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THE HEALTH STATUS OF WINTER DURUM WHEAT GRAIN IN RELATION TO FUNGICIDE TREATMENT[1]

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Abstract

The objective of an experiment made in 2005–2008 was to evaluate the colonization of grain of winter durum wheat, cultivar 'Komnata', by fungi, including cereal pathogens, after different chemical treatments. There were three fungicide treatments: 1 – seed treatment with Panoctine 300 LS before sowing, 2 – seed treatment with Panoctine 300 LS + 1 × spraying with Alert 375 SC + Talius 200 EC, 3 – seed treatment with Panoctine 300 LS before sowing + 2 × spraying with Alert 375 SC + Talius 200 EC and Acanto 250 SC + Charisma 207 EC. The three methods of chemical protection were similarly effective. The composition of fungi was similar but the frequencies of species differed with different treatments. The grain was strongly colonized both on the surface and internally, particularly by *Fusarium* species. The treatments did not protect effectively against *Fusarium* infection and colonization. Weather conditions affected the colonization of grain by *Fusarium* species. Most colonization was observed after a very warm and moist growing period in 2007. *Gibberella avenacea* (anamorph *Fusarium avenaceum*) was the most common fungus on/in durum wheat grain. Other *Fusarium* spp., including *F. culmorum, F. oxysporum, F. poae* and *F. sporotrichioides*, were less common. The high degree of colonization by *Fusarium* spp. did not affect grain development. *Aspergillus* and *Penicillium* species were present at low frequencies and so did not decrease grain quality. The mycotoxin content of the grain was low.

Key words: durum wheat, grain, fungi, fungicides, health, *Fusarium* spp.

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Introduction

In recent years, in Poland, there has been an increased interest in the cultivation of durum wheat (*Triticum durum*). This results from increased consumption of pasta, higher grain prices on the international market and changing climatic conditions which are more often favourable for durum wheat cultivation. Wheat breeders are being encouraged to develop new cultivars which are better adapted to Polish soils, European climatic conditions and provide grain of high quality (Rachoń et al. 2002, Obuchowski et al. 2007, Sulewska et al. 2007).

Durum wheat, as other cereals, is very susceptible to fungal pathogens. It is known to suffer from powdery mildew (*Blumeria graminis*), brown rust (*Puccinia recondita*), speckled leaf blotch (*Mycosphaerella graminicola*), Fusarium ear blight (*Gibberella avenacea* + *G. zeae* + *Fusarium culmorum* + *F. poae* + *Monographella nivalis*) and stem-base and root diseases. One of the most dangerous diseases is Fusarium ear blight. It may contribute to significant quantitative and qualitative losses in yield and accumulation of mycotoxins in grain (Rintelen 1995, Champeil et al. 2004, Jurado et al. 2006).

The objective of this study was to evaluate the health of grain of winter durum wheat, cultivar 'Komnata', grown in conditions of the Lower Silesia lowlands and subjected to different methods of plant protection using fungicides.

Materials and methods

The experiment on winter durum wheat, cultivar 'Komnata', grown in 2005––2008, was at the Agriculture Experimental Station in Pawłowice in the vicinity of Wrocław. The field experiment was established in 16.5 m^2 plots arranged in four randomized replicate blocks, on lessivê soil developed from silty clay, after winter oilseed rape.

The following fungicide treatments were applied and compared:

1. Seed treatment with Panoctine 300 LS (guazatine) applied before sowing, at the rate of 170 ml per 100 kg of grain.

2. Seed treatment with Panoctine 300 LS before sowing + 1 × spraying with Alert 375 SC (flusilazole + carbendazim) + Talius 200 EC (proquinazid) applied at the growth stage with second node swelling detectable (GS 32, Zadoks et al. 1974), at the rates of 0.8 l/ha + 0.15 l/ha, respectively.

3. Seed treatment with Panoctine 300 LS before sowing + 2 × spraying with Alert 375 SC + Talius 200 EC as in point 2 and Acanto 250 SC (picoxystrobin) + Charisma 207 EC (flusilazole + famoxat) applied at GS 32 and at the booting stage with first awns visible (GS 49), at the rates of 1 l/ha + 1.5 l/ha. In 2006, an additional spraying with insecticide Nurelle D 550 SC (chloropiryfos + cypermethrin) was applied at the rate of 0.6 l/ha, because of a massive occurrence of aphids and leaf beetles (*Chrysomelidae*).

4. Control with no treatments.

Alert 375 SC was applied against powdery mildew, speckled leaf blotch, Fusarium crown rot of wheat (*G. zeae, F. culmorum, F. pseudograminearum*), Fusarium ear blight, eyespot of wheat (*Oculimacula yallundae* + *O. acuformis*) and brown rust. Talius 200 EC was applied against powdery mildew, Acanto 250 SC against powdery mildew, tan spot (*Pyrenophora tritici-repentis*), brown rust, speckled leaf blotch and glume blotch (*Phaeosphaeria nodorum*), and Charisma 207 EC against Fusarium crown rot, powdery mildew, eyespot and speckled leaf blotch.

Tillage typical for winter cereals was performed each year. The winter durum wheat was sown on 23–26 October in 2005–2007. Quality seeds were planted (500 per 1 m^2) in rows 12.5 cm apart.

Phosphorus (P) and potassium (K) fertilizer was applied before sowing at 35 and 83 kg/ha, respectively, and the nitrogen (N) fertilizer was applied in two doses: 60 kg/ha at the pseudostem erect growth stage (GS 30) + 30 kg/ha at the second node swelling detectable stage (GS 32). The herbicide Cougar 600 SC (isoproturon + diflufenikan) was applied at the stage of seven leaves unfolded (GS 17). The same treatments were applied to the same plots each year. The phosphorus content in soil was high in 2005–2006 and medium in 2007. The potassium content was medium each year, and the magnesium (Mg) content was medium in 2006 and 2008, and high in 2007. The soil tended to be acidic in 2005–2006 and was acidic in 2007 (pH 6.5).

The wheat was sampled at the hard dough stage (GS 87–89) for mycological analysis. One hundred randomly chosen ears were collected from each plot. The grain health analysis was performed according to de Tempe (1970). Two hundred grains were randomly chosen from a pooled sample (from all four replicates) from each treatment. One hundred grains, with no surface disinfection, were placed on 2% malt agar in Petri dishes. Another hundred grains were surface-disinfected in sodium hypochlorite (1% available chlorine) for 10 min before being placed on 2% malt agar in Petri dishes. After incubation for 14 days at 22°C, the plates were examined microscopically. Subcultures on potato dextrose agar (PDA) slants were made for preservation of cultures. Sporulating fungi were identified on the basis of their morphology according to the available literature. The frequency of a single taxon (in %) was estimated on the basis of its contribution to the total number of isolates from a treatment. Fungi recorded from non-disinfected grains were considered to have grown, at least partly, from the surface of the grains, and those recorded from surface-disinfected grains were considered to have grown from inside of the grain.

The deoxynivalenol (DON), T-2 toxin and zearalenone (ZEA) in the grain was checked with gas chromatography equipment with electron content detector (ECD), and the aflatoxin and ochratoxin A contents by ELISA test, in 2008.

Results

The grain of the winter durum wheat was colonized at least by 27 species of fungi from 15 genera – 24 species on the grain surface and 23 species in the grain

interior. There were many non-sporulating fungi *in vitro*, which were up to 30% of the total. All the seeds were infected by fungi (Tables 1, 2).

There were more species on the grain surface (non-disinfected grain) than in the grain interior (surface-disinfected grain). *Alternaria alternata, Epicoccum nigrum* and *Fusarium* species were the most common fungi on/in grain. The frequency of *A. alternata* was 45.9% of isolates from non-disinfected grain and 38.0% from disinfected grain. Its lowest frequency often occurred in 2006, regardless of the fungicide treatment. Fungicide treatment seemed not to affect its frequency. *Epicoccum nigrum* was recorded more often from internal infections, after surface disinfection

Table 1

Frequency of fungi isolated from non-disinfected grain of winter durum wheat in relation to fungicide treatment (%)

No.	Taxon	Fungicide treatment 1			Fungicide treatment 2			Fungicide treatment 3			Control with no treatment		
		2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008
1	*Alternaria alternata*	38.7	46.4	50.2	39.8	46.7	54.6	48.4	37.3	50.0	46.5	46.6	47.0
2	*Aspergillus niger*	1.9	–	–	3.1	–	–	1.1	4.5	–	–	–	–
3	*Botrytis cinerea*	0.9	1.8	6.0	1.0	2.9	7.2	2.1	1.8	7.4	1.6	5.8	4.5
4	*Epicoccum nigrum*	11.3	0.9	8.5	9.2	5.7	9.7	8.4	4.5	5.9	10.1	1.9	9.1
5	*Fusarium culmorum*	0.9	3.6	1.0	1.0	1.9	1.9	–	5.5	0.5	–	–	–
6	*Fusarium merismoides*	1.9	–	–	2.0	–	–	5.3	–	–	0.8	–	–
7	*Fusarium oxysporum*	0.9	0.9	1.5	–	–	2.9	–	0.9	2.0	3.9	1.9	4.0
8	*Fusarium poae*	–	7.1	5.5	–	10.5	2.9	–	5.5	2.5	–	2.9	7.1
9	*Fusarium sporotrichioides*	–	5.4	–	–	6.7	–	–	3.6	0.5	–	9.7	1.0
10	*Gibberella avenacea*	8.5	14.3	6.5	7.1	15.2	8.7	12.6	14.5	11.4	15.5	12.6	9.6
11	*Gibberella intricans*	–	3.6	–	–	–	–	–	4.5	–	–	–	–
12	*Gibberella zeae*	–	–	–	–	–	1.0	–	–	1.5	–	–	–
13	*Khuskia oryzae*	–	–	1.0	–	–	–	–	–	0.5	–	–	–
14	*Microdochium bolleyi*	3.8	–	–	–	–	–	–	–	–	2.3	–	–
15	*Mucor hiemalis*	–	–	–	–	1.0	–	–	–	–	–	1.0	–
16	*Penicillium chrysogenum* var. *chrysogenum*	–	2.7	–	–	–	–	–	0.9	–	1.6	3.9	–
17	*Penicillium glabrum*	–	–	–	–	1.0	–	–	–	–	–	–	–
18	*Penicillium implicatum*	–	–	–	–	1.0	–	–	–	–	–	–	–
19	*Penicillium rugulosum*	–	–	–	–	–	2.9	–	–	–	–	–	–
20	*Rhizopus stolonifer*	–	1.8	–	4.1	2.9	0.5	4.2	–	1.5	–	3.9	1.0
21	*Sclerotinia sclerotiorum*	–	–	–	–	–	–	–	–	–	–	1.0	–
22	*Trichoderma harzianum*	2.8	2.7	–	3.1	–	–	1.1	1.8	–	5.4	3.9	–
23	*Ulocladium botrytis*	–	–	–	–	–	–	–	–	3.0	–	–	–
24	Non-sporulating fungi	28.3	8.9	19.9	29.6	4.8	7.2	16.8	14.5	13.4	12.4	4.9	16.7
25	Yeast colonies	–	–	–	–	–	0.5	–	–	–	–	–	–

Table 2

Frequency of fungi isolated from disinfected grain of winter durum wheat in relation to fungicide treatment (%)

No.	Taxon	Fungicide treatment 1			Fungicide treatment 2			Fungicide treatment 3			Control with no treatment		
		2006	2007	2008	2006	2007	2008	2006	2007	2008	2006	2007	2008
1	*Alternaria alternata*	34.7	33.0	37.9	28.0	37.5	47.9	33.0	35.4	48.1	44.9	39.0	37.9
2	*Aspergillus niger*	1.1	4.3	–	5.4	–	0.5	–	–	–	–	–	3.1
3	*Botrytis cinerea*	3.2	4.3	1.5	2.2	4.2	5.3	6.1	5.2	2.4	–	3.0	9.2
4	*Epicoccum nigrum*	8.4	12.8	12.6	7.5	7.3	9.5	7.0	11.5	11.7	9.0	9.0	14.4
5	*Fusarium culmorum*	–	2.1	2.5	–	2.1	–	7.0	3.1	–	1.3	4.0	–
6	*Fusarium oxysporum*	5.3	–	3.0	1.1	1.0	2.6	–	–	–	3.8	–	2.6
7	*Fusarium poae*	2.1	8.5	9.6	2.2	8.3	5.3	–	10.4	6.3	1.3	6.0	7.2
8	*Fusarium sporotrichioides*	–	–	–	–	10.4	–	–	–	–	–	2.0	0.5
9	*Fusarium tricinctum*	–	2.1	–	–	–	–	–	–	–	–	–	–
10	*Gibberella avenacea*	7.4	4.3	10.6	20.4	5.2	8.9	15.7	7.3	11.7	17.9	14.0	7.2
11	*Gibberella intricans*	–	3.2	–	–	–	–	–	1.0	0.5	–	6.0	–
12	*Gibberella zeae*	–	–	–	–	–	–	–	–	–	–	–	0.5
13	*Khuskia oryzae*	–	–	7.1	–	–	3.2	–	–	2.4	–	–	1.0
14	*Microdochium bolleyi*	6.3	–	–	–	–	–	–	–	–	–	–	–
15	*Penicillium chrysogenum* var. *chrysogenum*	17.9	1.1	–	18.3	–	–	9.6	–	–	7.7	–	–
16	*Penicillium thomii*	3.2	–	–	5.4	–	–	8.7	–	–	–	–	–
17	*Phoma eupyrena*	–	–	–	–	–	–	3.5	–	–	–	–	–
18	*Phoma medicaginis* var. *medicaginis*	–	–	1.0	–	–	–	–	–	–	–	–	–
19	*Rhizopus stolonifer*	–	–	–	–	–	–	–	–	–	–	1.0	0.5
20	*Trichoderma harzianum*	2.1	–	–	1.1	–	–	2.6	–	–	2.6	–	–
21	*Ulocladium botrytis*	–	–	–	–	–	–	–	–	2.4	–	–	2.6
22	Non-sporulating fungi	8.4	24.5	12.6	8.6	24.0	15.8	7.0	26.0	14.6	11.5	16.0	11.8
23	Yeast colonies	–	–	1.5	–	–	1.1	–	–	–	–	–	1.5

(on average 10.0% of all fungi recorded) than without disinfection (on average 7.1% of all fungi recorded). *Fusarium* species were recorded quite often on/in grain. Their frequency differed among years but was most in 2007, averaging 35% of isolates on grain from plants subjected to seed treatment with guazatine, and seed treatment with guazatine + 2 × spraying with flusilazole, carbendazim, proquinazid and picoxystrobin, flusilazole, famoxat, and 32% in disinfected grain from the control treatment.

Fungicide treatment tended to increase the frequency of *Fusarium* species in non-disinfected grain and to decrease their frequency in disinfected grain, compared with the control, only in 2007 (Tables 1, 2). In 2006 and 2008, the frequency

of *Fusarium* species in non-disinfected grain was less in all fungicide treatments than in the control.

Gibberella avenacea was the most common representative of the genus *Fusarium*. It occurred frequently on non-disinfected grain (on average 11.5% of all fungi recorded), and generally only slightly less frequently in the interior of grain, after disinfection (on average 10.8% of all fungi recorded). *Gibberella avenacea* was able to colonize the surface and the interior of grain of plants subjected to fungicide treatments (with frequencies amounted to 15.2–20.4%, Tables 1, 2). Its greatest frequencies were recorded on surface-disinfected grain of plants grown from seeds treated with guazatine and sprayed once with flusilazole, carbendazim, proquinazid or sprayed twice with flusilazole, carbendazim, proquinazid and picoxystrobin, flusilazole, famoxat. Fungicide treatments tended to decrease the frequency of *G. avenacea* on non-disinfected grain in 2006 and 2008. *Fusarium* species were most frequent and most variable in 2007, when grain colonization by *F. culmorum, F. oxysporum, F. poae* and *F. sporotrichioides* was favoured.

The total trichothecene content in the grain collected in 2008 was relatively low. An average deoxynivalenol content was 0.29 mg/kg and the T-2 toxin content was < 75 μg/kg of grain. Zearalenone, aflatoxin and ochratoxin A were not detectable.

Discussion

The mycological analysis showed that the grain of winter durum wheat was colonized mostly by fungi developing during crop growth in the field. The fungal communities recorded on the grain surface or internally after application of different fungicide treatments were similar qualitatively (consisting of the same species) and were different quantitatively (frequencies of individual species differed). This observation is in agreement with Chełkowski (1985) and Narkiewicz-Jodko (1998) who claim that, regardless of the cereal species and the origin of grain, the taxonomic composition of fungal communities is similar but their quantitative structures vary. The density of a community and frequencies of single species often result from the weather conditions during plant growth, tillage and chemical plant protection treatments applied (Kaniuczak and Lisowicz 2000, Wyczling et al. 2005, Woźniak 2006). The data provided show that the greatest density of fungi was on grain (non-disinfected) after a very warm and moist growing period in 2007 (Table 1). This indicates that temperature and moisture contribute to increased colonization of durum wheat grain by microorganisms.

Non-disinfected grain of winter durum wheat grain was more intensively colonized by fungi than disinfected grain (suggesting the smaller internal infection), regardless of the fungicide treatment. The presence of fungi in the inner tissues of cereal grains has been reported also by Narkiewicz-Jodko (1986), and Łukanowski and Sadowski (2002) and many others. *Alternaria alternata* colonized intensively both the surface and the interior of the grain. The fungus is potentially dangerous

because it can produce tenuazonic acid (TA) and alternariol. The former can inhibit the growth of roots and shoots of many plants and the latter inhibits the growth of seedlings (Bottalico and Logrieco 1998). Narkiewicz-Jodko (1998) reported after Stawicki (1967) that *A. alternata* should not exceed 46% of the fungal community on/in cereal grain. The frequency of *A. alternata* in the durum wheat grain was within this limit.

The durum wheat grain analysed had developed properly, no inhibition of grain formation was observed and plants did not show any visible symptoms of Fusarium ear blight. Grain was, however, strongly colonized by *Fusarium* species (on average more than 22% of all fungi recorded). It seems that "invisible Fusarium ear blight" could have occurred. In this case the fungi could have colonized (particularly a bran) during dough development or ripening (GS 80-95) (Miller 1995, Narkiewicz-Jodko 1998).

Considering the significance of the individual microorganisms, the mycological analysis of the cereal grains should provide not only the spectrum of fungal genera but also the spectrum of fungal species recorded. Some *Fusarium* species, including *F. culmorum* and *F. sporotrichioides*, can strongly decrease the germination abilities and stimulate the production of mycotoxins in grain. Detection of these important species contributes significantly to the process of evaluation of grain quality (Miller 1995).

The winter durum wheat grain was strongly colonized by *G. avenacea*. *Fusarium culmorum, F. oxysporum, F. poae* and *F. sporotrichioides* were less frequent. Mycotoxin analyses done in 2008 showed that *Fusarium* species, though occurring so often, did not decrease the quality of grain in this experiment. The concentration of deoxynivalenol (DON) produced in grain by *F. culmorum* and *G. zeae* was low and did not exceed the accepted limit of 125 mg/kg, and T-2 toxin produced by *F. poae* and *F. sporotrichioides* did not exceed 75 μg/kg. Zearalenone (ZEA), produced by many of the *Fusarium* species recorded, including *G. avenacea, F. culmorum, G. intricans, G. zeae, F. oxysporum, F. sporotrichioides* and *G. tricincta* (Chełkowski 1985), was not present at detectable concentrations. The low concentrations of mycotoxins in the grain were not affected by fungicide treatments.

The large amount of variation in frequencies of *Fusarium* species recorded on or within grain indicates the extent to which they may be affected by conditions, and their ability to tolerate fungicide treatments. Theoretically, the maximal effectiveness of fungicides against *Fusarium* spp. should be 70%. In practice, however, their effectiveness often reaches only up to 40% (Remlein-Starosta 1999), probably because of the difficulty of timing a fungicide to coincide with the dispersal or infection processes.

Chemical plant protection against powdery mildew, yellow rust (*Puccinia striiformis*), brown rust, tan spot, speckled leaf blotch and glume blotch on winter durum wheat is provided in the main regions of its cultivation (McIntosh et al. 1995, Nayar et al. 1996, Sharma et al. 1996, Udachin 1998, Bhardwaj et al. 1999, Mishra et al. 2001 a, 2001 b, Klindworth et al. 2006). The present results showed, however, that the usual and standard chemical treatments for powdery mildew, rusts and *Septoria* blotches do not provide the necessary control of *Fusarium*.

The potentially toxigenic *Aspergillus* and *Penicillium* species are the most common fungi among those colonizing stored grain. Losses caused by these fungi are related to decreased germination, discoloration, heating and mustiness, biochemical changes, possible production of toxins and loss in dry matter (Christensen 1972). Grain from the 'Komnata' cultivar studied was colonized only to an insignificant extent by *Aspergillus* and *Penicillium*. The results of mycological analysis were confirmed by results of chemical analysis. The durum wheat grain studied did not contain detectable amounts of aflatoxin and ochratoxin A, usually produced by *Aspergillus* and *Penicillium* species (Miller 1995). This indicates the proper storage conditions provided.

Conclusions

1. The grain of the durum wheat was colonized by *Fusarium* species, and fungicide treatments did not provide protection of plants against *Fusarium* infection.

2. Three combinations of fungicide treatments (1 – seed treatment with Panoctine 300 LS, 2 – seed treatment with Panoctine 300 LS + 1 × spraying with Alert 375 SC + Talius 200 EC, 3 – seed treatment with Panoctine 300 LS before sowing + 2 × spraying with Alert 375 SC + Talius 200 EC and Acanto 250 SC + Charisma 207 EC) resulted in similar grain infection by *Fusarium* spp. in durum wheat cultivar 'Komnata'.

3. The grain of durum wheat was particularly susceptible to infection by *G. avenacea*, which is a low toxigenic species.

4. The durum wheat grain contained low concentrations of mycotoxins (deoxynivalenol and T-2 toxin) which did not accumulate in the grain in conditions provided.

Streszczenie

ZDROWOTNOŚĆ ZIARNA OZIMEJ PSZENICY TWARDEJ
W ZALEŻNOŚCI OD SPOSOBU OCHRONY CHEMICZNEJ

Celem ścisłego doświadczenia polowego prowadzonego w latach 2005–2008 było określenie stopnia zasiedlenia ziarna ozimej pszenicy twardej odmiany 'Komnata', przy zróżnicowanym sposobie ochrony chemicznej, przez grzyby patogeniczne dla zbóż.

Ziarno pszenicy twardej – zarówno jego powierzchnia, jak i wnętrze – było dość podatne na zasiedlenie przez grzyby rodzaju *Fusarium*. Zaprawianie ziarna siewnego oraz dodatkowy jedno- lub dwukrotny oprysk fungicydami w czasie wegetacji roślin w zbliżonym stopniu zabezpieczały ziarno przed infekcją ze strony *Fusarium* spp. Warunki pogodowe miały duży wpływ na nasilenie występowania *Fusarium*

spp. Najsilniej było zasiedlone ziarno zebrane w bardzo ciepłym i wilgotnym roku 2007. Wysoki stopień kolonizacji ziarna przez *Fusarium* spp. nie wpłynął na pogorszenie jego jakości. Zawartość mikotoksyn w ziarnie była niewielka.

Najczęściej wyosabnianym gatunkiem *Fusarium* był *Gibberella avenacea* (syn. *F. avenaceum*). Inne fuzaria, m.in. *F. culmorum, F. oxysporum, F. poae* i *F. sporotrichioides,* występowały mniej licznie. Grzyby rodzajów *Aspergillus* i *Penicillium,* z uwagi na małą frekwencję, nie przyczyniły się do pogorszenia jakości ziarna.

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