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THE PATHOGENICITY AND TOXIGENIC PROPERTIES OF *Fusarium crookwellense* L.W. BURGESS, P.E. NELSON & TOUSSOUN DEPENDING ON WEATHER CONDITIONS

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ABSTRACT

Field inoculation experiments were performed during 2015–2017, in south-eastern part of Poland. The pathogenicity of *F. crookwellense* was estimated based on the kernels yield reduction of 10 selected oat genotypes. Panicles of oat were inoculated with conidial suspension of *F. crookwellense* strain No. 72 which caused a reduction in kernels yield by 28.54% and kernels number per panicle by 28.07%, compared to the control. The lowest yield reduction, as a result of the panicle inoculation with *F. crookwellense*, was found in the case of the cultivar 'Pablo' (18.73%), while the highest in the case of breeding line POB 961-1344/13 (36.4%) and cv. 'Kozak' (34.3%). Statistically the highest average yield reduction was observed in year 2016, when a higher amount of rainfall, especially in July (just after inoculation) and higher temperature, compared to the long-term standard, were observed. During the period between inoculation and harvest, *F. crookwellense* was able to produce nivalenol, fusarenone X and zearalenone in oat kernels at the average level of 0.065 mg·kg^{-1}, 0.026 mg·kg^{-1} and 0.015 mg·kg^{-1} respectively.

Key words: oat, cultivars, susceptibility, harmfulness, *Fusarium* toxins, yield reduction

INTRODUCTION

Fusarium is one of the most economically important genera of phytopathogenic fungi under diverse geographical conditions. The harmfulness of particular *Fusarium* species to plants depends on environmental factors, such as climate and soil, as well as on the susceptibility of cultivated genotypes and agrotechnical factors [Linkmeyer et al. 2013, Hofgaard et al. 2016].

These fungi can cause several diseases of plants in all growth stages, however *Fusarium* head blight (scab) of cereals is a major disease caused by a range of *Fusarium* species [Bottalico and Perrone 2002, Tamburic-Ilincic 2010, Hjelkrem et al. 2017]. *Fusarium graminearum* Schwabe, *F. avenaceum* (Fr.) Sacc., *F. culmorum* (Wm.G. Sm.) Sacc. and *F. poae* (Peck) Wollenw. were reported as the most common pathogens in plants from the *Poaceae* family, including oat. In addition *F. crookwellense* L.W. Burgess, P.E. Nelson & Toussoun, *F. sporotrichioides* Sherb., *F. equiseti* (Corda) Sacc. and *F. langsethiae* Torp & Nirenberg were often found [Bottalico and Perrone 2002, Tamburic-Ilincic 2010, Nielsen et al. 2011, Hofgaard et al. 2016, Mielniczuk 2018]. *Fusarium crookwellense* similarly as *F. avenaceum*, *F. culmorum* and *F. poae* is a common pathogen of cereals in the cooler temperate

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regions, while *F. graminearum* is a dominant species in the hotter regions [Bottalico and Perrone 2002] 7 of this cereal is very important.

Limited published information on *F. crookwellense* pathogenicity to cereals, especially to oat, and introducing new cultivars to agricultural practice, encouraged us to undertake studies on the effect of this species on yield reduction the and mycotoxins contamination in kernels of 10 oat genotypes after panicle inoculation with this species in different weather conditions.

MATERIALS AND METHODS

Field experiment

Ten oat genotypes, presented in Figure 1, were inoculated with *F. crookwellense* No. 72 under field conditions in Zamość, south-eastern part of Poland (50°45'03"N 23°14'33"E, WBR: Dystric Cambisols). The *F. crookwellense* strain No. 72 was chosen for the experiment on the basis of its pathogenicity and the inoculum was prepared according to method described by Kiecana et al. [2002]. The infectious material consisted of a conidial suspension with a density of 5×10^5 spores/ml. The studies were conducted over three years (2015–2017), using a randomized complete block design with four replications. Every year, 80 panicles of each oat genotype in the flowering stage (65 BBCH) – 20 panicles per replicate – were inoculated with *F. crookwellense*. The inoculum was applied with a sprayer. The same genotypes, sprayed with distilled water were used as a control group. Symptoms of the disease were evaluated according to Kiecana et al. [2002]. After harvest (1.08.2015, 3.08.2016, 7.08.2017) the number of kernels per panicle (NK) and the kernels yield (KY) (g) of the experimental groups were measured and compared to the control as in the studies described by Kiecana et al. [2002]. Weather conditions – temperature and rainfalls in analyzed years of studies differed from the long-term standard and are presented in Table 1.

Chemical analysis

Analysis of trichothecenes from group B. Kernels samples were analyzed for the presence of trichothecenes from group B according to Perkowski et al. [2003].

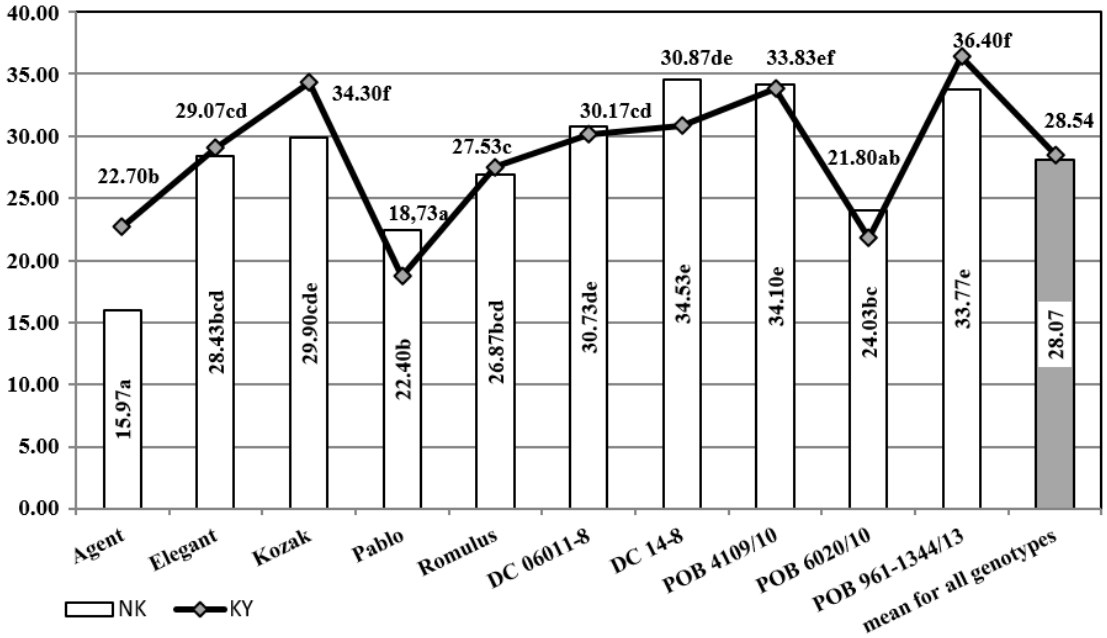

Fig. 1. Reduction (%) of kernels number (NK) and kernels yield (KY) after panicles inoculation with *F. crookwellense*, average after 3 years (2015–2017)

Values differ significantly ($P \leq 0.05$) if they are not marked with the same letter, $LSD_{0.05}$ NK – 5.020, KY – 3.282

Sub-samples (10 g) were extracted with acetonitrile/water (82 : 18) and cleaned up on a charcoal column [(Celite 545/charcoal Draco G/60/activated alumina neutral 4 : 3 : 4 (w/w/w)]. Nivalenol and fusarenone X were analyzed as TMS (trimethylsilyl ether) derivatives. To the dried extract the volume of 100 µl TMSI/TMCS (trimethylsilyl imidazole/trimethylchlorosilane, v/v 100/1) mixture was added. After 10 min 500 µl of isooctane were added and the reaction was quenched with 1 ml of water. The isooctane layer was used for the analysis and 1 µl of the sample was injected on a GC/MS system. The analyses were run on a gas chromatograph (Hewlett Packard GC 6890) hyphenated to a mass spectrometer (Hewlett Packard 5972 A, Waldbronn, Germany), using an HP-5MS 0.25 mm × 30 m capillary column. The injection port temperature was 280°C, the transfer line temperature was 280°C and the analyses were performed with programmed temperatures, the initial temperature was 80°C, held for 1 min, from 80°C to 200°C at 15°C/min, held for 6 min, and from 200°C to 280°C at 10°C/min, the final temperature being maintained for 3 min. The helium flow rate was held constant at 0.7 ml/min. Quantitative analysis was performed in the single ion monitoring (SIM) mode using the following ions for the detection of NIV 191 and 600, FUS X 103 and 570. Qualitative analysis was performed in the SCAN mode (100–700 amu). Recovery rates for the analyzed toxins were as follows: NIV 81 ±3.8% and FUS X 74 ±2.2%. The limit of detection was 0.001 mg·kg^{-1}.

Analysis of zearalenone. Each sample was extracted with a method similar to that described by Tanaka et al. [1985]. A portion of Florisil column eluate was subjected to analysis of ZEA by high-performance liquid chromatography with fluorescence detection. The chromatographic separation was performed using a silica gel column (LiChrosorb Si 60). The mobile phase was water saturated dichloromethane and 1-propanol (98.5 : 1.5 v/v). The column temperature and solvent flow rate set at 25°C and 2.0 min^{-1} respectively. The fluorescence programmable detector (HP 1046 A) was set at an emission wavelength of 450 nm, and the excitation wavelength was 236 nm. On the LiChrosorb SI 60 column, retention time for ZEA was 4.9 min. Average recovery of the ZEA was 96 ±2.8%. The detection limit was 0.001 mg·kg^{-1}.

Statistical analysis

Data were analyzed for significance by the analysis of variance using statistical program ARStat (developed in the Faculty of Applied Mathematics and Information Technology of the University of Life Sciences, Lublin). The means were compared to the use of the least significant differences based on the Tukey's test ($P \leq 0.05$).

RESULTS

Inoculation of oat panicles with *F. crookwellense* strain No. 72 proved to be effective, since the panicles exhibited typical symptoms of scab. On chaffs the etiological symptoms in the form of pink or orange sporodochia with macroconidia occurred. The infected kernels were shriveled, discoloured and often outgrown with mycelium, broke up easily or only chaffs

Table 1. Weather conditions in Zamość, in the seasons of oat vegetation on 2015–2017

Month	Mean for the years 1971–2005 (long-term standard)		Difference of air temperatures as compared to long-term standard (°C)			Percentage of rainfalls as compared to long-term standard		
	Air temperature (°C)	Rainfalls (mm)	2015	2016	2017	2015	2016	2017
April	7.9	44.1	–2.4	+2.3	+1.1	40.0	40.8	36.0
May	14.1	65.5	+1.1	0.0	–2.1	114.9	43.2	66.0
June	16.8	78.9	+1.8	+2.0	+0.7	54.9	83.1	46.9
July	18.4	98.4	+2.4	+1.2	+0.1	65.0	114.2	89.1
August	17.8	54.3	+3.7	+1.6	+4.7	4.9	55.2	34.5

Table 2. The influence of oat panicles inoculation with *F. crookwellense* on kernels number per panicle and yield in 2015–2017

Cultivars and lines of oat	Kernels number		Yield (g)	
	control	F. cr.	control	F. cr.
Agent	71.11	58.83*	26.16	20.74*
Elegant	69.90	51.20*	26.49	19.52*
Kozak	79.58	56.29*	29.38	19.64*
Pablo	71.37	54.29*	25.48	20.64*
Romulus	74.75	55.55*	28.37	20.86*
DC 14-8	73.50	48.50*	24.58	17.24*
DC06011-8	91.30	66.75*	32.11	23.52*
POB 4109/10	67.64	44.97*	24.41	16.53*
POB 6020/10	84.92	64.34*	26.48	20.36*
POB 961-1344/13	73.55	50.14*	28.12	18.70*

F. cr. – *F. crookwellense*

* Mean values differ significantly compared to the control at $P \leq 0.05$

Table. 3. Content of mycotoxins in oat kernels after panicles inoculation with *F. crookwellense* during 2015–2017

Genotypes of oat	Average concentration of *F. crookwellense* toxins (min.–max.) in $mg \cdot kg^{-1}$		
	Nivalenol	Fusarenone X	Zearalenone
Agent	0.065ab (0.020–0.106)	0.018a (0.011–0.032)	0.012a (0.000–0.260)
Elegant	0.042ab (0.015–0.075)	0.017a (0.009–0.030)	0.010a (0.001–0.025)
Kozak	0.028ab (0.008–0.050)	0.028ab (0.011–0.040)	0.020a (0.002–0.029)
Pablo	0.014a (0.008–0.019)	0.026ab (0.009–0.039)	0.014a (0.001–0.017)
Romulus	0.108c (0.057–0.209)	0.019a (0.019–0.037)	0.015a (0.001–0.035)
DC 06011-8	0.187d (0.048–0.384)	0.043c (0.021–0.066)	0.019a (0.005–0.033)
DC 14-8	0.049ab (0.038–0.069)	0.029ab (0.012–0.042)	0.014a (0.005–0.027)
POB 4109/10	0.044ab (0.007–0.069)	0.022a (0.011–0.032)	0.011a (0.000–00.32)
POB 6020/10	0.043ab (0.035–0.053)	0.038bc (0.012–0.070)	0.020a (0.003–0.031)
POB 961-1344/13	0.069bc (0.044–0.117)	0.020a (0.011–0.031)	0.011a (0.003–0.025)
Mean for all genotypes	0.065	0.026	0.015
$LSD_{0.05}$	0.052	0.012	0.037

Values in columns differ significantly ($P \leq 0.05$) if they are not marked with the same letter

without kernels were formed. Differences in the kernels number per panicle and in the yield, compared to the control, were found in all oat genotypes (Tab. 2). The mean reduction of kernels number and yield, as a result of oat panicles inoculation with *F. crookwellense*, was 28.07% and 28.54%, respectively (Fig. 1). Compared to the control, the lowest reduction of the yield was observed in the cultivar 'Pablo' (18.73%), and the decrease of kernels number in a panicle for this cultivar was the 22.40%. In the case of both the breeding line POB 961-1344/13 and the cultivar 'Kozak' the greatest losses of kernels yield were observed (36.40% and 34.30% respectively), while the reduction of kernels number in a panicle for those genotypes was 33.77% and 29.90%, respectively (Fig. 1).

The chemical analysis of oat kernels infected with *F. crookwellense* revealed the presence of nivalenol (NIV), fusarenone X (FUS X) and zearalenone (ZEA). The mean content of NIV in the oat kernels, after three years of studies, ranged from 0.014 mg·kg^{-1} (cv. 'Pablo') to 0.187 mg·kg^{-1} (line DC 06011-8), the average concentration of NIV established for all oat genotypes was 0.065 mg·kg^{-1} (Tab. 3). On the other hand, the mean concentration of fusarenone X and zearalenone, after three years of research was on the level of 0.026 mg·kg^{-1} and 0.015 mg·kg^{-1} respectively and differed in individual years (Tabs 3, 4). The concentration of *Fusarium* toxins in inoculated kernels depended on the genotype too. The highest content of FUS X was detected in line DC 06011 (0.043 mg·kg^{-1}), while ZEA in kernels of POB 6020/10 and cv. 'Kozak' (0.020 mg·kg^{-1}). The lowest concentrations of FUS X and ZEA were found in cv. 'Elegant' (0.017 and 0.010 mg·kg^{-1} respectively) – Table 3.

For all genotypes – the highest average reduction of yield was observed in year 2016 (Tab. 4), when a higher amount of rainfall, especially in July (just after inoculation) and higher temperature compared to the long-term standard were observed (Tab. 1). In 2016, fusarenone X and zearalenone concentration in kernels of oat was also higher than in 2015 and 2017 (Tab. 4).

DISCUSSION

Fusarium crookwellense was considered one of the main causes of *Fusarium* head blight of wheat cultivated in Japan, Canada and Slovakia [Sugiura et al. 1993, Šrobarová et al. 2008, Amarasinghae et al. 2015]. This species was also isolated from barley kernels in China and Argentina [Zhang et al. 2011, Castañares et al. 2013]. The obtained results confirm the pathogenicity of *F. crookwellense* to cereals. To investigation different aspects of *Fusarium* head blight, the most effective inoculation methods was selected [Mielniczuk et al. 2004, Tamburic-Ilincic 2010, Tekle et al. 2012]. In the present study, oat panicles were sprayed with a suspension of. *F. crookwellense* macroconidia, a procedure that resemble the natural infection. The method of inoculation used in our experiments was successful, and the panicles of the tested oat genotypes infected with *F. crookwellense* exhibited typical scab symptoms

Table 4. Average reduction of kernels number, kernels yield and content of mycotoxins in oat kernels for all cultivars after panicles inoculation with *F. crookwellense* in each year 2015, 2016, 2017

Year of studies	Kernels number reduction (%)	Kernels yield reduction (%)	Nivalenol (mg·kg^{-1})	Fusarenone X (mg·kg^{-1})	Zearalenone (mg·kg^{-1})
2015	20.61b	21.38b	0.077b	0.012a	0.003a
2016	50.14c	49.82c	0.046a	0.034b	0.028b
2017	13.47a	14.42a	0.072b	0.033b	0.013a
Mean for all years	28.07	28.54	0.065	0.026	0.015
$LSD_{0.05}$	3.420	4.821	0.029	0.014	0.011

Values in columns differ significantly ($P \leq 0.05$) if they are not marked with the same letter

with a higher percentage of diseased spikelets, besides a significant reduction of kernels number in the panicle was noted, similarly to observed in the case of oat infection by other *Fusarium* species [Kiecana et al. 2002, 2012, Tamburic-Ilincic 2010, Tekle et al. 2012, Mielniczuk et al. 2015]. The obtained results and *Fusarium* panicle blight symptoms in oat were also comparable with previous observations typical for scab of wheat [Goliński et al. 2010], barley [Perkowski and Kiecana 1998] and rye [Kiecana et al. 2010].

The inoculation of oat panicles with *F. crookwellense* No. 72 at full anthesis, reduced the yield on an average of 28.54%. The tested strain, proved to be slightly less harmfulness to oat panicles than the *F. crookwellense* strain No. 47 and *F. avenaceum* No. 122, in similar growing conditions [Kiecana et al. 2002, Mielniczuk et al. 2004], while the inoculation of selected oat cultivars by *F. culmorum* No. KF 350 was the cause of a smaller reduction in yield as compared with the tested strain of *F. crookwellense* [Perkowski and Kiecana 1997]. A greater reduction in yield was already noted in the case of spring barley heads inoculation with *F. crookwellense* [Perkowski and Kiecana 1998].

The studies carried out in Canada also showed significant virulence of six isolates of *F. crookwellense* in relation to heads of wheat genotypes [Xue et al. 2004].

The presented results confirm a significant differences of individual oat genotypes in susceptibility to infection with *Fusarium* spp., as in other cereals [Mielniczuk et al. 2004, Linkmeyer et al. 2013, Martinelli et al. 2014].

The chemical analysis indicate that kernels from oat panicles inoculated with *F. crookwellense* were contaminated with nivalenol at lower concentration level, if compared with kernels of wheat cultivated in Japan, spring barley in Poland and oat in UK [Sugiura et al. 1993, Perkowski and Kiecana 1998, Edwards 2009, Hofgaard et al. 2016]. On the other hand, a concentration of NIV in analyzed samples was higher, than in samples of wheat kernels inoculated with *F. crookwellense* No. O 45 as previously described [Christ et al. 2011].

That amount of FUS X and ZEA in kernels of analyzed genotypes was higher than detected in the samples of naturally infected oat kernels in Poland, when it was 0.002 and 0.01 $mg \cdot kg^{-1}$ respectively, in the 2014 growing season [Bryła et al. 2016], but lower than in oat in Norway during 2004–2009 (FUS X – 0.08, ZEA 0.05 $mg \cdot kg^{-1}$) [Hofgaard et al. 2016]. In our experiment, the level of ZEA contamination in oat kernels was also much lower, compared with barley and wheat kernels after heads inoculation with *F. crookwellense* and *F. graminearum* [Perkowski and Kiecana 1998, Christ et al. 2011]. Particularly high concentration of ZEA was found in wheat kernels after heads inoculation with *F. graminearum* under the same climatic conditions as in the case of the presented studies [Goliński et al. 2010].

Our results confirms that humid weather, during flowering and just before harvest, significantly affects the pathogenicity of *Fusarium* spp., development of *Fusarium* head blight and the content of some mycotoxins in kernels [Xu et al. 2008, Bernhoft et al. 2012, Mielniczuk 2018].

In conclusion, the presented results showed that *F. crookwellense* is pathogenic to panicles of oat, and that fungus infection determined a significant reduction of the kernels yield.

Oat cultivars used in the study were high variable in susceptibility to panicles infection with *F. crookwellense* – the cultivar 'Pablo' was characterized by the lowest yield reduction and low content of mycotoxins and was recognized as the most resistant to the scab. Thus the cultivar may be recommended in plant breeding and cultivation in the conditions conductive to infection with *Fusarium* spp.

Inoculation of oat with *F. crookwellense* creates a risk of its contamination with nivalenol, fusarenone X and zearalenone, however, genotype and weather conditions, (especially in flowering stage) proved to have a significant impacts on the yield reduction and the concentration of *Fusarium* mycotoxins in oat kernels.

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