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COLONIZATION OF WHEAT GRAIN BY FUSARIA IN TWO CROP
MANAGEMENT SYSTEMS VARYING IN INTENSITY
OF PRODUCTION TECHNOLOGY

ABSTRACT

The purpose of this study was to investigate the effects of two cropping systems (intensive and integrated) on infection level of winter and spring wheat kernels by *Fusarium* species. Field experiments were conducted with winter wheat 'Tonacja' and spring wheat 'Bombona' during two consecutive growing seasons (2007/2008 and 2008/2009 - winter wheat, and 2008 and 2009 - spring wheat). A rainfall level in 2009 from the last decade of May to the first decade of July was 2.5-times higher than that in 2008. After the harvest, kernels were surface disinfected with 1.5% NaOCl solution for 2 min and then analysed for the infection level by different species of *Fusarium*. Fusaria were isolated on CZID medium and identified on the basis of macro- and micro-morphology on three media (PDA, SNA and a medium containing tannin). Our results demonstrate that the wheat grain infection by *Fusarium* depended mainly on a rainfall level. The intensive cropping system was more conducive to the grain infection by fusaria in comparison to the integrated one. The most frequent species were *F. avenaceum* in 2008, and *F. graminearum*, *F. avenaceum* and *F. poae* in 2009.

Key words: wheat, grain infection level, *Fusarium* species, crop management system, rainfall level

INTRODUCTION

Fusarium head blight (FHB), caused by several fungal species (including *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae*) is a significant threat to wheat production world-wide. The distribution and predominance of the FHB pathogens is, to a large extent, determined by climatic factors, particularly tem-

perature and moisture (Doohan *et al.* 2003). Under weather conditions favourable for the growth of fusaria in the field, FHB causes heavy yield losses and reduce grain quality, especially due to the presence of mycotoxins (Champeil *et al.* 2004a, Doohan *et al.* 2003, Parry *et al.* 1995, Xu *et al.* 2005) which have adverse effects on animal and human health (Bennett and Klich 2003). Cereal grain infection by fusaria depends (besides the environmental factors and the level of resistance of the host variety) on agricultural practices *e.g.* land preparation, use of fertilizers and pesticides as well as crop rotation, so a crop production technology may significantly affect the plant health and yield quality (Champeil *et al.* 2004a, Edwards 2004 Jalli and Parikka 2007, Łukanowski and Sadowski 2006, Pirgozliw *et al.* 2003, Podolska 2007). Therefore, the purpose of this study was to investigate the effects of two crop management systems (intensive conventional and less intensive integrated one) on infection level of winter and spring wheat kernels by *Fusarium* species. The aim of the conventional agriculture system is to achieve the highest net income by maximizing yield by the use of big amounts of fertilizers and pesticides. The integrated system, in which both input levels of fertilizers and pesticides are generally lower, is have regard to maximize net income with preserving the environment from an excess of the chemicals (Krasowicz 2007, Kuś *et al.* 2007, Podolska 2008).

MATERIAL AND METHODS

Field experiment

Field experiments were conducted with winter wheat (*Triticum aestivum* L.) ‘Tonacja’ and spring wheat ‘Bombona’ during two consecutive growing seasons (2007/2008 and 2008/2009 or 2008 and 2009 in the case of winter wheat and spring wheat, respectively). Both wheat cultivars were grown in a four-field rotation experiment (winter rapeseed, winter wheat, spring wheat, spring cereals) located on a lessive soil (Luvisol) of good wheat complex at the Experimental Station in Osiny (51°27’ N, 22°2’ E, Lublin province) of the Institute of Soil Science and Plant Cultivation – State Research Institute in Puławy. The field experiment consisted of two crop management systems: intensive (conventional) and integrated. In the case of the intensive system, mineral NPK fertilizers, herbicides, fungicides, insecticides and retardants were applied according to the high input technology generally used by farmers in Poland, and in the integrated system, these substances were used after preliminary evaluation of nutrient content in soil and plants and in according to integrated pest management based on the threshold-action (Table 1). The systems also varied in the method of preceding crop residue management. In the case of the intensive system the straw after harvest was chopped and incorporated into soil with

the stubble by 22 cm plowing whereas in the integrative one the straw after harvest was removed from the field and only the stubble was plowed under. Both wheat cultivars were seeded in two seeding rates: Tonacja 300 and 500 kernels m^{-2} , and Bombona 450 and 600 kernels $\times m^{-2}$.

Grain sampling and microbial analyses

The grain samples were taken after harvest with a combine in 2008 and 2009. To determine the internal infection of the wheat grain by fusaria, the wheat kernels randomly selected were disinfected by energetic hand shaking with sodium hypochlorite solution (1.5% of active chlorine) for 2 min and rinsed five times for 20 s by energetic hand shaking with sterile water. For each sample, 105 surface-disinfected kernels (7 kernels per each of 15 Petri dishes) were placed on the surface of selective CZID agar medium (Samson *et al.* 1992). Fusaria from all growing colonies on CZID after incubation at 27 °C for 4-6 days were transferred to PDA agar medium (½ strength). *Fusarium* species were identified according to Kwaśna *et al.* (1991) and Leslie and Summerell (2006) on the basis of their morphological (macro- and micro-morphology) and cultural characteristics on three media - ½ PDA, SNA (Samson *et al.* 1992) and a medium containing tannin (Thrane 1986). No growth of *F. poae* and *F. langsethiae* was observed on the tannin medium.

Statistical analysis

For statistical evaluation of significant difference between the *Fusarium* infection of wheat grain originated from different crop management systems and growing seasons, confidence intervals of the proportions of wheat kernels in which fusaria were detected in groups of the total kernels tested were calculated according to the equation (Oktaba 1966):

where:

$$\frac{2Y + u_{\alpha}^2 - K}{2(n - u_{\alpha}^2)} < p < \frac{2Y + u_{\alpha}^2 + K}{2(n - u_{\alpha}^2)}$$

p is a confidence interval,

$$K = u_{\alpha} \times \sqrt{x} \quad \text{and}$$

$$x = u_{\alpha}^2 + 4Y \times (1 - Y/n),$$

Y is the number of infected wheat kernels,

n is the total number of wheat kernels tested,

u_{α} is the Student's t value obtained from tables for an infinite number of degrees of freedom (1.645 for 90% confidence intervals, 1.96 for 95% confidence intervals and 2.576 for 99% confidence intervals). In Tab. 3 are presented 95% confidence interval limits as percentages ($p \times 100$).

RESULTS

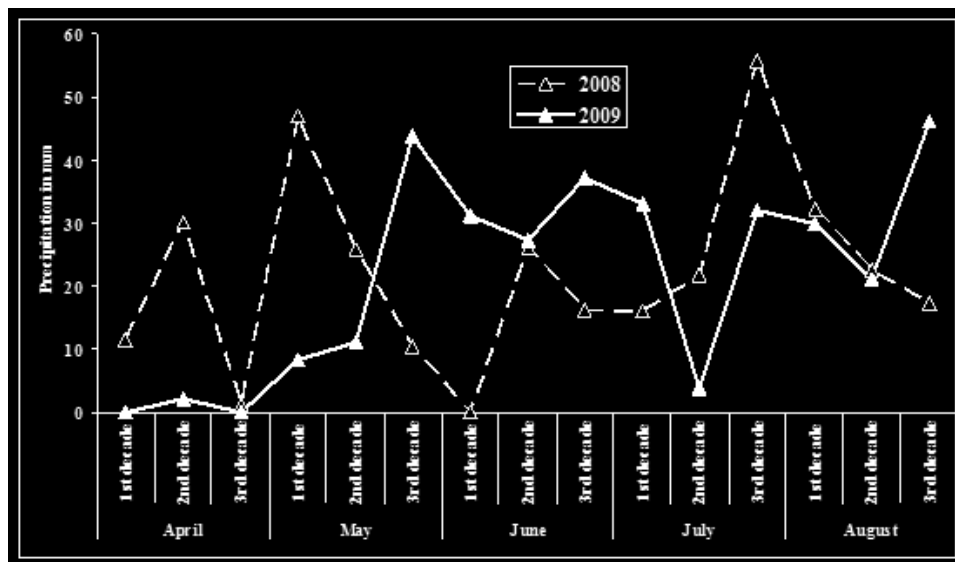


Fig. 1. Total decadal rainfall during the periods from April to August in 2008 and 2009 in Osiny

Weather in 2009 was very unfriendly for the wheat growth, especially of the spring wheat because of strong deficiency of rain in April and two first decades of May (the sum of precipitation in this period equals only 21.5 mm in comparison to 115.7 mm in 2008) and abundant rainfall during the period from third decade of May to first decade of July (172.6 mm in comparison to 68.9 mm in 2008) (Fig. 1). The decadal temperature during those periods ranged from 14.1 to 19.0°C (av. 17.4°C) in 2008 and from 12.8 to 20.2°C (av. 15.6°C) in 2009 (data not presented). In comparison to 2008, the grain yields in 2009 were lower of about 20-30% and 30-40% in the case of winter wheat and spring wheat, respectively (Table 2). Also the weights of 1000 kernels of the spring wheat were distinctly lower (of about 30-40%) than those in 2008 (Table 2).

The main differences between the crop management systems were in N, P and K fertilization as well as in the straw disposal. The plants grown in the integrated system obtained (in comparison to the intensive one) less N by about 20%, less P by about 20-25% and less K by about 15-40%, according to the year and the cultivar (Table 1).

Table 1
Usage of fertilizers, pesticides, plant growth regulators and straw obtained after harvest in the field experiments

Treatment	Winter wheat						Spring wheat		
	Integrated system		Intensive system		Integrated system		Intensive system		
	2008	2009	2008	2009	2008	2009	2008	2009	2010
N fertilizers (NH ₄ NO ₃)	152 kg/ha (in 4 doses)	140 kg/ha (in 4 doses)	180 kg/ha (in 4 doses)	180 kg/ha (in 4 doses)	120 kg/ha (in 3 doses)	120 kg/ha (in 3 doses)	150 kg/ha (in 3 doses)	150 kg/ha (in 3 doses)	150 kg/ha (in 3 doses)
P ₂ O ₅ fertiliz-	59 kg/ha	60 kg/ha	80 kg/ha	80 kg/ha	59 kg/ha	65 kg/ha	80 kg/ha	80 kg/ha	80 kg/ha
K ₂ O fertiliz-	80 kg/ha	78 kg/ha	100 kg/ha	100 kg/ha	60 kg/ha	85 kg/ha	100 kg/ha	100 kg/ha	100 kg/ha
Fungicides	Tilt Plus + Unix 75 WG (1.1+0.7 kg), Artea 330 EC (0.4 l)	Tilt Plus + Unix 75 WG (1.1+0.7 kg), Amistar + Artea 330 EC	Tilt Plus + Unix 75 WG (1.1+0.7 kg), Amistar + Artea 330 EC	Tilt Plus + Unix 75 WG (1.1+0.7 kg), Amistar + Artea 330 EC	Olímpus 250 EW + Artea 330 EC (1.8 l + 0.4 l)	Olímpus 250 EW + Artea 330 EC (1.8 l + 0.4 l)	Tilt Plus (1 l), Olímpus 250 EW + Artea 330 EC (1.8 l + 0.4 l)	Tilt Plus (1 l), Olímpus 250 EW + Artea 330 EC (1.8 l + 0.4 l)	Tilt Plus (1 l), Olímpus 250 EW + Artea 330 EC (1.8 l + 0.4 l)
Herbicides	Maraton 375 SC (4 l)	Maraton 375 SC (4 l)	Maraton 375 SC (4 l)	Maraton 375 SC (4 l)	Mustang 306 SC (0.6 l)	Mustang 306 SC (0.6 l)	Mustang 306 SC (0.6 l)	Mustang 306 SC (0.6 l)	Mustang 306 SC (0.6 l)
Insecticides	-	Decis 250 EC (0.3 l x 2)	-	Decis 250 EC (0.3 l x 2)	Decis 250 EC (0.3 l x 2)	Decis 250 EC (0.3 l x 2)	Decis 250 EC (0.3 l)	Decis 250 EC (0.3 l)	Decis 250 EC (0.3 l)
Retardants	CCC <i>Stefes</i> 720 SL (1 l)	Modus 250 EC (0.4 l)	CCC <i>Stefes</i> 720 SL (1 l)	Modus 250 EC (0.3 l x 2)	Modus 250 EC (0.3 l)	Modus 250 EC (0.3 l)	Modus 250 EC (0.3 l)	Modus 250 EC (0.3 l)	Modus 250 EC (0.3 l)
Forecrop straw	Removed from the field	Removed from the field	Chopped and buried by	Chopped and buried by	Removed from the field	Removed from the field	Removed from the field	Removed from the field	Chopped and buried by

* superphosphate

** KCl

Table 2

Grain yields and weights of 1000 kernels of winter and spring wheat

Crop management system	Winter wheat 'Tonacja'				Spring wheat 'Bombona'			
	2008		2009		2008		2009	
	Seeding rate [per m ²]							
	300	500	300	500	450	600	450	600
Ear numbers [per m ²]								
Integrated	660 (100%)	584 (100%)	448 (100%)	592 (100%)	329 (100%)	500 (100%)	608 (100%)	639 (100%)
Intensive	676 (102%)	672 (115%)	528 (118%)	656 (111%)	400 (122%)	408 (82%)	537 (88%)	603 (94%)
Grain yields [t × ha ⁻¹]								
Integrated	9.481 (100%)	8.491 (100%)	6.607 (100%)	6.980 (100%)	5.821 (100%)	5.716 (100%)	3.598 (100%)	3.353 (100%)
Intensive	10.074 (106.3%)	9.218 (108.6%)	6.995 (105.9%)	6.843 (98.0%)	5.749 (98.8%)	5.414 (94.7%)	4.375 (121.6%)	4.070 (121.4%)
1000 kernel weights [g]								
Integrated	48.6 (100%)	48.6 (100%)	43.7 (100%)	44.15 (100%)	43.05 (100%)	44.6 (100%)	29.75 (100%)	27.15 (100%)
Intensive	48.35 (99.5%)	44.65 (91.9%)	43.98 (100.6%)	44.05 (99.8%)	42.65 (99.1%)	42.15 (94.5%)	31.3 (105.2%)	29.3 (107.9%)

The grain of both winter and spring wheat cultivars grown in 2009 was much more infected by fusaria than that obtained in 2008. The differences in the level of grain infection of the particular cultivars growing in each cropping system between the years are statistically significant at $P \leq 0.05$ (Table 3). In the case of both years and both wheat cultivars, grain obtained from the intensive crop management system was infected by fusaria in the higher level (Table 3) (Tonacja - 11% versus 1% and 26% vs. 21% in 2008 and 2009, respectively, Bombona - 3% vs 1% in 2008 and 21% vs. 9% in 2009). The differences between the cropping systems were statistically significant at $P \leq 0.05$ in the case of Tonacja in 2008 and Bombona in 2009 (Table 3).

Table 3 shows that in 2008 the main *Fusarium* species in wheat grain from all experimental series was *F. avenaceum* (1% and 3-6% of the wheat kernels in integrated and intensive system, respectively). In 2009 a wide range of *Fusarium* species were identified in the wheat kernels (Table 3). The most common species were *F. graminearum* (2-8% and 9% of wheat kernels in integrated and intensive system, respectively), *F. avenaceum* (5-6% and 4-9%), *F. poae* (1-5% and 1-6%), *F. tricinctum* (0.5-1.5% and 0.5-2%). Moreover in the case of intensive system some isolated fusaria were identified as: *F. sporotrichioides* (1.5% of kernels) in grain of both wheat cultivars; *F. culmorum* (2%) and *F. chlamy-*

dosporum (0.5%) in kernels of Tonacja; *F. crookwellense* (0.5%) in kernels of Bombona (Table 3). One strain of *F. equiseti* was also isolated from the grain of Bombona cultivated according to the rules of integrated system in 2009.

Table 3
The effect of cropping system on occurrence frequency of *Fusarium* species in the wheat kernels

<i>Fusarium</i> species	Winter wheat 'Tonacja'				Spring wheat 'Bombona'			
	2008		2009		2008		2009	
	Integrated	Intensive	Integrated	Intensive	Integrated	Intensive	Integrated	Intensive
	Numbers of isolates ***							
<i>F. graminearum</i> Schwabe	0	0	16	19	0	0	4	19
<i>F. avenaceum</i> (Fries) Saccardo	1	6	13 (8+5)	9 (4+5)	1	3	11 (5+6)	19 (14+5)
<i>F. poae</i> (Peck) Wollenweber	0	0	11 (7+4)	13 (8+5)	0	0	2 (2+0)	2 (1+1)
<i>F. tricinctum</i> (Corda) Saccardo	0	3	3 (2+1)	5 (4+1)	0	0	1 (0+1)	1 (1+0)
<i>F. sporotrichioides</i> Sherbakoff	0	0	0	3 (1+2)	0	0	0	3 (2+1)
<i>F. culmorum</i> (W.G. Smith) Saccardo	0	0	0	4 (3+1)	0	0	0	0
<i>F. crookwellense</i> Burg., Nels. & Touss.	0	0	0	0	0	0	0	1 (0+1)
<i>F. solani</i> (Martius) Appel & Wollenw. emend. Snyder & Hansen	0	1	0	0	0	0	0	0
<i>F. equiseti</i> (Corda) Saccardo	0	0	0	0	0	0	1 (1+0)	0
<i>F. chlamydosporum</i> Wollenw. & Reink.	0	0	0	1 (1+0)	0	0	0	0
<i>Fusarium</i> spp.	0	1	1 (1+0)	1 (0+1)	0	0	0	0
Total number of isolates***	1	11	44 (23+21)	55 (30+25)	1	3	19 (12+7)	45 (28+17)
Percentage of infected grains and the confidence limits at $P=0.05$	1.0 a**	10.5 bc	21.0 cd	26.2 d	1.0 a	2.9 ab	9.0 b	21.4 cd
	0.2-5.2	6.0-17.8	16.0-27.0	20.7-32.5	0.2-5.2	1.0-8.1	5.9-13.7	16.4-27.5

Although the observed differences were not statistically significant at $P \leq 0.05$, in all experimental series in 2009 increasing the seeding rate (what caused increase of canopy density from 5 to 32% <av. 18.4%>) tended to lower the percentage of wheat kernels infected by fusaria (from 11-29% <av. 22.1%> to 7-24% <av. 16.7%>) (Table 3).

DISCUSSION

Our results demonstrate that a level of wheat grain infection by fusaria depended mainly on a rainfall level. The grain colonization by fusaria, especially by *F. graminearum*, *F. avenaceum* and *F. poae*, was much stronger in 2009 than that in 2008 because of, in contrast to 2008, abundant precipitation in 2009 during the period from the third decade of May to the first decade of July. In comparison, the period from heading (GS 50 acc. to Zadoks) to wax maturity (GS 80 Zadoks) lasted from 29 May to 18 July and from 17 June to 20 July in the case of the winter wheat and the spring wheat, respectively. Furthermore, the plants, especially the spring wheat were weakened in 2009 by the drought during April and two decades of May (Fig 1). Similar conclusions - that weather conditions were the stronger factors influencing infection of wheat grain by *Fusarium* species, deoxynivalenol (DON) content in the grain or FHB severity than different cropping systems - have been drawn by Birzele *et al.* (2002) and Champeil *et al.* (2004b). Doohan *et al.* (2003) reported that risk assessment and disease forecasting models for FHB are usually based on climatic conditions around the flowering to the early milky-ripe stage. Wheat plants are most susceptible to the disease during anthesis (GS 60-69 acc. to Zadoks), especially when warm and wet conditions prevail during the period (Parry *et al.*, 1995, Xu, 2003). For wheat, wetness periods of at least 24 h and temperatures above 15°C (20-25°C are optimal) are required for significant infection of heads by fusaria including *F. graminearum*, *F. culmorum*, *F. poae* and *F. avenaceum* (Doohan *et al.* 2003). Similarly, Champeil *et al.* (2004a) reported that infection of the cereal ears by macroconidia of these fusaria, takes place over 24-60 h in optimal conditions (100% relative humidity and 25°C).

The main differences between the crop management systems were in N, P and K fertilization and in the method of disposal of the preceding crop straw (Table 1). Probably higher input of N-fertilizers was the strongest factor increasing the colonization of wheat kernels by fusaria in the intensive system. Martin *et al.* (1991), Lemmens *et al.* (2004) and Teich (1989) cited by Edwards (2004) observed that the increasing amount of nitrogen applied to cereals resulted in increased incidence of fusarium-infected grain or FHB as well as DON content. Nitrogen application also significantly increased the predisposition of wheat to attacks by *F. avenaceum* and *Microdochium nivale* (Champeil *et al.* 2004a after cited Deadman and Cooke 1997). Furthermore, a high versus low N fertilizer rate increased food rot of wheat (Colbach *et al.* 1996), and the greater abundance of *F. culmorum* in soil with a high input of fertilizers also suggests that these conditions are likely to favour fusarium disease in cereals (Bateman and Coşkun 1995). However, Fauzi and Paulitz (1994) and Lori *et al.* (2009) demonstrated that FHB levels in spring wheat were similar in the presence and

absence of applications ammonium nitrate ($140 \text{ kg} \times \text{ha}^{-1}$) or different doses of urea.

The influence of application of P and K fertilizers on infection of wheat grain by fusaria and FHB severity is unclear. Teich and Nelson (1984) reported that the application of phosphorus limited FHB. However, the result obtained in the second year of the study was not confirmed (Teich and Hamilton 1985). Similarly, potassium applications do not appear to affect the incidence of FHB (Teich and Hamilton 1985) but higher applications of mineral N, P, K, Mg fertilizers increased the number of *F. culmorum* propagules in soil (Bateman and Coşkun 1995).

The practice of incorporating straw into soil may be more at risk from fusarium diseases. The results obtained by Bateman *et al.* (1998) suggested that propagules of *F. culmorum* were more abundant in the upper 10 cm of soil in which chopped straw had been incorporated than in soil in which amounts of straw had been reduced by burning after harvest. Furthermore, Bateman *et al.* (1998) reported that the evidence from other experiments at Rothamsted suggests that incorporating straw into soil can lead to increased fusarium foot rot. Similarly, the intact stubble incorporation into soil significantly increased the percentage of recovery of *F. graminearum* from plants at 3 out of 4 locations/years in comparison to the burned stubble incorporation (Summerel *et al.* 1990). Moreover, Smagacz and Sowiński (2005) found that the incidence of stem-base diseases (including fusariosis) of winter wheat was higher when the straw was plowed under in comparison to the control object in which the straw was removed. However, Colbach *et al.* (1996) reported that removal or burial of straw did not affect the foot rot of winter wheat. Similarly, the method of utilization of after-harvest residues of spring oilseed rape or winter wheat (straw was removing or plowing under) did not have a clear effect on the intensity of infection of the roots and base of winter wheat by fusaria (Majchrzak *et al.* 2005) and the numbers of *Fusarium culmorum* propagules in soil (Bateman and Coşkun 1995), respectively.

The observed in 2009 tendency to lower the percentage of wheat kernels infected by fusaria by increase of the seeding rate could not be accidental. May *et al.* (2004) reported that increasing of seeding rate from 150 to 300 m^{-2} significantly decreased the level of durum wheat kernels damaged by *Fusarium* at 4 out of 7 grain samples of different cultivars growing in three consecutive years. Champeil *et al.* (2004a) suggested that if the inoculum is dispersed primarily by splashing, as it happened during the rain, a high density of the canopy may increase the number of obstacles, limiting the vertical dispersal of spores towards the ear. Alternatively, the high density is likely to increase the humidity of the canopy what can favour the development of foot rot and FHB (Champeil *et al.* 2004a). Colbach *et al.* 1996) reported that high plant density of winter wheat increased early foot rot and levels of *M. nivale* but decreased late food rot and the levels of *Fusarium* spp. (Colbach *et al.* 1996).

CONCLUSIONS

1. A level of the wheat grain infection by fusaria depended mainly on a rainfall level;
2. The most frequent species were *F. graminearum*, *F. avenaceum* and *F. poae* in 2009 – a year favourable for the wheat grain colonization by fusaria because of the abundant precipitation during the anthesis periods of winter and spring wheat, and *F. avenaceum* in 2008, when almost no rain was noticed during the plant stage;
3. The intensive cropping system (with the higher N fertilizers input and the practice of incorporating straw into soil) was more conducive to the grain infection by fusaria in comparison to the integrated one (with the lower use of N fertilizers and the practice of the straw removing from the field).

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