This capability of *Fusarium* species could have significant influence on our results, as mycotoxins can be produced by isolates grown on agar media and infiltrate the medium (Hestbjerg *et al.* 2002, Clear *et al.* 2006). Therefore, symptoms observed in present experiment might be mainly the result of phytotoxic action of metabolites produced by *Fusarium* isolates.

Published results showed that one of the crucial factor of aggressiveness of pathogenic *Fusarium* species is their ability to produce mycotoxins. The most extensive work was done as regards *F. graminearum,* causing ear rot of maize and head blight of wheat. Proctor *et al.* (2002) analyzed pathogenicity of TRI5 disruption mutants non-producing trichothecene toxins (e.g. deoxynivalenol). They found that trichothecene production contributes to high levels of aggressiveness of *F. graminearum* on wheat and maize. Results also indicated that trichothecenes were aggressiveness factors rather than pathogenicity factors. Trichothecenes increase the severity of disease caused by *F. graminearum*, but in the absence of their production the fungus can still cause low levels of disease. The low levels of disease caused by the trichothecene-non-producing mutants demonstrate that other factors contribute to the ability of the *F. graminearum* to cause disease. It was proved that the same mechanism of aggressiveness is present in the *Fusarium* seedling blight pathogenesis (Wojciechowski *et al.* 1996, Wiśniewska and Buśko 2005). Deoxynivalenol has strong phytotoxic effect on wheat seedlings by inhibition of protein synthesis and growth of wheat coleoptile tissue and seedlings (Miller 1989, Bruins *et al.* 1993, Wakuliński

1989). Thus, the level of mycotoxin production can be regarded as main factor determining *Fusarium* isolate/species aggressiveness on cereal seedlings or heads.

On average, at the seedling stage winter rye appeared to be the most susceptible and winter wheat the most resistant to *Fusarium* blight. These results correspond to findings reported by Mańka (1989). Winter triticale response was intermediate in comparison to the other species. However, notable differences in resistance among individual cultivars were observed.

Concerning the inoculum composition used for screening of breeding materials, it should be pointed out, that no statistically significant interaction between pathogen isolates/species and cereal genotypes was found. In contrast, significant interactions between *Fusarium* spp. isolates causing head blight and wheat cultivars were reported by other authors (Mesterhazy 1987, Snijders and Van Eeuwijk 1991, Gilbert *et al.* 1993). However, low stability of resistance/aggressiveness patterns over experiments or years was observed. Genotype ranking was influenced more by environmental conditions than by *Fusarium* isolates. Additionally, Mesterhazy (1988, 2002) found an interaction between host genotype and disease severity, which can be a component of environmental effect. In our study, the interaction of host genotype by disease severity is evidenced by weak correlations of the cultivar resistance in both experiments. Mean disease level appeared 2-fold higher in the present experiment than in the previous one (Arseniuk *et al.* 1993). Van Eeuwijk *et al.* (1995) presented results from studies of *Fusarium* head blight of wheat carried out in different countries over several years. The latter authors proved the lack of host specificity for *F. culmorum* and *F. graminearum*. Our findings suggest that the lack of host specificity is an attribute of a larger number of *Fusarium* spp. attacking cereals. Moreover, Van Eeuwijk *et al.* (1995) did not find any evidence for a geographical diversity in expression of virulence genes. These observations correspond closely to the results reported presently. In our previous experiment (Arseniuk *et al.* 1993) we found isolates of *F. subglutinans* and *F. poae* to be higher aggressive to triticale than to rye and wheat. Isolates of the other species showed the highest aggressiveness to rye and the lowest to wheat. However, this pattern was not confirmed in this experiment. In the present study we observed ranking differences for other isolates/species. It is to add that such differences were found only for a few isolates and cereal cultivars.

All above findings show low range of variability in the pathogen population regarding aggressiveness. According to Van der Plank's (1984) definition and principles only horizontal resistance was found in tested sets of cereal cultivars. Appearance of non-specificity in *Fusarium* - cereals pathosystem would suggest to use a single, aggressive isolate for resistance testing (Mesterhazy 1988, Snijders and Van Eeuwijk 1991). But, the above described considerable influence of environmental conditions on expression of resistance requires more complex inoculum for resistance testing. The occurrence of instability of aggressiveness *Fusarium* isolates over years was observed by Mesterhazy (1987, 2002) in head blight resistance study of wheat. He stated, that since aggressiveness level highly influences disease severity, it is impossible to determine the amount of host resistance using single isolate. The author proposed to use a mean reaction to several isolates as more stable and closer to natural conditions.

We found that in both experiments a mixture of isolates revealed the highest variation among cultivars. In the present test high variation between cultivars was pro-

duced by medium aggressive isolates as well. It is to mention that mixture of isolates possessed about the same aggressiveness regardless of experimental conditions. Moreover, the ranking of cereal cultivars in both experiments was more constant when tested with a mixture of isolates than one based on mean reactions to single isolates. This phenomenon suggests that for assessing seedling resistance mixtures of isolates/species of different aggressiveness should be used instead of a single isolate of medium or high aggressiveness. This opinion is also in accord with the results of *Fusarium* head blight screening presented by Van Eeuwijk *et al.* (1995).

However, when using mixture of isolates of different species, we should remember that their mean aggressiveness is also result of competition between species, not only simple averaging of their aggressiveness. Dawson *et al.* (2004) found that *F. equiseti* isolates decreased DON on wheat inoculated with *F. culmorum* by more than 70%. Other *F. equiseti* isolate decreased the percentage of diseased grains by 92% and DON by 94% on wheat inoculated with *F. graminearum.* Mixture of isolates in our experiments contained 2 *F. equiseti* isolates, so their medium aggressiveness could be the result of competition *F. equiseti* versus *F. graminearum* and/or *F. culmorum*. However, it is to mention that aggressiveness of 2 *F. equiseti* isolates was not significantly different from both *F. graminearum* and one *F. culmorum* isolates. Hestbjerg *et al.* (2002) found that some *F. equiseti* isolates can produce nivalenol and other trichothecenes. Hence, they can be highly aggressive on cereal seedlings.

The synthesis of mycotoxins is an another reason supporting the use of isolate mixtures for resistance screening in cereals. Logrieco *et al.* (1990) observed no differences in mean aggressiveness between different chemotypes of *Fusarium graminearum*. However, within each chemotype, authors found notable variability in aggressiveness among isolates. On the other hand, aggressiveness of isolates was not correlated with their ability to produce mycotoxins *in vitro*. Mańka *et al.* (1985) found a relationship between aggressiveness of *Fusarium* isolates and production of DON and zearalenone, though some aggressive isolates of *F. graminearum* did not produce above mentioned toxins. Wiśniewska and Buśko (2005) observed that *F. culmorum* isolate causing severe wheat head infection and producing DON revealed high aggressiveness to wheat seedlings.

Because of the above reasons single isolates should not be treated as pathogenically equivalent. This conclusion is in accordance with a similar suggestion of Miller *et al.* (1985) and Mesterhazy *et al.* (1999).

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