

Susceptibility of oat genotypes to *Fusarium crookwellense* Burgess, Nelson and Toussoun infection and mycotoxin accumulation in kernels

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Field experiments with 10 cultivars and 4 lines of oat were performed during three years (1996, 1997 and 1998) in South-Eastern part of Poland. Panicles of oat were inoculated with a conidial suspension of *Fusarium crookwellense*, which caused a reduction in yield by 32%, the 1000 kernel weight (TKW) by 23% and the number of kernels per panicle (NK) by 24%. *F. crookwellense* was able to accumulate nivalenol (NIV) in kernels at an average level of 0.15 mg/kg (from 0.1 to 0.37). The highest susceptibility to scab caused by *F. crookwellense* was found for genotypes Farys, German and STH 2795, whereas cv. Slawko exhibited the highest resistance to *F. crookwellense* in terms of yield, TKW and NK reductions after inoculation.

Key words: *Fusarium* spp., scab, mycotoxins, nivalenol, resistance to pathogens.

Introduction

Oat has been more often used in human nourishment in recent years because of the highest amount of dissoluble fibre, especially β -glucan in its kernels, that is considered to be necessary for any rational diet (WOOD et al., 1990).

Fusarium spp. are pathogens directly affecting oat panicles and kernels (VEISZ et al., 1997). Infection of plant heads by fungi of *Fusarium* genus has a direct negative effect on both the yield size causing its decrease and the quality of

the kernels (KIECANA, 1994; LANGSETH et al., 1995; MIELNICZUK, 2001; KIECANA et al., 2002). *Fusarium crookwellense* was described in Poland by KWASNA & CHEŁKOWSKI (1988).

In the South-Eastern part of Poland fusariosis of oat was frequently observed; *F. avenaceum*, *F. culmorum*, *F. poae*, *F. sporotrichioides* and *F. crookwellense* were reported as the most common pathogens in oat (KIECANA & PERKOWSKI, 1998; KIECANA & MIELNICZUK, 2001; MIELNICZUK, 2001).

F. crookwellense can usually produce ni-

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valenol and fusarenone-X, zearalenone and its derivatives (BOTTALICO, 1998), as well as fusarin C (GOLIŃSKI et al., 1988). In the field conditions it may also produce DON and its derivatives (SUGIURA et al., 1993; BOTTALICO, 1998; PERKOWSKI & KIECANA, 1998; CHEŁKOWSKI et al., 2001).

Besides, as a result of infection by *Fusarium* spp., accumulation of mycotoxins, which are harmful to human and animals, takes place in the infected kernels (VESONDER & GOLINSKI, 1989; PERKOWSKI et al., 1997; GOLIŃSKI et al., 1999).

Limited information on *F. crookwellense* pathogenicity to oat encouraged us to undertake studies concerning the effect of *F. crookwellense* on reduction of the yield, kernel weight and mycotoxin accumulation in kernels after panicles inoculation with this fungus in 14 oat genotypes.

Material and methods

Field experiment

The experiments were carried out in Zamość region (South-Eastern Poland). Ten oat cultivars most common in Poland (Boryna, Borys, Dukat, Farys, German, Halny, Komes, Kwant, Santor, Sławko) and four lines (CHD 1171, CHD 1296, STH 2594, STH 2795) were inoculated with *F. crookwellense* No. 47. This isolate used for inoculation was obtained from a culture collection in the Department of Phytopathology, Agricultural University of Lublin, Poland, and was isolated from oat kernels. *F. crookwellense* No. 47 isolate was chosen for the experiment on the basis of strain pathogenicity determined by the method of MISHRA & BEHR (1976). The strain reduced kernel germination ability of Ducat cv. by 9%.

The inoculum was prepared according to the modified method described by MESTERHAZY (1978). The growing medium (1 L) composed from water extract from 0.5 kg oat leaves and selective medium – SNA, was autoclaved for 1 h at 121 °C and 1.21 atm (0.12 MPa) and then inoculated with culture of a two-week old *F. crookwellense* isolate No. 47. Then it was incubated for two weeks at 18–20 °C in a 12 h period of natural light (KIECANA, 1988). After incubation, the inoculum was stirred for 10 min and filtered through a cheesecloth; and the supernatant of the conidial suspension (5×10^5 spores per mL) was used for the inoculation.

All oat cultivars and lines were studied in one location of Zamość region over three years (1996, 1997 and 1998). Each year the experiments were carried out in a randomized complete block design with four replications. Eighty panicles of oat (20 panicles per replicate) were inoculated with *F. crookwellense* No. 47 four days after the anthesis of a minimum of 50% of plants (21 June – 8 July 1996; 25–30 June 1997; and 25–28 June 1998). The inoculum (2 mL per panicle) was applied with a laboratory sprayer. Control plants were sprayed with 2 mL of distilled water. After inoculation or water spraying (control plants), the panicles were

protected with plastic bags for 24 h to avoid water evaporation and the spread of the inoculum.

Weather conditions somewhat differed from the multiannual averages but not significantly. Mature panicles were collected during August (11 August 1996; 14–16 August 1997; and 7 August 1998) and threshed manually. The yield (Y), number of kernels per panicle (NK) and the 1000 kernel weight (TKW) were measured and compared with controls.

Symptoms of the disease (scabby kernels) were evaluated according to KIECANA et al. (2002).

Chemical analysis of the mycotoxins

All kernel samples of 20 g each were ground in the same way in a special laboratory mill WZ-1 (for grinding grain samples) produced at the Research Institute for Baking Industry Ltd., Bydgoszcz, Poland. Next, each sample was placed in a 200 mL Erlenmeyer flask with 100 mL of acetonitrile-water 82:18, vol/vol. After being shaken vigorously for 15 min, the mixture was left for 12 hr, and then shaken again for 15 min. The samples were filtered under vacuum with a Büchner funnel through Whatman No. 5 filter paper. The extracts were then purified on a 5-mL column of mixed alumina (neutral activated, 70–230 mesh, Merck), activated carbon Darco G 60 100 mesh (Aldrich), and Celite 545 (Serva), (5:9:4 wt/wt/wt). Charcoal columns were prepared as follows: a small woolen ball was placed into the filtration column (6 mL-polypropylene filtration tube, Supelco), and acid-washed Celite (0.25 g) plus a mixture containing: 0.84 g of alumina + 1.50 g of charcoal Darco G 60 + 0.67 g of Celite was added. Prepared columns were washed with 15 mL of acetonitrile-water (82:18) mixture. After the vacuum has been disconnected, a clean filter flask was inserted and collected extract was put on the column. After 5 min, vacuum was again applied, providing a flow rate through the column of 1 mL/min. The extracts volume was filtered to reach the glass wool in the bottom of column, and 30 mL of acetonitrile-water (82:18) was added. The extracts were evaporated to dryness using a rotary evaporator. The residue was dissolved and transferred to a vial using two aliquots of 2 mL ethyl acetate and 2 mL of chloroform-acetonitrile 4:1 [vol/vol].

The trichothecenes group B (DON, NIV, FUS-X, 3-AcDON, 15-AcDON) were analysed as TMS (trimethylsilyl silyl ethers) derivatives. 100 µL of TMSI/TMCS (trimethylsilyl imidazole/trimethylchlorosilane v/v 100/1) mixture was added to the dried extract. After 10 min, 500 µL of isooctane was added, and the reaction was quenched with 1 mL of water. Isooctane layer was used for the analysis and 1 or 2 µL of sample was injected on a gas chromatograph-mass spectrometer (GC/MS) (Hewlett Packard GC 6890, MS 5972 A, Waldbronn, Germany) using a HP-5MS (0.25 mm × 30 m) capillary column. The injection port temperature was 280 °C and the transfer line temperature was 280 °C; the analysis was performed with a programmed temperature (from 80 °C to 280 °C at 25 °C/min) where the final temperature was being held for 10 min. Helium flow rate was held constant at 0.7 mL/min. Each sample was run twice: in full scan mode

Table 1. Average number of kernels per panicle, 1000 kernel weight and yield of 14 genotypes inoculated with *F. crookwellense* No 47.

Cultivars and lines	Kernel number		1000 kernel weight (g)		Yield (g)	
	control	F. cr.	control	F. cr.	control	F. cr.
Boryna	65.51	53.14	26.73	20.74*	17.21	11.03*
Borys	64.65	55.24	25.16	19.83*	16.27	11.46*
Dukat	54.82	47.39	28.87	23.47	15.18	11.46*
Farys	59.49	40.90*	28.62	19.90*	16.10	8.53*
German	50.52	49.09	35.29	22.24*	17.15	11.63*
Halny	58.25	42.98*	31.50	25.80	16.70	11.40*
Komes	64.18	43.71*	23.96	19.30	15.18	8.54*
Kwant	60.78	50.53	28.55	20.42*	15.43	10.53*
Santor	55.60	49.35	32.18	20.53*	16.21	10.28*
S3awko	58.79	52.97	28.61	26.49	16.43	14.07
CHD 1171	74.49	61.61*	25.97	22.29	19.29	14.40*
CHD 1236	73.19	53.09*	23.21	18.81	16.94	11.05*
STH 2594	54.76	41.57	29.12	22.88	14.86	9.95*
STH 2795	71.36	32.37*	27.69	18.80*	14.94	8.62*

* Means differ significantly ($P < 0.05$).

(100–600 amu) for identification in per foses and in SIM mode for quantification. In order to confirm the identities of the trichothecenes in samples, full-scan analysis in the range of 100 to 600 amu was performed for all samples with comparison of the sample spectra to the DON, 3-AcDON, 15-AcDON, NIV standard (Sigma) spectrum.

The following ions were used for trichothecenes detection: deoxynivalenol- m/z 235, 422, fusarenone X-103, 570, 3-acetyldeoxynivalenol-117, 482; 15-acetyldeoxynivalenol-193, 482; nivalenol-585, 289. The first ion in each set was used for quantification.

Recovery test for toxins ($n = 8$) was carried out according to the above method giving the following yields $84\% \pm 3.8$ for DON, $81\% \pm 4.4$ for NIV, $86\% \pm 5.4$ for FUS-X, $74\% \pm 2.2$ for 15-AcDON, and $78\% \pm 4.8$ for 3-AcDON. The limit of detection for analysed trichothecenes group B was $10 \mu\text{g}/\text{kg}$.

Statistical analysis

The number of kernels per panicle (KN), the 1000 kernel weight (TKW) and yield (Y, per 10 panicles) were calculated for each genotype using statistical analyses – multiple confidence interval T-Tukey (OKTABА, 1972).

Results

Kernels of inoculated oat panicles exhibited typical symptoms of scab; they were smaller, shriveled and discoloured.

During three years of the experiment significant differences between all tested oat genotypes were observed. Experimental groups, when compared with the control, exhibited a significant diversity in TKW and the yield reduction during the entire period of investigation.

On the basis of grand mean value, significant differences in the yield were found in all the oat genotypes examined with an exception of Slawko cv. (Tab. 1). Significant differences in TKW were observed in cultivars: Boryna, Borys, Farys, German, Kwant and Santor as well as in STH 2795 (Tab. 1). In comparison with control plants significant differences in the number of kernels per panicle were found only in Farys, Halny and Komes as well as in lines CHD 1171, CHD 1296 and STH 2795 (Tab. 1).

Reduction in the size of yield and of 1000 kernel weight after panicle inoculation with *F. crookwellense* was 32% and 23%, respectively (Figs. 1,2). The decrease in the number of kernels per panicle remained at the level of 24% (Fig. 3). The greatest loss in the yield was for Farys cv. (46.5%) and line STH 2795 (48.7%) (Fig. 1), while the loss of 1000 kernel weight for those genotypes was 29.1% and 29%, respectively; higher reduction of TKW was observed in the cultivars German (37%) and Santor (33%) (Fig. 2). The reduction in the number of kernels per panicle for the entire period of studies ranged from 16.4% in Kwant cv. to 48.5% for line STH 2795 (Fig. 3).

The chemical analysis of kernels inoculated with *F. crookwellense* revealed the presence of nivalenol (NIV) only. The mean concentration of this metabolite for three years of studies ranged from 0.1 mg/kg (cv. Borys and Farys) up to 0.37 mg/kg (line STH 2594), with an average value 0.15 mg/kg (Tab. 2).

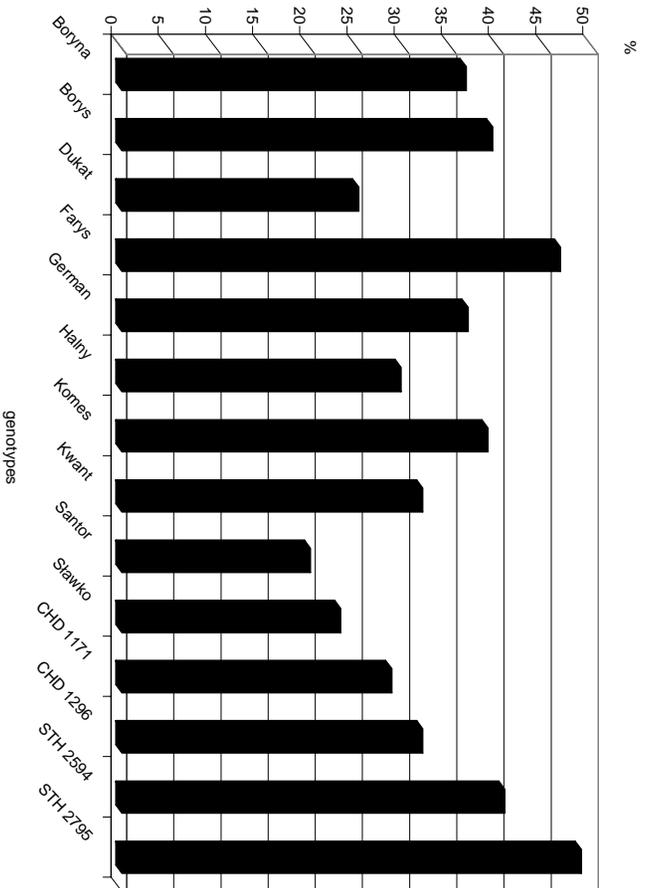


Fig. 1. Mean reduction (%) of yield compared to the control after oat panicles inoculation with *F. crookwellense*.

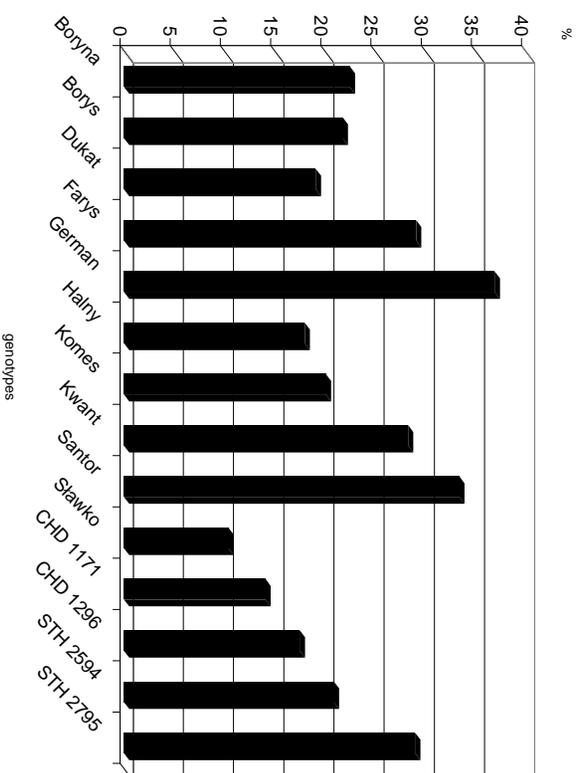


Fig. 2. Mean reduction (%) of TKW compared to the control after oat panicles inoculation with *F. crookwellense*.

Discussion

The inoculation (described in the Material and methods section) was successful in our experiments and the panicles of experimental groups exhibited scab symptoms typical for natural infection with a higher percentage of diseased spikelets.

Branes in contact place with spikelet axels along the nervure exhibited etiological symptoms of salmon pink or orange sporodochia producing conidia of *F. crookwellense*. Sporodochia were also visible on internal and external glumes of all kernels in infected spikelet. The infected kernels were shriveled and discoloured. Kernels were often out-

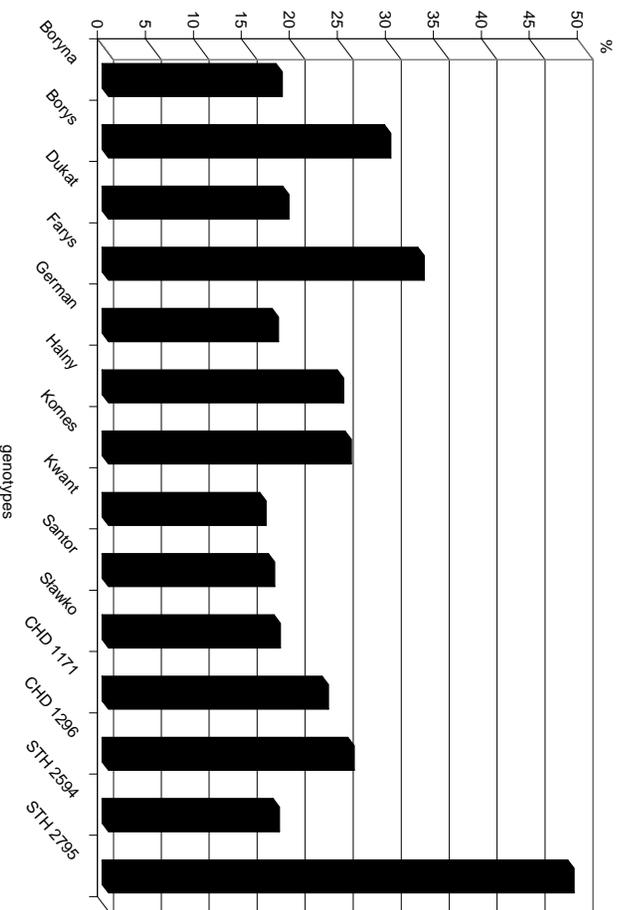


Fig. 3. Mean reduction of number of kernels per panicle compared to the control after oat inoculation with *F. crookwellense*.

Table 2. Mycotoxin content (mg/kg) in oat kernels inoculated with *F. crookwellense*.

Genotypes	NIV concentrations (mg/kg)
Boryna	0.05
Borys	0.01
Dukat	0.06
Farys	0.01
German	0.06
Halny	0.26
Korzes	0.30
Kwant	0.08
Santor	0.28
Slawko	0.35
CHD 117	0.03
CHD 1236	0.10
STH 2594	0.37
STH 2795	0.08
Mean	0.15

grown with mycelium, they broke up easily or only chaffs without kernels were formed.

Different methods of inoculation have been described in literature (TAKEDA & HETA, 1989). In our experiment, according to the methods described by MESTERHAZY (1978), STACK & MC-

MULLEN (1985) or KIECANA (1994), panicles were sprayed with a water suspension of *F. crookwellense* macroconidia and this procedure, in our opinion, resembles the natural infection.

The presence of *F. crookwellense* on wheat spikelets, oat panicles and maize ears in Poland (PERKOWSKI & CHEKOWSKI, 1993; PERKOWSKI et al., 1997; MIELNICZUK, 2001) as well as its significant importance in causing diseases of generative organs of cereals and maize in different countries (SUGIURA et al., 1993; CLEAR et al., 1996; BOTTALICO, 1998; LAUREN & MENNA, 1999; CROMERY et al., 2001) caused that this problem has been undertaken in the present studies.

The three-year-long studies described in the present paper were conducted on the same experimental plot with the use of identical agricultural treatments. No statistically significant differences within the analysed years were observed. Hence, in our paper only the mean results concerning the whole three years of studies were presented.

On the basis of the results obtained in our experiment we can conclude that the inoculation of panicles at full anthesis reduced the yield (on average by 32%) and the infected kernels were small, shriveled and discoloured. The obtained results of fusarium panicle blight in case of oat were comparable with earlier observations typical for scab of wheat (MESTERHAZY, 1978), barley (KIECANA,

1994), triticale (KIECANA, 1988), oat (KIECANA et al., 2002) and rye (PARRY et al., 1995).

It follows from the studies that *F. crookwellense* had a similar effect on the reduction of the oat yield to *F. avenaceum* (KIECANA et al., 2002). Besides, *F. crookwellense* also showed similar pathogenicity towards oat seedlings to *F. culmorum* (KIECANA & KOCYLAK, 1999). This fungus was also considered as a dangerous pathogen of spring barley heads. In South-Eastern part of Poland *F. crookwellense* reduced the TKW for this cereal from 34.7% to 68.6% after heads inoculation (KIECANA & PERKOWSKI, 1998).

The harmfulness of *F. crookwellense* towards cereal heads or panicles is increased by the accumulation of mycotoxins dangerous to human health and farm animals in the infected kernels.

In the laboratory conditions, toxigenic isolates of *F. crookwellense* produce a number of harmful fungus metabolites. NIV, FUS-X and ZEA and its derivatives belong to those that are most frequently produced (BOTTALICO, 1998). Fusarin-C (GOLIŃSKI et al., 1988) and 4-acetylnivalenol (SUGIURA et al., 1993) are also formed in the process of biosynthesis. In field conditions, besides of NIV, FUS-X and ZEA, also DON and its derivatives were found in the inoculated kernels (PERKOWSKI & KIECANA, 1998). That was probably caused by secondary infection by fungi *F. graminearum* or *F. culmorum*.

The profiles of the toxins produced by *F. crookwellense* are similar to those produced by *F. graminearum*. BOTTALICO (1998) reported that within *F. graminearum* two populations designated as Group 1 and Group 2 were characterized, with almost the same toxigenic potential. The Group 1 very rarely forms perithecia in nature and mainly causes crown rot of cereals and grasses, whereas the Group 2 readily forms abundant perithecia in nature and mainly causes head blight of cereals kernels, stalk and ear rot of maize. Studies on genetic diversity indicated that *F. graminearum* Group 2 have greater affinity to *F. culmorum* and *F. crookwellense* than to *F. graminearum* Group 1. In addition, the toxigenic strains of *F. graminearum* were classified in two chemotypes: DON and NIV producers, according to the main type B trichothecenes synthesized. Furthermore, DON-chemotype strains of *F. graminearum* were subclassified into two chemotypes: 3-AcDON and 15-AcDON producers. Ecological differences in chemotype distribution may contribute to characterizing a regional kernels contamination. Toxigenic strains of *F. culmorum* can be divided into two types: DON and NIV chemotypes, according

to the trichothecenes type B produced. DON-type strains produced also AcDON.

In the course of the present studies, only NIV was detected in the analysed oat kernels among the analysed toxins. Its mean concentration was 0.15 mg/kg. That concentration was higher than that for the samples of naturally infected oat kernels in Poland, which was 0.063 mg/kg in the years 1997–1999 (BASIŃSKI & PERKOWSKI, 2002). However, it was much lower than for barley kernels after heads inoculation with *F. crookwellense* (PERKOWSKI & KIECANA, 1998) or wheat cultivated in Japan (Sugiura et al., 1993). It is certainly associated with the data presented by GAREIS et al. (1989), PETERSSON (1996) and SMITH et al. (1994), who reported that the concentration of fusariosic toxins in oat in relation to other cereals is usually lower. Strains of *Fusarium* spp. differ in their ability to toxins production (PERKOWSKI et al., 1996; CHEŁKOWSKI et al., 1999; PERKOWSKI, 1999). Low level of NIV in oat kernels showed that isolate of *F. crookwellense* No. 47 used for inoculation was weakly toxigenic. The present studies, however, point out that although relatively small quantities of NIV were detected in the oat kernels infected by the fungus *F. crookwellense*, its strongly toxic properties make some danger since the kernels of this cereal are used in dietetic nutrition.

General conclusions resulting from present studies can be summarized as follows:

(1) Inoculation of oat panicles with *F. crookwellense* had a similar effect on the decrease of the elements of the yield structure like in other experiments concerning the harmfulness of the discussed species towards cereals.

(2) The concentration of the accumulated toxins in infected kernels was smaller in relation to other similar studies conducted with the use of fungi from the *Fusarium* genus.

(3) Comparing the susceptibility of the examined cultivars and lines with regard to field studies describing the yield structure and the production of fusariosic toxins in the kernels, it was found out that the cultivars Farys and German as well as line STH 2795 were the most sensitive to panicle infection by *F. crookwellense*, whereas Sławko cv. was the least sensitive.

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