European Journal of Plant Pathology **108:** 245–251, 2002.

Scab response and moniliformin accumulation in kernels of oat genotypes inoculated with *Fusarium avenaceum* in Poland

I. Kiecana[1], E. Mielniczuk[1], Z. Kaczmarek[2], M. Kostecki[3] and P. Golinski[2,3,*]
[1]*Department of Phytopathology, Agricultural University of Lublin, ul. Leszczyńskiego 7, 20-069 Lublin, Poland;* [2]*Institute of Plant Genetics, Polish Academy of Sciences, ul. Strzeszynska 34, 60-479 Poznan, Poland;* [3]*Department of Chemistry, August Cieszkowski Agricultural University, ul. Wojska Polskiego 75, 60-625 Poznan, Poland;* [*]*Author for correspondence (Phone: +4861 8487837; Fax: +4861 8487824; E-mail: piotrg@owl.au.poznan.pl)*

Accepted 3 January 2002

Key words: Fusarium head blight, *Fusarium* pathogens, resistance to pathogens, mycotoxins

Abstract

Inoculation experiments with 14 genotypes of oats (10 cultivars and 4 lines) were performed during 1996, 1997 and 1998 in Sitaniec, South-Eastern Poland. Panicles of oats were inoculated with a conidial suspension of *Fusarium avenaceum*, which caused a reduction in yield by 33% and in 1000 kernel weight (TKW) by 21%. During the period between inoculation and harvest, *F. avenaceum* was able to accumulate moniliformin (MON) in kernels at an average level of 0.13 mg kg^{-1} (μg g^{-1}). The highest reduction of yield components caused by the *F. avenaceum* inoculation was found for cv. Santor, followed by lines CHD 1171, STH 2795 and cvs: Kwant and Farys, while cvs Slawko, Dukat, Borys and Komes exhibited the highest resistance to the disease in terms of TKW and yield reductions after inoculation.

Introduction

Oats are one of the cereals commonly cultivated in Poland, but in the north and south of the country their production is more intensive since the yield of the crop is significantly dependent on higher soil moisture content. World literature on oats diseases draws attention to pathogens that attack generative organs. However, there is limited information concerning the pathogenicity of the genus *Fusarium* and especially the effect of *Fusarium avenaceum* (Fr.) Sacc. on scab development on oat panicles (Langseth et al., 1995). In a recent report (Mielniczuk, 2001), a significant incidence of oat *Fusarium* panicle blight incidence was reported in the region of Lublin (South-Eastern Poland). In years with weather conditions favourable for panicle infection with *Fusarium* and for pathogen development, the disease incidence of *F. avenaceum* observed in fields of oats was up to 17%. *Fusarium* head blight (FHB), of cereals other than oats was studied in detail in earlier papers (Golinski et al., 1996a; 1999). Symptoms of the disease are similar in all small grain cereal crops; however, differences between cereal species are observed in field experiments with inoculated cereals (Parry et al., 1995). Scab severity depends on several climatic, agronomic and genetic factors. The disease can result in *Fusarium* damaged kernels (FDK); these are smaller, shrivelled and discoloured (white to pale pink), and result in yield reduction and the accumulation of toxic secondary metabolites. Moisture levels (rainfall), especially during anthesis, play an important role in head infection and colonization, as does the subsequent FHB severity (Parry et al., 1995).

According to the literature (Lacicowa, 1989; Mihuta-Grimm and Forster, 1989; Clear and Patrick, 1990; Kiecana, 1994; Golinski et al., 1996a,b; 1999), *F. avenaceum* is international pathogen of oats. Isolates of *F. avenaceum* vary in virulence and temperature optima (Booth, 1971). Additionally, the mycotoxin potential – the ability of different *F. avenaceum* species to produce moniliformin (MON) – has been described in detail (Golinski et al., 1996b). Limited published

information on *F. avenaceum* pathogenicity to oats encouraged us to undertake studies on the reduction of yield, 1000 kernel weight (TKW) and MON accumulation in kernels of 10 oats cultivars and 4 lines after panicle inoculation with *F. avenaceum*.

Materials and methods

Field experiment

Ten cultivars (Boryna, Borys, Dukat, Farys, German, Halny, Komes, Kwant, Santor, Slawko) and four lines (CHD 1171, CHD 1236, STH 2594, STH 2795) of oats were inoculated under field conditions at the experimental station in Sitaniec near Zamosc (South-Eastern Poland), situated on leached brown soil (Dystric Cambisols) formed on loess deposits (FAO, 1998). Oat cultivars and lines, used in the inoculation experiments, were originally developed and introduced in Poland. The *F. avenaceum* isolate No. 121 was obtained from the Plant Pathology Department at the Agricultural University of Lublin, Poland and was originally isolated from oat kernels. *F. avenaceum* No. 121 isolate was chosen for the experiment on the basis of strain pathogenicity determined by the method of Mishra and Behr (Kiecana, 1994). The strain reduced cv. Dukat seed germination by up to 3%. Inoculum was prepared according to the modified method of Mesterházy (1978). The growing medium (1 : 1) was composed of water extract from 0.5 kg oat leaves, selective medium – SNA, autoclaved for 1 h at 121 °C and 1 atm and inoculated with mycelium of a two-week-old culture of *F. avenaceum* isolate No. 121 incubated for two weeks at 18–20 °C with a 12 h period of natural light. After incubation, the inoculum – stirred for 10 min – was filtered through cheesecloth, and the supernatant of the conidial suspension (5×10^5 spores ml^{-1}) was used for the inoculation.

Fourteen oat cultivars and lines were studied in one location (Sitaniec) over 3 years (1996, 1997 and 1998), using a randomized complete block design with four replications. Eighty panicles of oats (20 panicles per replicate) were inoculated with *F. avenaceum* 4 days after the anthesis of a minimum of 50% of plants (on 21.06–8.07.1996, 25–30.06.1997 and 25–28.06.1998). The inoculum (2 ml per panicle) was applied with a laboratory sprayer. The same cultivars and lines, sprayed with 2 ml distilled water instead of a conidial suspension, were used as a control (non-inoculated) group. After inoculation or water spraying, the panicles were protected with plastic bags for 24 h to avoid water evaporation and the spread of the inoculum.

Weather conditions did differ, but not significantly, from the multiannual averages. Mature panicles were collected during August (11.08.1996, 14–16.08.1997, 7.08.1998) and threshed manually. Yield and TKW of the experimental groups were measured and compared with controls. Symptoms of the disease (scabby kernels) were evaluated according to Golinski et al. (1996a).

Statistical analysis

The crop yield traits were analysed statistically (Golinski et al., 1996b). Two-factor univariate analyses of variance were used to examine the differences between years and between oat cultivars with regard to TKW and yield reductions (% of control group). Hypotheses about the equality of years and all cultivars and the interaction among years and cultivars for both yield parameters were tested at significance levels of $P = 0.05$ and $P = 0.01$. After the rejection of the general hypothesis of no differences between cultivars, contrasts between the cultivar of the lowest susceptibility and the others were estimated and statistically verified. As a result of the analysis of variance, homogenous groups of cultivars of the lowest susceptibility in particular years were distinguished. To study the differences and interrelations between cultivars with respect to the reductions of yield and TKW jointly, the bivariate analysis of variance with Mahalanobis distances and the shortest dendrite was applied (Calinski and Kaczmarek, 1973). For a graphical presentation of the tested cultivars for both the investigated parameters, a canonical analysis was performed (Morrison, 1976). Finally, in order to investigate the relationship between TKW and yield reductions and MON content in kernels, the simple coefficients of correlation were calculated.

Chemical analysis

The MON chemical analysis was performed according to Golinski et al. (1999) and may be summarized as follows: finely ground kernels were extracted with acetonitrile–methanol–water (16 : 3 : 1 v/v/v) using 5 ml of the solvent per 1 g of sample. Extracts, defatted with *n*-hexane (3 × 50 ml), were concentrated and purified on Florisil columns (Golinski et al., 1999). MON content was estimated on

Merck 5554 silica gel thin-layer chromatography plates (Merck, Darmstadt, Germany) with the developing solvent 2-propanol–butanol–water–ammonium hydroxide (12 : 4 : 1 : 1 v/v/v/v). The colour of spots was developed with 3-methyl-2-benzo-thiazolinone-hydrochloride (MBTH) (Chelkowski et al., 1990). The intensity of dark spots on the chromatogram was compared to that of the metabolite standard. A precise quantification of MON was obtained by high performance liquid chromatography (HPLC) using the Waters 501 apparatus (Waters Division of Milipore, Milford, MA, USA) with a C-18 Nova Pak column (3.9 × 300 mm) and a Waters 486 UV detector (λ_{max} = 229 nm). Acetonitrile–water (15 : 85 v/v) buffered with 10 ml of 0.1 M K_2HPO_4 in 40% *t*-butyl-ammonium hydroxide in 1 l of solvent (Sharman et al., 1991) was used as a mobile phase (flow rate 0.6 ml min^{-1}). The retention time of MON was 11.5 min with 90% recovery and detection limit of 25 ng g^{-1}.

Results

Kernels of inoculated oat panicles exhibited typical symptoms of scab, and were smaller, shrivelled and discoloured compared to the control (non-inoculated) group. During the 3 years of the experiment, significant differences between all the tested forms of oats (10 cultivars and 4 lines) were observed. Experimental groups, when compared to the control, exhibited significant variation in TKW reductions and yield during all 3 years of field studies (Figures 1 and 2). The distribution of results indicates cultivars and lines with significant reductions of yield components (cvs Santor, Farys and lines CHD 1171, STH 2795) and those resistant to the disease (cvs Slawko, Dukat, Komes and Borys).

The chemical analysis of kernels of 10 cultivars and 4 lines inoculated with *F. avenaceum* revealed the presence of MON. The only exceptions were cvs German and Komes, and lines CHD 1171 and STH 2594, where the toxin was not detected, while cv. Farys exhibited trace amounts of the metabolite. The detected MON concentration ranged from 0.06 mg kg^{-1} (line CDH 1236) up to 0.34 mg kg^{-1} (cv. Slawko), with an average value of 0.13 mg kg^{-1} (Table 1).

The mean values (over the 3 years of the experiment) of TKW and yield reductions, followed by the MON accumulation calculated for each tested cultivar and line are shown in Table 1. The hypothesis for equality of cultivars and lines was rejected for both parameters (TKW and yield) (F = 5.06 and 3.62, respectively, with $F_{0.01}$ = 2.28). Additionally, the years × cultivars

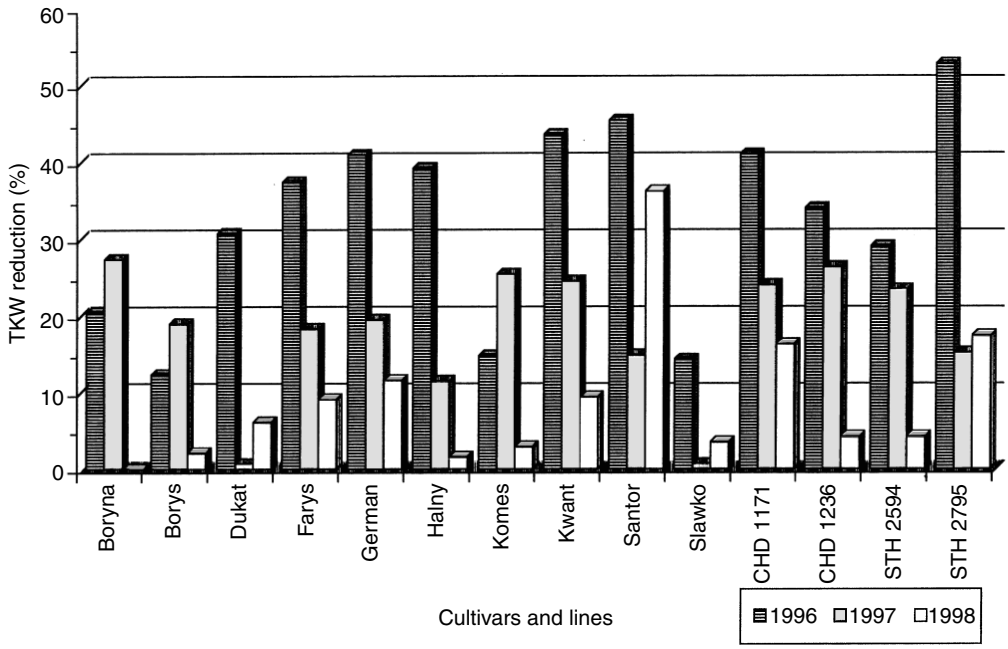

Figure 1. Reductions (%) of TKW compared to controls after oat panicle inoculation with *F. avenaceum* in 1996–1998.

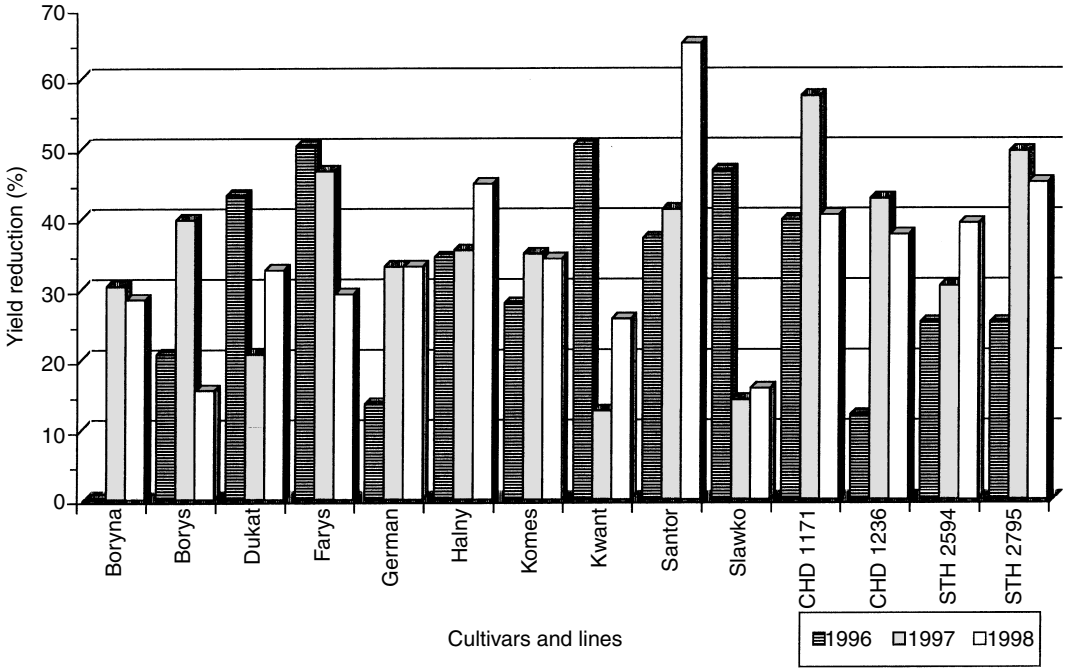

Figure 2. Reductions (%) of kernel yield (Y) compared to controls after oat panicle inoculation with *F. avenaceum* in 1996–1998.

Table 1. Mean values of TKW, yield reduction and MON content in kernels of oat cultivars and lines inoculated with *F. avenaceum* (means for 3 years)

No.	Cultivar/line	TKW reduction (%)	Yield reduction (%)	MON content (mg kg^{-1})
1	Boryna	15.6	19.8	0.14
2	Borys	10.2	21.2	0.15
3	Dukat	11.1	30.4	0.25
4	Farys	20.6	40.7	TR
5	German	22.3	26.5	ND
6	Halny	16.7	38.0	0.18
7	Komes	14.1	31.1	ND
8	Kwant	24.9	24.2	0.17
9	Santor	31.4	47.6	0.21
10	Slawko	5.5	25.2	0.34
11	CHD 1171	26.5	45.8	ND
12	CHD 1236	20.9	31.5	0.06
13	STH 2594	18.5	28.5	ND
14	STH 2795	27.8	39.8	0.31
	Mean	19.0	32.2	0.13

TR – trace amounts of MON (close to the detection limit 0.025 mg kg^{-1}); ND – not detected.

interactions were significant for reductions of both TKW and yield at $\alpha = 0.01$ level ($F = 2.67$ and 2.51, respectively, with $F_{0.01} = 1.91$), indicating that oat cultivars and lines differed during the years of the experiment. Thus, the reaction of different cultivars and lines of oats to weather conditions during the years of the experiment differed; the weather of each year influenced the reduction of yield parameters. Since the relationship between years (annual weather conditions) and scab response has been investigated in detail for cereals other than oats and reported in earlier work (Golinski et al., 1996a; 1999), the current work only assessed general trends, without any analyses of the correlation between yearly temperature, rainfall and disease symptoms. Since the hypothesis for equality of oat cultivars/lines was rejected and years × cultivars interactions were statistically significant, the homogenous groups of cultivars and lines of lowest susceptibility to the disease were distinguished in terms of TKW and yield reductions for each year over the 3 year period (Table 2). Over the 3 years of the experiment, cvs Slawko, Borys, Dukat and Komes were least susceptible to scab on the basis of TKW, but on the basis of yield reduction, five additional cultivars/lines should be included, namely Boryna, Kwant, German, STH 2594 and CHD 1236. Taking into consideration the reductions in both TKW and yield, the group of least susceptible cultivars consisted of Slawko, Dukat, Borys and Komes (Figure 3), with cv. Slawko being the least susceptible. However,

Table 2. Homogenous groups (with the lowest susceptibility to scab) of oat cultivars and lines inoculated with *F. avenaceum* based on reductions in TKW and yield of kernels

Parameter	Year			1996–1998
	1996	1997	1998	
	Borys Slawko Komes Boryna	Slawko Dukat Halny Santor STH 2795	Boryna Halny Borys Dukat STH 2594 Komes Slawko CHD 1236 German Kwant Farys	Slawko Borys Dukat Komes
TKW reduction				
	Boryna German CHD 1236 STH 2594	Kwant Slawko Dukat Boryna	Borys Slawko Kwant	Boryna Borys Kwant Slawko
Yield reduction	Borys STH 2795 Komes	STH 2594 German Komes		German STH 2594 Dukat Komes CHD 1236

cv. Slawko exhibited the highest accumulation of MON in kernels.

On the basis of the bivariate analysis of variance, F-values for TKW and yield reductions, and the reduction in both parameters, for the three cultivars only (Dukat, Borys and Komes) showed no significant differences to cv. Slawko (Table 3). A graphical interpretation of these results is shown in Figure 4, where Mahalonobis distances between cv. Slawko and the other cultivars/lines of oats are presented for TKW and yield reductions jointly. The Mahalanobis distances between cvs Slawko and Dukat, Slawko and Borys, Slawko and Komes are shorter than the critical distance $D_{0.05}$. The confirmation of the results presented in Table 3 is the configuration of the cultivars in the space of the first two canonical variates, with the shortest dendrite connecting the nearest cultivars (Figure 3). Cultivars similar to Slawko are shown in the circle.

Discussion

The inoculations were successful and panicles which exhibited typical scab symptoms, with salmon pink or orange sporodochia producing conidia of *F. avenaceum*. Sporodochia were also visible on internal and external glumes of all kernels in infected spikelets. Diseased kernels were small, soft and light. In our experiment, using the methods of Mesterházy (1978) and Kiecana (1994), panicles were sprayed with a suspension of *F. avenaceum* macroconidia in water. On the basis of the results obtained, we can conclude that inoculation of panicles at full anthesis reduced the yield, on average, by 33%; the infested kernels were small, shrivelled and discoloured. The percentage of scabby kernels correlated with yield reduction. The results obtained and the *Fusarium* oat panicle blight symptoms produced were comparable with earlier observations for scab of wheat (Mesterházy, 1978), barley (Kiecana, 1994), triticale and rye (Parry et al., 1995). The results of a two-factor analysis of variance allowed us to reject the hypothesis for no differences between years for TKW reduction only ($F = 65.72$ with $F_{0.01} = 4.78$); however, the average reduction (%) for TKW (31.8, 17.7, 7.4) and yield (29.4, 34.5, 32.6) of all tested oat cultivars/lines, for the 3 years of the experiment indicate an influence of weather conditions on severity of the disease symptoms. Since this relationship has been investigated for other cereals, in this study we were interested in general trends only, without any analyses of correlations between yearly temperature, rainfall and oat scab symptoms. It can thus be concluded that oat panicle infestation was significantly dependent on the relationship between pathogen, environment and oat genotype, confirming a similar tendency in other cereals (Chelkowski et al., 2000; Golinski et al., 1996a; 1999). *F. avenaceum* isolates have been frequently found in naturally contaminated oat panicles (Mielniczuk, 2001). The incidence of occurrence in cereal panicles and heads under different weather conditions confirms the tolerance of the pathogen to temperature and humidity and explains why the disease occurs internationally (Kiecana, 1994).

The results of chemical analyses indicate that kernels of oat panicles inoculated with *F. avenaceum* were contaminated with MON at a lower concentration level when compared to other cereals (Chelkowski et al., 1990; 2000; Golinski et al., 1996a,b; 1999). Our findings confirm an earlier report by Sharman et al. (1991) indicating a low level of the mycotoxin in naturally contaminated oat field samples. A low correlation between the reduction of yield traits and the accumulation of toxic secondary metabolites in kernels was reported earlier (Golinski et al., 1996a; 1999), and can be explained by a complex reaction of the plant to infestation with the pathogen. Four types

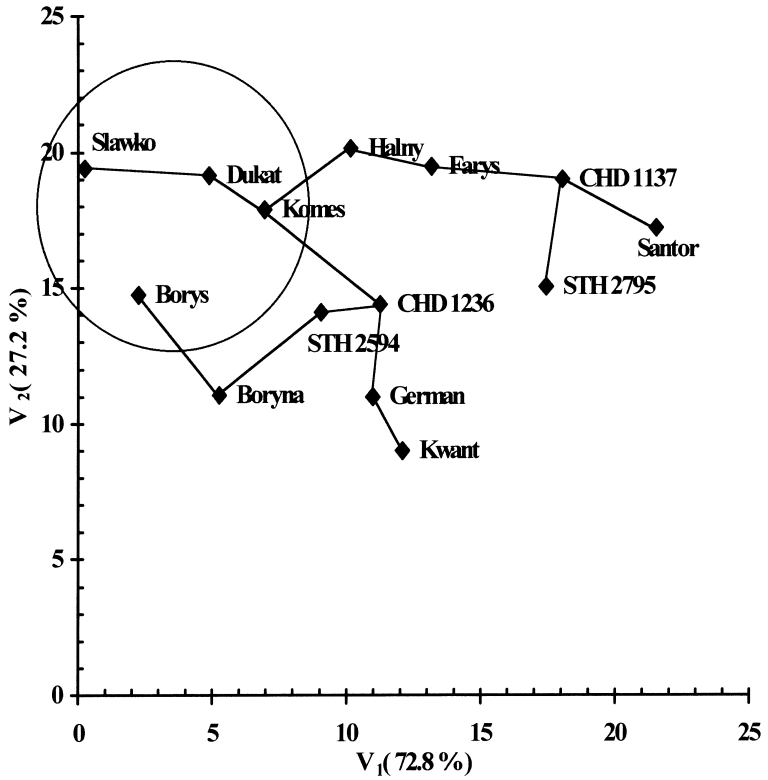

Figure 3. Configuration of oat cultivars and lines characterized by reductions (% control) in TKW and yield in the space of the first two canonical variates with the shortest dendrite connecting the nearest cultivars/lines.

Table 3. Differences between oat cultivars/lines tested and cv. Slawko (lowest susceptibility) for TKW and yield reduction

Cultivar/line	Differences for		F-value for both parameters jointly
	TKW reduction	Yield reduction	
Boryna	10.1*	−5.4	3.56*
Borys	4.7	−4.0	0.97
Dukat	5.6	5.2	0.81
Farys	15.0**	15.5*	6.26**
German	16.8**	1.3	6.97**
Halny	11.2*	12.8	3.70**
Komes	8.6	5.9	1.78
Kwant	19.4**	−1.0	9.85**
Santor	25.9**	22.4**	17.14**
CHD 1171	21.0**	20.6**	11.88**
CHD 1236	15.4**	6.3	5.48*
STH 2594	13.0**	3.3	3.97*
STH 2795	22.3**	14.6*	11.81**
Critical values	$F_{0.05}$		3.07
	$F_{0.01}$		4.78

*Difference significant at $P = 0.05$. **Difference significant at $P = 0.01$.

of mechanisms of plant resistance to scab have been suggested, with resistance of the host plant to infection with pathogenic fungi as the first mechanism, and resistance of the host to the process of pathogen spread in plant tissue as another. The latter concept was further extended to include two additional mechanisms: susceptibility of the plant to *Fusarium* mycotoxin degradation in kernels and tolerance of the host tissue to a high concentration of toxic metabolites formed by the pathogen (Wang and Miller, 1988).

Conclusions from this work indicate inoculation of oat panicles with *F. avenaceum* resulted in kernels which were smaller, and exhibited typical scab symptoms associated with a reduction in yield traits. Susceptibility of oats to scab after inoculation with the fungus was genotype dependent. Cultivars Slawko, Dukat, Borys and Komes showed the lowest reductions in yield traits and were the most resistant to the disease. Cultivars Santor, Kwant and Farys, and lines STH 2795 and CHD 1171 were highly susceptible to *Fusarium* panicle blight with significant reductions of yield components. The average yield reduction was 32% with a high tendency to MON accumulation.

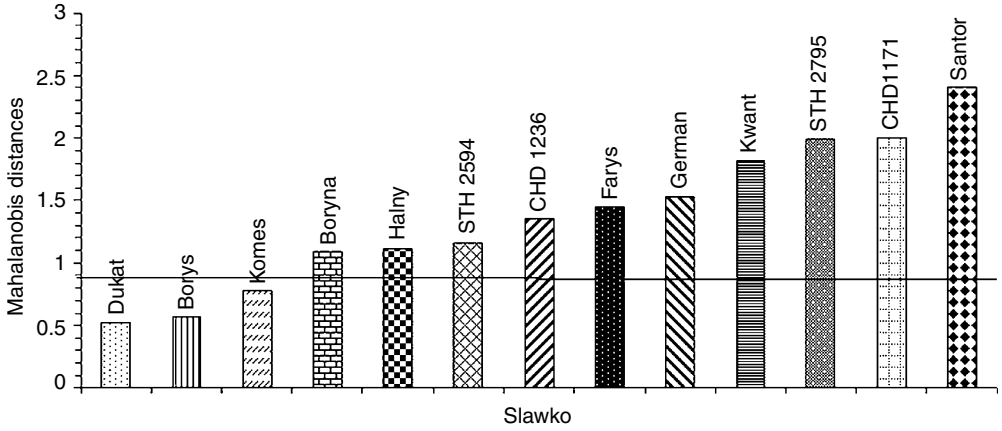

Figure 4. Mahalanobis distances between cv. Slawko and other tested oat cultivars and lines for reductions (% control) in TKW and yield jointly, after inoculation with *F. avenaceum*.

Acknowledgement

Technical assistance of Ewa Rymaniak and Iwona Rissmann is very much appreciated.

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