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The effect of soil tillage and herbicide treatments on the incidence of *Fusarium* fungi genus in the grain of rye

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ABSTRACT

The reduced tillage in cereals contributes to intensive weed growth and thus encourages better conditions for the development of *Fusarium* fungi. The aim of the study was to determine the effect of different tillage systems and herbicide applications, on the grain infestation by *Fusarium* spp., in two rye cultivars. The cultivar factor significantly affected the infestation of the grain by *F. avenaceum* and *F. culmorum*. Cv. Dańkowskie Złote was more susceptible to the infection by *Fusarium* spp. than cv. Picasso. The reduced tillage encouraged higher grain infestation than the conventional one, irrespectively of the cultivar. Chlorsulfuron, applied in autumn, contributed to a greater grain infestation by *F. avenaceum* and *F. culmorum*. In cv. Picasso, the grain infection by *Fusarium* spp. was equally well prevented by the autumn or spring application of iodosulfuron + mesosulfuron. All *Fusarium* species responded differently to variable environment conditions mediated by cultivar, tillage system and herbicide. *Fusarium avenaceum* infected most intensively cv. Dańkowskie Złote, grown in the reduced tillage system and protected by chlorsulfuron applied in autumn.

Keywords: *Secale cereale*; resistance; hyphomycetes; weed control

One of the main recommendations issued for European Community states with reference to sustainable agriculture concerns the application of the reduced tillage systems. It has been commonly recognized that the reduced soil tillage may considerably lessen the costs of production and prevent soil erosion (Derpsch et al. 2014). However, the reduced tillage systems often significantly alter physical, chemical and microbiological properties of soils. Moreover, they usually provoke changes in the communities of weeds in crop canopy (Carvalho and Lourenco 2014). Despite the routine applications of herbicides in rye, increased weed infestation are recently not an exception. Furthermore, for the increased moisture and organic matter content in the upper layers of soil, the abundance of the *Fusarium* spp. fungi has been higher (Gilbert and Haber 2013). These fungi develop and sporulate well on different sorts of soil substrate, including crop residues, as well as monocots and dicots weeds (Scherm et al. 2013).

Research conducted in many countries demonstrates that such a profound rearrangement of soil habitat as the one observed in reduced tillage systems affects crop health (Bateman et al. 2007, Suproniėnė et al. 2012). Modern herbicides interact not only with the target organisms but also non-target components of the ecosystem. Among the others, herbicides may affect fungal pathogens and antagonistic fungi present in environment, or alter trophic relation between the two, which in turn could affect crop health (Fernandez et al. 2008). Unfortunately, few studies focus on the latter issue.

The rye is highly variable in respect to its susceptibility to *Fusarium* spp. In the North-East Europe the species most frequently isolated from the rye grain are *Fusarium avenaceum* and *Fusarium cul-*

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morum. Recently *Fusarium oxysporum* has been found on the rye grain as well. In Scandinavia, northwestern Russia, Siberia and the Russian Far East the most common *Fusarium* species are the *F. avenaceum*/*arthrosporioides*/*tricinctum*, *F. graminearum*/*culmorum*/*cerealis* and F. *poae*/*sporotrichioides*/*langsethiae* species groups based on morphology (Yli-Mattila 2010).

The aim of the present work was to determine the effect of different soil tillage systems and herbicide applications on the extent of the grain infestation by *Fusarium* spp. in selected winter rye cultivars.

MATERIAL AND METHODS

Field experiments. The research was performed during 2011–2013 in monoculture of winter rye in the fields located 30 km from Wrocław (the South-West Poland). The experiment was set up in a split-plot design in four replicates, with the plot area of 20 m^2. The rye was grown on Haplic Luvisol.

The primary experimental factor was the tillage system: 1. conventional (plough) and 2. reduced. The reduced tillage system included, after harvesting the preceding crop, the stubble breaking at the depth of 0.1 m, followed by one passage of spring cultivator aggregated with the string-type crumble roller. In contrast, with the conventional (plough) system, the post-harvest stubble breaking at the depth of 10 cm was followed by deep (25 cm) ploughing and only in the seedbed preparation the cultivator + crumbler roller aggregate was used. In the conventional tillage system, after the harvest of preceding crop straw was chopped into pieces of 5–10 cm length and then distributed on field surface whereas, in reduced tillage, it was incorporated into the soil at the depth of 10 cm. Two rye cultivars (1. F1 hybrid cv. Picasso, 2. population cv. Dańkowskie Złote) were used as the secondary factor. The third factor was herbicide treatment including untreated object, herbicide Atlantis 04 WG, herbicide Glean 75 WG. Herbicide Atlantis 04 WG (iodosulfuron-methyl-sodium 0.6% + mesosulfuron-methyl 3.0%) was applied at the rate of 0.15 kg/ha in tank mixture with adjuvant Actirob 842 EC (1.0 L/ha). Herbicide Glean 75 WG (chlorsulfuron 75.0%) was applied at the rate of 25 g/ha. Each herbicide was used at two terms: in autumn, at two leaves stage (12 BBCH) and in spring, at five leaves stage of rye (25 BBCH).

Meteorological data. Autumn of 2010 was characterized by low rainfall and temperatures in October that slowed down rye emergence (Table 1). Also, inhibition of autumn growth occurred relatively early. Spring and summer of 2011 were warm with significantly lower rainfall compared to other years. The level of rainfall rose significantly in the last decade of September. In the second decade of October, temperature considerably decreased. This season was characterized by mild winter in December and January, whereas in early February heavy frost occurred, which lasted for about 10 days. However, this situation did not affect rye overwintering. The spring of 2012 was warm, however, it was characterized by high rainfall in May, June and July. The autumn of 2012 was warm and humid, which was conducive to rye emergence and growth. Winter had a relatively mild course with heavy snowfall and short periods of low temperatures. The start of spring growth occurred early – at the beginning of April. The

Table 1. Weather conditions for the experimental period

Year	Weather factor	Month											
		IX	X	XI	XII	I	II	III	IV	V	VI	VII	VIII
2010/2011	t	15.5	7.0	3.7	3.6	1.0	–1.9	6.0	9.7	15.5	17.1	19.9	19.1
	R	24.4	2.6	0.0	34.5	46.8	27.5	13.9	19.6	15.9	92.9	26.6	27.4
2011/2012	t	13.1	14.4	6.1	–5.3	0.4	–3.4	4.3	11.5	14.3	18.8	17.9	19.1
	R	112.3	20.0	61.9	3.0	28.3	6.5	26.6	26.5	42.1	71.9	105.4	76.4
2012/2013	t	14.6	8.8	5.6	–0.9	–1.7	0.0	–0.8	8.9	14.3	16.1	20.4	19.3
	R	43.8	37.0	23.9	14.2	39.5	23.2	24.6	13.6	31.5	110.0	78.3	68.5
Mean	t	14.4	10.1	15.1	–0.9	–0.1	–1.8	3.2	10.0	14.7	17.3	19.4	19.2
	R	60.2	10.9	28.6	17.2	38.2	10.1	32.5	19.9	29.8	91.6	70.1	57.4

t – monthly mean temperature (°C); R – monthly sum of precipitation (mm)

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spring of 2013 was cool, which slowed down rye growth and development. In the first decade of June, high precipitation occurred.

Mycological analysis. In each year of the study the fungi of *Fusarium* spp. were isolated from the grain of tested cultivars sampled from every experimental treatment. From every general sample (i.e. 4 samples of all replicates of a treatment, mixed together) 100 seeds were taken randomly. The seeds were placed directly on PDA (potato dextrose agar) medium. After incubation, the grown colonies of mycelium were further inoculated on PDA slants and then were identified taxonomically using monographic keys (Leslie and Summerell 2006).

Statistical analysis. Log-linear analysis was used in order to test the significance of the effect of experimental factors on the abundance of *Fusarium* spp. in the grain (Goodman 1978). Such analysis enables to estimate the variation in fungi abundance even in case of their absence at one or more experimental treatments. In this analysis, any significant deviations of the observed values from the expected ones indicate relations between the investigated variables. After the logarithmic transformation of the expected values the model takes the form of a linear relation, represented by the following formula:

$$\mathrm{Ln}(E_{ij}) = M + \lambda_i^{X} + \lambda_j^{Y} + \lambda_{ij}^{XY}$$

Where: E_{ij} – expected values; M – general mean based on equal number of observations in every treatment; λ_i^X – effect of the i^{th} observation value taken by the variable X; λ_j^Y – effect of the j^{th} observation value taken by the variable Y; λ_{ij}^{XY} – effect of the interaction between the i^{th} value of X and the j^{th} value of the Y.

In order to adequately represent the variation of the experimental factors, the analysis of correspondence was applied (Hill 1974).

RESULTS AND DISCUSSION

Two species of *Fusarium* (*F. avenaceum* and *F. culmorum*) were isolated from the rye grain in majority of the analyses, whereas *F. oxysporum* was the less frequent. The number of isolates of each *Fusarium* species was significantly different depending on the experimental factors (Table 2). Cv. Picasso showed higher resistance to grain infections compared to cv. Dańkowskie Złote yet,

Table 2. Array boundary number of *Fusarium* species (pcs.) isolated from rye grain (total of 3 years)

Tillage system	Cultivar	*Fusarium* species	Untreated	12 BBCH		15 BBCH		Total
				Atlantis 04 WG + Actirob 842 EC	Glean 75 WG	Atlantis 04 WG + Actirob 842 EC	Glean 75 WG	
Conventional tillage	Dańkowskie Złote	*F. a.*	31	25	38	31	28	153
		F. c.	24	31	3	16	19	93
		F. o.	3	3	3	8	3	19
		total	58	59	44	55	49	265
	Picasso	*F. a.*	4	28	22	13	14	81
		F. c.	22	1	17	14	22	76
		F. o.	3	3	1	1	2	11
		total	29	32	40	28	29	168
Reduced tillage	Dańkowskie Złote	*F. a.*	31	33	48	29	32	173
		F. c.	48	27	47	71	52	245
		F. o.	1	0	12	6	2	21
		total	80	60	107	106	86	439
	Picasso	*F. a.*	21	11	37	30	32	131
		F. c.	9	33	13	15	27	97
		F. o.	2	4	4	2	2	14
		total	32	48	54	4	61	242
Total			199	199	245	236	225	1114

F.a. – *Fusarium avenaceum*; *F.c.* – *Fusarium culmorum*; *F.o.* – *Fusarium oxysporum*

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Table 3. The occurrence of *Fusarium* species (pcs.) in rye grain in experimental years

Fusarium species	2011	2012	2013	Total
Fusarium avenaceum	189	280	69	538
Fusarium culmorum	85	174	252	511
Fusarium oxysporum	19	30	16	65
Total	293	484	337	1114

the infection of both cultivars was significantly greater in the reduced tillage system. Irrespective of tillage system, species the most isolated from the grain was *F. avenaceum*. The exception was cv. Dańkowskie Złote harvested from the conventional tillage plots, in the grain of which *F. culmorum* dominated. *Fusarium oxysporum* occurred rarely; therefore no statistically valid conclusions were drawn from available data.

The highest number of *Fusarium* spp. isolates were recorded in grain harvested from the plots treated with Glean 75 WG in autumn (Table 2). Within that category, cv. Dańkowskie Złote grown in the reduced tillage system, was the most prone to infection. Irrespective of cultivar and tillage system, the lowest number of *Fusarium* spp. isolates were obtained from reference plots and from plots treated with Atlantis 04 WG in autumn. It seems that a significant interaction was observed between the fungi species on the one hand and the tillage system or weediness after the herbicide spraying, on the other hand. This apparently points to the different responses of the fungi to the tillage systems.

As Table 3 shows, *Fusarium* species appeared in rye grain the most numerously in the year 2012 that was related to high rainfall in May, June and July. In contrast, poor precipitation in these months in 2011 contributed to lower grain infestation.

The significance of the main factors and interactions between them was verified using Chi^2 test (Table 4). The high values of this test allow for rejection of the null hypothesis that assumed no effect of the experimental factors on the number of fungi species in the grain. However, based on the log-linear analysis no interaction was found between the cultivar and other factors i.e. tillage system, herbicide and fungi species. It means that the cultivars affected the fungi abundance independently of the other factors.

Figure 1 shows the variable incidence of the fungi species depending on the tillage system and herbicide. The wide spacing between the points

Table 4. Tests of main effects, marginal and partial associations and interactions between experimental factors

Object	Degrees of freedom	Chi^2 partial relation	*P*	Chi^2 marginal relation	*P*
1. Years	2	49.89	0.0000	49.89	0.0000
2. Tillage	1	59.56	0.0000	59.56	0.0000
3. Cultivars	1	85.66	0.0000	85.66	0.0000
4. Herbicides	4	13.21	0.0103	13.20	0.0103
5. Fungi	2	436.04	0.0000	436.04	0.0000
1 × 2	2	9.90	0.0070	17.98	0.0001
1 × 3	2	26.68	0.0000	32.07	0.0000
1 × 4	8	19.64	0.0117	28.97	0.0003
1 × 5	4	165.71	0.0000	188.91	0.0000
2 × 3	1	0.11	0.7361	0.12	0.7283
2 × 4	4	8.31	0.0808	7.17	0.1270
2 × 5	2	7.82	0.0200	15.87	0.0003
3 × 4	4	4.55	0.3365	5.16	0.2704
3 × 5	2	0.73	0.6923	6.12	0.0468
4 × 5	8	16.71	0.0332	18.84	0.0157

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representing the tillage system confirm the variable response of the fungi to this experimental factor. The most similar incidence of the pathogens was observed between the reference plots with reduced tillage and the plots with herbicide (Glean 75 WG in spring or Atlantis 04 WG in autumn).

Although the rye is tolerant to recurrent cultivation, in persistent monoculture the increased grain infestation by *Fusarium* spp. may occur. This phenomenon was also observed in this study. The results of the present study proved the frequent incidence of *F. avenaceum* and *F. culmorum*, irrespectively of the rye cultivar and the herbicide treatment.

F. culmorum produces chlamidospores that are able to survive in soil for few years. Under reduced tillage, increased microbiological activity in the upper layer of soil can provoke faster decomposition of resting spores than ploughing at the depth of 25 cm. Conventional tillage does not destroy spores of these fungi species whereas *F. avenaceum* spreads by ascospores that are destroyed by being incorporated by ploughing into soil at the depth of 20–25 cm. In this study, cultivation of rye in monocrop system and low precipitation in August contributed to poor decomposition of crop residues under reduced-tillage system. Therefore, *Fusarium* spp. occurring on plant residues caused greater infection of plants growing under reduced-tillage than under conventional system.

The pesticides and cultural methods only partially prevent the infections by *Fusarium*. The cultivation of resistant cultivars coupled with a proper harvesting technology is the best way to restrict the fungi incidence. In our study, rye cultivars proved different susceptibility to *Fusarium* – the population cv. Dańkowskie Złote was more infested than the cv. Picasso. Bułatowicz et al. (2009) report, that the cultivars are usually more prone to infections than the cultivars.

In this study, the reduced tillage system was more predisposed to increase weediness, than conventional one. Kordas (2004) claims that the conventional tillage equips the crop canopy in a greater ability to weeds suppression. Also the mycological analysis showed higher incidence of the *Fusarium* fungi in the grain harvested from the reduced tillage treatments. In general, the opinions on the effect of reduced tillage on crop health are numerous and provide ambiguous results (Pląskowska et al. 2009, Małecka et al. 2012).

Alterations in the soil properties resulting from different tillage systems may affect the incidence of fungi-related plant diseases. In the reduced tillage systems, the crop residues left on the soil surface constitute a direct source of disease (Bateman et al. 2007, Małecka et al. 2012). In this tillage system, the skimming, performed just after harvest, removes the crop residues and destroys the volunteer crops, disrupting life cycle of many pathogenic fungi. Displacing the residues into the top layer of soil results in their faster decomposition, leading to dominance of the saprophytic fungi over the pathogenic taxa. Nevertheless, the reduced tillage does not destroy the weeds, frequently being pathogen reservoirs (Małecka et al. 2012). On the other hand, the pre-planting ploughing, used in conventional tillage, covers all the residues of plants that had been infected during the post-harvest period. Still, once the same crop species is grown next year, the pre-planting ploughing, used one next time, causes the so far non-decayed plant parts, along with fungal structures on them, to re-emerge, so that the pathogens are able to infect the new crop (Korbas et al. 2008).

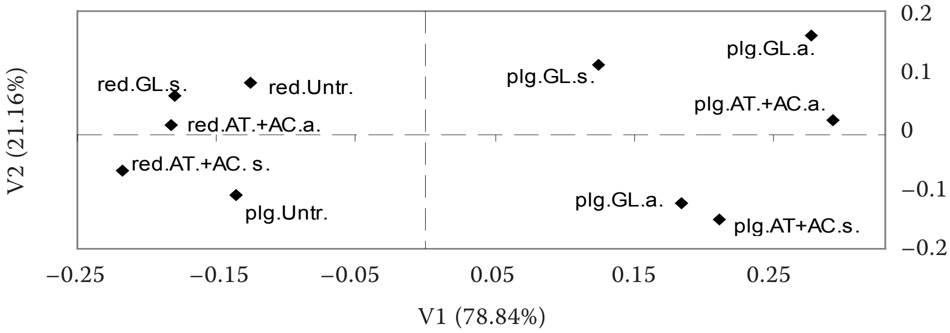

Figure 1. Variation in the abundance of *Fusarium* spp. in particular herbicide treatments and in tillage systems. Red. – reduced tillage; plg. – ploughing; GL. – Glean 75 WG; AT + AC. – Atlantis 04 WG + Actirob 842 EC; s. – spring; a. – autumn

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So far, only a limited number of studies have been known that deal with the effect of herbicide on the health of cereal grain. They proved that herbicides affect the fungi incidence more than genetic traits of the crop, environment, fertilization or grain storage (Damszel et al. 2008, Fernandez et al. 2008). Herbicides, however, alter the direction and the intensity of biochemical processes in soil and, therefore, they are not neutral to the microorganisms that infest plants (Damszel et al. 2008). The results of this study conform to that rule: Glean 75 WG used in autumn, in the highest degree contributed to the increased grain infestation by *Fusarium* spp.

In contrast, applying no herbicides results in a low-density crop with the low air humidity within the canopy that does not favour fungi growth in the upper part of plants. Furthermore, the weeds in crop canopy may act as a barrier for the spread of the pathogenic spores. The weed infestation may therefore affect plant disease incidence in different, sometimes ambivalent ways (Munger et al. 2014).

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