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SUSCEPTIBILITY OF WINTER TRITICALE CULTIVARS TO *RHIZOCTONIA CEREALIS* (SHARP EYESPOT) AND *R. SOLANI*

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Abstract: In the field study period from 2006 to 2010, the incidence and severity of sharp eyespot caused by *Rhizoctonia* were recorded on 36 cultivars of triticale at the milk ripe growth stage. Four localities in north-central Poland were included: Chrząstowo, Dębina, Kończewice and Minikowo. The susceptibility of the seedlings of 30 cultivars of triticale to *R. cerealis* (AG-D subgroup I) and *R. solani* (AG-5) was studied in the laboratory. There was much variation in incidence and severity of sharp eyespot between years and locations. The disease was most intense at Chrząstowo. At this location, the mean percentage of diseased stems on 28 cultivars was 2.6–35.7 (–55.0), and the mean disease index was 0.7–15.6 (–24.5), with the lowest and highest values in 2008 and 2009, respectively. At Minikowo, the disease was least intense. At this location, the mean percentage of diseased stems on 23 cultivars was 1.0–4.6 (–18.0), and the mean disease index was 0.3–1.4 (–6.3), with the lowest and highest values in 2006 and 2007, respectively. The cultivars with least intense disease were Tulus and Atletico (Chrząstowo), Grenado and Zorro (Dębina), Krakowiak and Tornado (Kończewice), and Woltario and Constans (Minikowo). The cultivars with most intense disease were Alekto (Chrząstowo), Baltiko (Dębina), Pawo (Kończewice) and Borwo (Minikowo). Mostly *R. cerealis* was isolated from the diseased stems; *R. solani* was isolated only sporadically. There was a wide variation in the susceptibility of triticale cultivars to *Rhizoctonia*. Most triticale seedlings inoculated with *R. cerealis* produced symptoms typical of sharp eyespot. Seedlings inoculated with *R. solani* formed extended lesions with no defined borders. Most symptoms developed on coleoptiles, with less symptoms on the leaves and the least on the roots. There was much variation in susceptibility of triticale cultivars to both *Rhizoctonia* species. Cultivars were grouped into six categories according to the intensity of seedling infection. Categories 1, 2 and 3, representing low, moderate and high susceptibility to *R. cerealis*, included 17, 10 and 3 cultivars, respectively. Categories 4, 5 and 6, representing low, moderate and high susceptibility to *R. solani*, included 3, 12 and 15 cultivars, respectively. Cultivars Baltiko and Zorro had low, and cv. Cultivo had high susceptibility to both *Rhizoctonia* species. No cultivar was resistant to *Rhizoctonia*. There was a positive correlation between infection by *R. cerealis* and *R. solani.* Infection of coleoptiles by *R. cerealis* or *R. solani* was significantly correlated with infection of leaves. No correlation between intensity of sharp eyespot on triticale plants in the field and on seedlings in controlled conditions was found.

Key words: cultivar, resistance, *Rhizoctonia cerealis*, *R. solani*, sharp eyespot, tolerance, winter triticale

INTRODUCTION

Sharp eyespot is one of the stem-base diseases of cereals. It is mainly caused by the soil-borne fungus *Rhizoctonia cerealis* van der Hoeven (teleomorph: *Ceratobasidium cereale* D. Murray & L.L. Burpee). *R. cerealis* may be accompanied by *R. solani* J.G. Kühn [teleomorph: *Thanatephorus cucumeris* (A.B. Frank) Donk].

The pathogen destroys phloem tissues in leaf sheaths and stems of the host plant, thus disturbing the transport of water and nutrients. Early infection may cause pre- and post-emergence damping-off and seedling shoot death. Infection at later development stages of cereals is seen more often. Severe disease on mature stems may induce lodging and cause premature spike senescence or ripening, which results in the grain shriveling (Clarkson and Cook 1983).

Sharp eyespot has not been considered one of the most important diseases of cereals. This disease usually had little effect on yield. Locally, however, because of its increased incidence, considerable yield losses could occur (Cromey *et al*. 2002), particularly in cultivars susceptible to *R. cerealis* (Clarkson and Cook 1983).

Until 30 years ago, *R. cerealis* had been reported as a pathogen of wheat in only a few regions of the world. Since then, the disease appears to have become gradually more severe in areas of wheat, rice and grass cropping in China, Italy, New Zealand, Russia, Turkey, the Ukraine and the United Kingdom (Rossi *et al*. 1995; Colbach *et al*. 1997; Kryuchkova 2000; Etheridge *et al*. 2001; Cromey *et al*. 2002; Tunali *et al*. 2008; Zhalieva 2008; Chen *et al*. 2010; Hamada *et al*. 2011). The increase has been mostly related

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to a wider distribution of plants, including cultivars of wheat, susceptible to *R. cerealis* (Hamada *et al*. 2011).

The disease usually occurs on winter wheat (Żółtańska 2005; Kurowski and Adamiak 2007; Lemańczyk 2012b). *R. cerealis* attacks numerous *Poaceae* species, including rye, barley and oats, although wheat appears most susceptible. Sharp eyespot has recently been found to occur in winter triticale (x *Triticosecale* Wittmack) in Poland (Lemańczyk 2010a). The possibility of occurrence of *Rhizoctonia* on triticale in Poland was reported by Wachowska (2000). In Poland, a recent increase in sharp eyespot incidence has been observed particularly on winter triticale and more rarely on spring triticale (Lemańczyk 2010a, b).

Two possible strategies for decreasing sharp eyespot are application of fungicides and breeding for resistance (Bateman *et al*. 2000; Hamada *et al*. 2011; Li *et al*. 2011). The former strategy may be less satisfactory because of the possibility of resistance development. The latter strategy is considered safer. So far, however, no cereal cultivars with permanent, stable and environmentally-independent resistance to *R. cerealis* have been developed. No cereal cultivars entirely resistant to sharp eyespot are listed in the Polish Cultivar Registration Book (Tokarski 2011). Traditional breeding techniques have so far not been useful, particularly since no mechanisms of resistance to *Rhizoctonia* have yet been identified. The advent of modern techniques of genetic modification, being developed mainly in wheat breeding (Ren *et al*. 2007), raises some hopes (Li *et al*. 2011).

There is no information on the susceptibility of triticale to *R. cerealis*. The aim of this research was to survey and compare: (i) commercial crops of winter triticale grown in Poland from the point of view of incidence and severity of sharp eyespot, (ii) cultivars of winter triticale from the point of view of their susceptibility to *R. cerealis* and *R. solani*.

MATERIALS AND METHODS

Field survey

In the 2006–2010 time period, commercial crops of the 36 winter triticale cultivars were surveyed at four locations: Chrząstowo, Dębina, Kończewice and Minikowo in north-central Poland (Kuyavia-Pomerania and the Vistula Marshlands). The locations were 15–150 km apart (Fig. 1, Table 1). The surveyed cultivars were recommended for growing in north and central Poland. The triticale seed was drilled in the last 10 days of September.

Incidence and severity of sharp eyespot were determined at the milk ripe stage (GS 75–77 – Zadoks *et al*. 1974). One hundred plants were collected at random along a diagonal transect across each of four plots (11 m x 1.5 m each) (only one plot at Minikowo) located at similar intervals along a diagonal transect across each field. Disease on sampled plants was assessed from symptoms on leaf sheaths and stems of each main shoot and tiller, according to a 0–4 severity scale (Lemańczyk 2012a). The percentage of diseased stems in each score category was recorded. A disease index (DI) was calculated on a % scale using the Townsend and Heuberger formula (Wenzel 1948).

Fig. 1. Location of surveyed fields in Pomerania (with Vistula Marshland at the top right, Dębina) and Kuyavia-Pomerania

Weather data, soil type, and management procedures for each location are presented in figure 1.

Isolation and identification of fungi

In 2007, mycological analysis of 100 stem pieces which were 2 cm-long and cut from 100 plants of each cultivar, was done. Pieces of stem collected at the milk ripe stage (GS 75–77) were rinsed for 45 min in running water, disinfected in a 1% $AgNO_3$ solution for 15 s and rinsed three times for 1 min in sterile distilled water. Then, the pieces were placed on potato dextrose agar (PDA; 40 g filtered white potatoes, 20 g agar, 1 l distilled water, 50 mg of streptomycin per 1 l of medium, pH = 7) in Petri dishes. Fungi were counted. Representative cultures were identified by their morphology on PDA and synthetic nutrient agar (SNA; 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose, 20 g agar, 10 mg chlorotetracycline, 50 mg dihydrostreptomycin sulphate, 1 l distilled water) using available literature. Hyphal staining was used to help identify the *Rhizoctonia* species (Bandoni 1979).

To confirm the correct identification of the *Rhizoctonia* isolates based on their morphology, the polymerase chain reaction (PCR) was performed using the specific Sequence Characterized Amplified Region (SCAR) primers Rc2 F/R for *R. cerealis* (Nicholson and Parry 1996) and ITS1/GMRS–3 for *R. solani* (Johanson *et al*. 1998). Total DNA was isolated using the modified method of Doyle and Doyle (1990). Amplification reaction was performed with the *Taq* PCR Core Kit (QIAGEN Inc., USA).

The frequency (defined as percentage of isolates of an individual species in the total number of fungal isolates) of *Rhizoctonia* species in each fungal community was determined.

Table 1. Weather data, soil type, and management procedures for triticale crops in north-central Poland in the 2005–2010 time period

Location	Chrząstowo			Dębina			Kończewice			Minikowo		
Geographic coordinates	53°09′N 17°35′E			54°07′N 19°02′E			53°11′N 17°33′E			53°10′N 17°44′E		
Weather conditions	average temp. [°C]	precipitation [mm]		average temp. [°C]	precipitation [mm]		average temp. [°C]	precipitation [mm]		average temp. [°C]	precipitation [mm]	
2005/2006	–	–		6.1	434		7.2	378		7.1	372	
2006/2007	9.4	590		9.1	670		9.4	629		9.6	591	
2007/2008	7.9	387		7.6	531		8.5	406		8.0	343	
2008/2009	7.3	508		–	–		–	–		7.6	423	
2009/2010	6.7	471		–	–		–	–		–	–	
Kind o soil	silty loam			silty loam			loam			loam		
Preceding crop	yellow lupin – 2007, 2008, 2010 narrow-leaved lupin – 2009			winter oilseed rape			winter oilseed rape			winter oilseed rape		
Level of mineral fertilization [kg/ha]	N	P	K	N	P	K	N	P	K	N	P	K
2005/2006	–	–	–	142	38	50	103	25	47	103	30	47
2006/2007	120	36	54	167	40	70	105	25	47	105	25	47
2007/2008	120	31	47	153	34	28	110	30	54	110	30	50
2008/2009	120	53	76	–	–	–	–	–	–	108	25	47
2009/2010	120	35	70	–	–	–	–	–	–	–	–	–
Fungicide[1] applied												
2006 GS 30–31[2]	–			Alert 375 SC			Alert 375 SC			Charisma 207 EC		
GS 49–59	–			Charisma 207 EC			Amistar 250 SC			Amistar 250 SC		
2007 GS 30–31	Tilt Plus 400 EC + Sarfun 500 SC			Wirtuoz 520 EC			Alert 375 SC			Juwel TT 483 SE		
GS 49–59	Amistar 250 SC + Artea 330 EC			Escudo Forte 375 CS			Amistar 250 SC			Amistar 250 SC		
2008 GS 30–31	Tilt Plus 400 EC + Sarfun 500 SC			Escudo Forte 375 CS			Tilt Plus 400 EC			Charisma 207 EC		
GS 49–59	Amistar 250 SC + Artea 330 EC			–			Artea 330 EC			Amistar 250 SC		
2009 GS 30–31	Alert 375 SC			–			–			Opera Max 147,5 SE		
GS 49–59	Amistar 250 SC + Artea 330 EC			–			–			Fandango 200 EC		
2010 GS 30–31	Mirage 450 EC			–			–			–		
GS 49–59	Amistar 250 SC + Artea 330 EC			–			–			–		

[1] fungicide active ingredients:
Alert 375 SC (125 g/l flusilazole + 250 g/l carbendazim), DuPont de Nemours (France) S.A.S.
Amistar 250 SC (250 g/l azoxystrobin), Syngenta Crop Protection AG, Switzerland
Artea 330 EC (250 g/l propiconazole + 80 g/l cyproconazole), Syngenta Crop Protection AG, Switzerland
Charisma 207 EC (106.7 g/l flusilazole + 100 g/l famoxadone), DuPont de Nemours (France) S.A.S.
Escudo Forte 375 CS (250 g/l flusilazole + 125 g/l carbendazim), DuPont de Nemours (France) S.A.S.
Fandango 200 EC (100 g/l prothioconazole + 100 g/l fluoxastrobin), Bayer CropScience AG, Germany
Juwel TT 483 SE (83 g/l epoxiconazole + 83 g/l kresoxim-methyl + 317 g/l fenpropimorph), BASF SE, Germany
Mirage 450 EC (450 g/l prochloraz), Makhteshim Agan, Israel
Opera Max 147, 5 SE (85 g/l pyraclostrobin + 62.5 g/l epoxiconazole), BASF SE, Germany
Reveller 280 SC (200 g/l picoxystrobin + 80 g/l cyproconazole), DuPont, Switzerland
Sarfun 500 SC (500 g/l carbendazim), Sarzyna, Poland
Tilt Plus 400 EC (275 g/l fenpropidin + 120 g/l propiconazole), Syngenta Crop Protection AG, Switzerland
Wirtuoz 520 EC (320 g/l prochloraz + 160 g/l tebuconazole + 40 g/l proquinazid), DuPont, Switzerland

[2] fungicides were applied at the beginning of stem elongation (GS 30–31) and during heading (GS 49–59)

Comparing susceptibilities of triticale cultivars to *R. cerealis* and *R. solani* in laboratory pathogenicity tests

Mycelium of *R. cerealis* (Ww 542, belonging to AG-D subgroup I) and *R. solani* (Ww 11, belonging to group AG-5) grown on PDA was used to inoculate germinating grains of triticale. Both *Rhizoctonia* isolates were obtained from winter wheat stems. In preliminary pathogenicity tests, *Rhizoctonia* isolates were more virulent than other isolates on various cereals. Both isolates are in the Culture Collection of the Department of Phytopathology and Molecular Mycology, the University of Technology and Life Sciences in Bydgoszcz, Poland.

Grain of each of the 30 cultivars was surface-disinfected in mercury (II) chloride (1% $HgCl_2$) for 5 min, rinsed in sterile distilled water 6x10 min, placed on sterile wet blotting paper in Petri dishes and incubated at 20–22°C for 72 h. Germinated grains were transferred to 10-mm PDA discs, cut from 10-day-old *R. cerealis* or *R. solani* cultures, on three sheets of sterile, wet (with 16 ml of water) blotting paper in Petri dishes (200x30 mm) and incubated at 20°C. Infection on coleoptiles, leaves, and roots was assessed after 14 days for *R. cerealis* and after 10 days for *R. solani* according to a 0–4 severity scale (Demirci 1998). A disease index (DI) was calculated on a % scale using the Townsend and Heuberger formula (Wenzel 1948). Each *Rhizoctonia* isolate was tested on 80 grains of each cultivar in four Petri dishes.

Statistical analyses

Data for sharp eyespot incidence (percentage of diseased plants) and disease severity (disease index), after arcsin square-root transformation, were subjected to one-way analysis of variance (ANOVA) with a random variable. After proving significant differences between triticale genotypes ($p \leq 0.05$), the k-means analysis was applied. This grouped 30 triticale cultivars into six categories according to their susceptibility (low, moderate and high) to *R. cerealis* and *R. solani.* Calculations were based on disease indices for coleoptiles, leaves, and roots.

Coefficients of variation (C_V%) were calculated to compare extent of variability among cultivars and among years in each location according to the formula:

$$c = \frac{\sigma}{\mu}$$

where:
σ – standard deviation
μ – mean

This shows the extent of variability in relation to the mean of the population. Because of the considerable variation in particular years and different numbers of cultivars evaluated, values were converted to the T scale (McCall 1922; Clarke 1984; Gondko *et al*. 1994; Wardlaw 2000; Kotwica 2008); using the formulae:

$$T = 10\,Z + 50$$

$$Z = (d_i - d_x)/S$$

$$d_i = (x_i - x)/n$$

where:
Z – standardization
d_i – deviation for cultivar
d_x – average deviation
S – standard deviation
x_i – mean from all years' DI for cultivar
x – overall mean of DI from years and cultivars
n – total number of cultivars

Coefficients of Pearson's correlation were calculated to compare the relationship between the intensity of sharp eyespot on different plant parts (coleoptiles, leaves, roots) and susceptibility of seedlings to infection by *R. cerealis* and *R. solani.* The statistical analyses were done with Excel 2007 (Microsoft Corporation) or with Statistica v. 10 (StatSoft Polska).

RESULTS

Field survey

There was much variation among years and locations as far as incidence and severity of sharp eyespot were concerned. The greatest intensity of the disease was observed at Chrząstowo, where the mean percentage of diseased stems on 28 triticale cultivars was 2.6–35.7 (–55.0), and the mean disease index was 0.7–15.6 (–24.5). The lowest and highest values were in 2008 and 2009, respectively (Table 2). The intensity of the disease was very slight at: (i) Dębina, where the mean percentage of diseased stems was 3.67–10.0 (–15.0) on 10 triticale cultivars. The mean disease index was 1.02–2.53 (–3.8), with no symptoms in 2006 and highest values in 2008 (Table 3); (ii) Kończewice, where the mean percentage of diseased stems was 2.67–4.67 (–9.0) on 11 triticale cultivars. The mean disease index was 0.72–1.30 (–3.0), with the lowest and highest values in 2006 and 2007, respectively (Table 4). Disease occurred the least at Minikowo, where the mean percentage of diseased stems was 1.00–4.57 (–18.0) on 23 triticale cultivars. The mean disease index was 0.33–1.36 (–6.3), with the lowest and highest values in 2006 and 2007, respectively (Table 5). Figure 2 presents the disease severity (DI) in particular years and locations (see top, median, and bottom quartiles).

At all four locations, there was a wide variation in disease on individual triticale cultivars over the five year period (2006–2010, based on the disease index). This was indicated by the coefficient of variation values, which were 65.1–154.5% at Chrząstowo, 98.7–141.4% at Dębina, 0–141.4% at Kończewice, and 55.0–156.3% at Minikowo (Tables 2–5). The disease was least intense on cvs Tulus and Atletico at Chrząstowo, Grenado and Zorro at Dębina, Krakowiak and Tornado at Kończewice, and Woltario and Constans at Minikowo. The disease was most intense on cv. Alekto at Chrząstowo, Pawo at Kończewice, and Borwo at Minikowo (see T scale, Tables 2, 4, 5).

Less variation in disease (based on the disease index) among years occurred at Chrząstowo (C_V% = 41.0–56.3%) than at Dębina, Kończewice or Minikowo, which had C_V% = 0–89.2%, C_V% = 46.2–132.2% and C_V% = 66.2–150.2%, respectively (Tables 2–5).

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Table 2. Incidence and severity of sharp eyespot on stem base of winter triticale, at Chrząstowo, in the 2007–2010 time period

No.	Cultivar	Percentage [%] of stems with sharp eyespot symptoms							Disease index [%]						
		2007	2008	2009	2010	mean	C_V%	T scale	2007	2008	2009	2010	mean	C_V%	T scale
1.	Alekto	–	–	52.0	21.0	36.5	60.1	71.8	–	–	22.3	7.8	15.1	68.1	69.6
2.	Algoso	–	–	30.0	15.0	22.5	47.1	51.4	–	–	16.3	5.5	10.9	70.1	58.1
3.	Aliko	35.0	4.0	29.0	16.0	21.0	65.9	49.2	11.0	1.0	10.0	5.5	6.9	66.8	47.0
4.	Atletico	–	–	–	10.0	10.0	–	33.2	–	–	–	2.8	2.8	–	35.8
5.	Baltiko	6.0	–	21.0	18.0	15.0	52.9	40.5	1.5	–	10.3	5.3	5.7	77.4	43.7
6.	Borwo	–	–	52.0	7.0	29.5	107.9	61.6	–	–	21.5	2.3	11.9	114.1	60.8
7.	Fidelio	24.0	1.0	–	–	12.5	130.1	36.8	6.8	0.3	–	–	3.6	129.5	38.0
8.	Gniewko	35.0	4.0	20.0	20.0	19.8	63.9	47.5	12.5	1.0	8.5	7.3	7.3	65.1	48.1
9.	Grenado	–	4.0	33.0	4.0	13.7	122.2	38.6	–	1.0	12.3	1.0	4.8	136.9	41.3
10.	Hewo	21.0	3.0	–	–	12.0	106.1	36.1	5.8	0.8	–	–	3.3	107.1	37.1
11.	Hortenso	25.0	1.0	43.0	17.0	21.5	81.2	49.9	8.5	0.3	20.5	6.0	8.8	96.4	52.3
12.	Janko	27.0	0	–	–	13.5	141.4	38.3	8.0	0	–	–	4.0	141.4	39.1
13.	Kazo	40.0	1.0	–	–	20.5	134.5	48.5	13.0	0.3	–	–	6.7	135.0	46.5
14.	Leontino	–	–	53.0	8.0	30.5	104.3	63.0	–	–	24.0	3.0	13.5	110.0	65.2
15.	Magnat	31.0	4.0	30.0	–	21.7	70.5	50.2	9.3	1.0	10.3	–	6.9	74.3	47.0
16.	Moderato	54.0	3.0	4.0	7.0	17.0	145.4	43.4	22.0	0.8	1.0	2.8	6.7	154.5	46.5
17.	Pawo	52.0	4.0	43.0	13.0	28.0	82.5	59.4	16.0	1.5	21.0	3.8	10.6	89.1	57.2
18.	Pigmej	–	–	34.0	17.0	25.5	47.1	55.8	–	–	15.0	4.8	9.9	72.9	55.3
19.	Pizarro	–	–	55.0	16.0	35.5	77.7	70.3	–	–	24.5	4.8	14.7	95.1	68.5
20.	Sorento	24.0	3.0	44.0	20.0	22.8	73.9	51.8	8.3	0.8	18.3	6.8	8.6	84.9	51.7
21.	Todan	40.0	3.0	35.0	19.0	24.3	69.0	54.0	11.8	0.8	15.5	6.5	8.7	74.1	52.0
22.	Tornado	16.0	–	–	–	16.0	–	41.9	5.0	–	–	–	5.0	–	41.8
23.	Trigold	–	–	41.0	18.0	29.5	55.1	61.6	–	–	22.3	6.8	14.6	75.3	68.2
24.	Trismart	–	–	45.0	1.0	23.0	135.3	52.1	–	–	17.5	0.3	8.9	136.7	52.5
25.	Tulus	–	–	–	19.0	19.0	–	46.3	–	–	–	6.8	2.3	–	34.4
26.	Witon	32.0	1.0	31.0	7.0	17.8	90.3	44.5	9.5	0.3	14.5	3.0	6.8	93.9	46.8
27.	Woltario	35.0	3.0	18.0	–	18.7	85.6	45.9	12.5	0.8	7.0	–	6.8	86.5	46.8
28.	Zorro	26.0	–	–	–	26.0	–	56.5	7.5	–	–	–	7.5	–	48.7
	Mean	30.8	2.6	35.7	13.7	20.7	74.0		9.9	0.7	15.6	4.6	7.7	84.1	
	C_V%	39.0	54.0	37.6	44.2	33.2			47.0	56.3	41.0	47.0	44.9		

C_V% – coefficients of variation; T scale – explained in materials and methods

Table 3. Incidence and severity of sharp eyespot on stem base of winter triticale, at Dębina, in the 2006–2010 time period

No.	Cultivar	Percentage [%] of stems with sharp eyespot symptoms						Disease index [%]					
		2006	2007	2008	mean	C_V%	T scale	2006	2007	2008	mean	C_V%	T scale
1.	Baltiko	0	2.0	15.0	5.67	143.6	64.2	0	0.8	3.8	1.50	130.7	64.1
2.	Dinaro	0	3.0	–	1.50	141.4	44.9	0	0.8	–	0.38	141.4	44.7
3.	Fidelio	0	9.0	–	4.50	141.4	58.8	0	2.5	–	1.25	141.4	59.8
4.	Grenado	0	0	–	0	–	38.0	0	0	–	0	–	38.2
5.	Madilio	–	2.0	–	2.00	–	47.2	–	0.5	–	0.50	–	46.8
6.	Magnat	0	2.0	5.0	2.33	108.0	48.7	0	0.5	1.3	0.58	109.3	48.2
7.	Moderato	0	9.0	–	4.50	141.4	58.8	0	2.5	–	1.25	141.4	59.8
8.	Sorento	0	1.0	–	0.50	141.4	40.3	0	0.3	–	0.13	141.4	40.4
9.	Woltario	0	5.0	10.0	5.00	100.0	61.1	0	1.3	2.5	1.25	98.7	59.8
10.	Zorro	0	–	–	0	–	38.0	0	–	–	0	–	38.2
	Mean	0	3.67	10.00	4.56	111.0		0	1.02	2.53	1.19	107.5	
	C_V%	–	90.5	50.0	47.4			0	89.2	49.4	48.8		

C_V% – coefficients of variation; T scale – explained in materials and methods

Table 4. Incidence and severity of sharp eyespot on stem base of winter triticale, at Kończewice, in the 2006–2010 time period

No.	Cultivar	Percentage [%] of stems with sharp eyespot symptoms						Disease index [%]					
		2006	2007	2008	mean	C_V%	T scale	2006	2007	2008	mean	C_V%	T scale
1.	Aliko	–	4.0	4.0	4.00	0	55.4	–	1.0	1.0	1.00	0	54.2
2.	Borwo	–	–	3.0	3.00	–	49.8	–	–	0.8	0.75	–	49.3
3.	Gutek	–	4.0	–	4.00	–	55.4	–	1.0	–	1.00	–	54.2
4.	Hewo	0	6.0	–	3.00	141.4	49.8	0	1.5	–	0.75	141.4	49.3
5.	Janko	2.0	–	–	2.00	–	44.3	0.5	–	–	0.50	–	44.4
6.	Krakowiak	0	–	–	0	–	33.1	0	–	–	0	–	34.5
7.	Pawo	7.0	9.0	2.0	6.00	60.1	66.6	2.0	3.0	0.5	1.83	68.8	70.5
8.	Pigmej	–	–	4.0	4.00	–	55.4	–	–	1.0	1.00	–	54.2
9.	Todan	–	3.0	5.0	4.00	35.4	55.4	–	0.8	1.3	1.00	33.7	54.2
10.	Tornado	0	–	–	0	–	33.1	0	–	–	0	–	34.5
11.	Witon	7.0	2.0	1.0	3.33	96.5	51.7	1.8	0.5	0.3	0.83	93.6	50.8
	Mean	2.67	4.67	3.17	3.50	29.7		0.72	1.30	0.82	0.94	33.3	
	C_V%	129.0	53.6	46.4	35.4			132.2	69.3	46.3	38.2		

C_V% – coefficients of variation; T scale – explained in materials and methods

Table 5. Incidence and severity of sharp eyespot on stem base of winter triticale, at Minikowo, in the 2006–2010 time period

No.	Cultivar	Percentage [%] of stems with sharp eyespot symptoms							Disease index [%]						
		2006	2007	2008	2009	mean	C_V%	T scale	2006	2007	2008	2009	mean	C_V%	T scale
1.	Alekto	–	–	–	4.0	4.00	–	56.4	–	–	–	1.3	1.30	–	57.4
2.	Algoso	–	–	2.0	0	1.00	141.4	40.6	–	–	0.5	0	0.25	141.4	40.7
3.	Aliko	2.0	1.0	1.0	–	1.33	43.3	42.3	1.0	0.3	0.3	–	0.53	75.8	45.2
4.	Baltiko	–	3.0	1.0	–	2.00	70.7	45.9	–	0.8	0.3	–	0.55	64.3	45.5
5.	Borwo	–	–	–	7.0	7.00	–	72.3	–	–	–	2.5	2.50	–	76.4
6.	Constant	–	–	1.0	0	0.50	141.4	37.9	–	–	0.3	0	0.15	141.4	39.1
7.	Fidelio	0	0	2.0	–	0.67	115.5	38.8	–	0	0.5	–	0.25	141.4	40.7
8.	Fleurs	–	–	2.0	–	2.00	–	45.9	–		0.5	–	0.50	–	44.7
9.	Gniewko	–	18.0	2.0	1.0	7.00	136.3	72.3	–	6.3	0.5	0.3	2.37	144.0	74.3
10.	Grenado	2.0	3.0	3.0	–	2.67	21.7	49.4	0.5	1.5	0.8	–	0.93	55.0	51.5
11.	Hortenso	0	11.0	1.0	1.0	3.25	159.6	52.5	0	3.0	0.3	0.3	0.90	156.3	51.0
12.	Kazo	2.0	5.0	–	–	3.50	60.6	53.8	0.5	1.3	–	–	0.90	62.9	51.0
13.	Madilo	–	–	2.5	0	1.25	141.4	41.9	–	–	0.8	0	0.40	141.4	43.1
14.	Magnat	1.0	2.0	3.5	–	2.17	65.5	46.8	0.3	0.5	1.0	–	0.60	60.1	46.3
15.	Mieszko	–	–	3.5	–	3.50	–	53.8	–	–	1.3	–	1.30	–	57.4
16.	Moderato	0	4.0	1.0	–	1.67	124.9	44.1	0	1.0	0.3	–	0.43	118.4	43.6
17.	Pigmej	–	–	–	1.0	1.00	–	40.6	–	–	–	0.3	0.30	–	41.5
18.	Sorento	–	5.0	–	–	5.00	–	61.7	–	1.3	–	–	1.30	–	57.4
19.	Todan	–	4.0	–	–	4.00	–	56.4	–	1.0	–	–	1.00	–	52.6
20.	Trismart	–	–	5.0	–	5.00	–	61.7	–	–	1.3	–	1.30	–	57.4
21.	Witon	–	3.0	–	–	3.00	–	51.1	–	0.8		–	0.80	–	49.4
22.	Woltario	–	1.0	0	–	0.50	141.4	37.9	–	0.3	0	–	0.15	141.4	39.1
23.	Zorro	0	4.0	–	–	2.00	141.4	45.9	0	1.0	–	–	0.50	141.4	44.7
	Mean	1.00	4.57	2.13	1.75	2.36	65.4		0.33	1.36	0.58	0.59	0.72	62.8	
	C_V%	99.1	102.1	66.0	142.4	80.1			113.5	116.7	66.2	150.2	88.1		

C_V% – coefficients of variation; T scale – explained in materials and methods

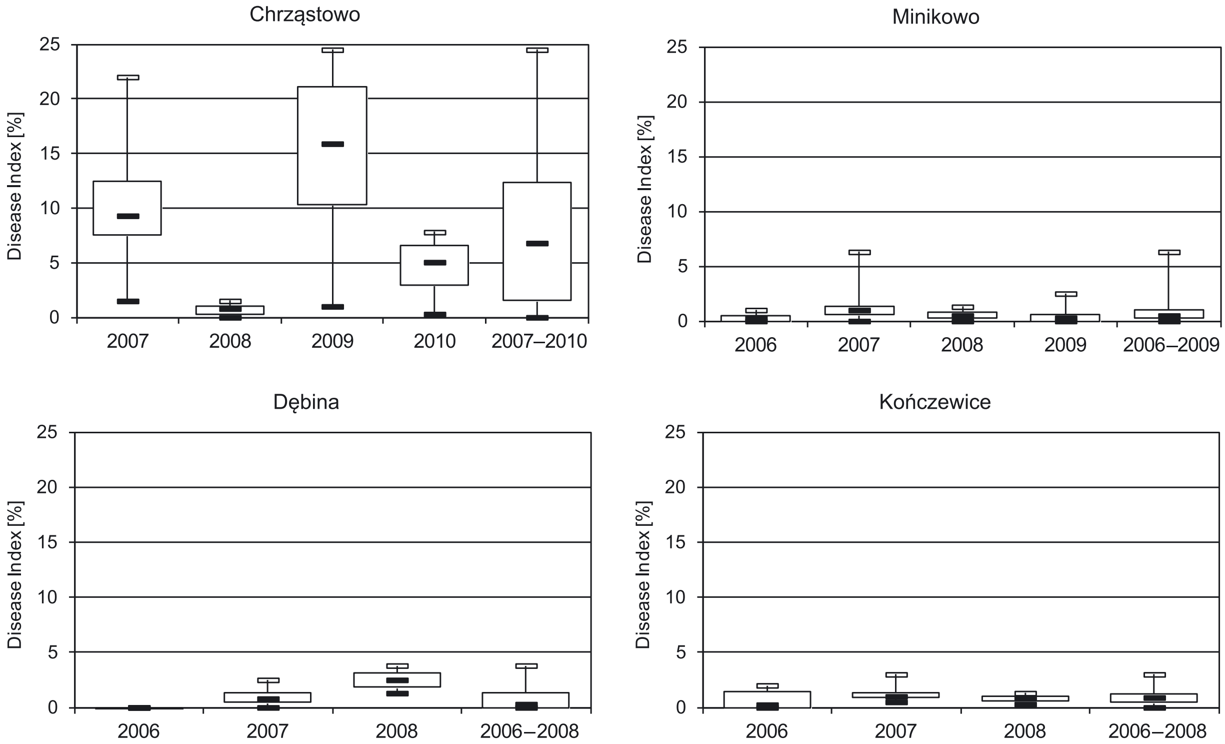

Fig. 2. Sharp eyespot severity in different years and locations (top, median, and bottom quartiles)

Isolation and identification of fungi

The *in vitro* growth rate and morphology of mycelium isolated from lesions confirmed the initial diagnosis of sharp eyespot made in the field. Mostly *R. cerealis* was isolated from triticale stems (Fig. 3). The highest percentage of stems colonized by *R. cerealis* was recorded at Chrząstowo, particularly on cvs Moderato (39%), Todan (29%) and Pawo (23%). The percentages of stems colonized by *R. cerealis* at Dębina (0–2%), Kończewice (0–7%) and Minikowo (0–3%) were much smaller. *Rhizoctonia solani* was isolated only sporadically. Its frequency in the fungal community was only 0–1% at Chrząstowo, Dębina and Minikowo, and 0–2% at Kończewice. The *Rhizoctonia* species were not isolated from cv. Baltiko at any location.

The PCR (polimeraze chain reaction) primers specific for *R. cerealis* (SCAR Rc2 F/R) and *R. solani* (ITS1/GMRS–3) produced amplified DNA products from stem base samples and from the isolated *Rhizoctonia* species of 800-bp and 550-bp, respectively. This confirmed the identification based on morphology.

Comparing susceptibilities of triticale cultivars to *R. cerealis* and *R. solani* in laboratory pathogenicity tests

Most triticale plants inoculated with *Rhizoctonia* produced symptoms typical of sharp eyespot. Symptoms developed mostly on coleoptiles. There were fewer symptoms on leaves and the least on roots. Coleoptiles and leaves inoculated with *R. cerealis* showed elliptical, cream-tan coloured, silver-grey or brown lesions with sharply defined dark brown borders. Sometimes coleoptile lesions enlarged and formed irregular girdling patches. Coleoptiles of plants inoculated with *R. solani* were uniformly brown or showed elliptical, brown lesions with silver-grey centres and with no defined dark brown borders. Lesions on leaves were elongated with no defined borders. Roots of plants inoculated with either of the *Rhizoctonia* species became brown and rotted. Infection of coleoptiles and leaves resulted in seedling death. Death occurred much more quickly after inoculation with *R. solani* than with *R. cerealis*. This explains the difference in the duration of the incubation period before disease assessment, *i.e.* 10 days for *R. solani* and 14 days for *R. cerealis*. Koch's postulates were fulfilled by re-isolation of the *Rhizoctonia* isolates used for inoculation.

The amount of the disease resulting from the inoculation with each *Rhizoctonia* species, varied quite a bit among triticale cultivars. After inoculation with *R. cerealis*, disease indices were 38.8–92.9% for coleoptiles, 15.8–81.7% for leaves and 18.8–61.3% for roots (Table 6). *R. solani* resulted in disease indices of 30.8–88.3% for coleoptiles, 12.3–77.0% for leaves and 1.3–18.8% for roots (Table 7).

Triticale cultivars were grouped into six categories. The grouping was according to the severity of disease on coleoptiles, leaves, and roots of seedlings resulting from inoculation with *R. cerealis* or *R. solani*. Categories 1, 2 and 3, which indicate low, moderate and high levels of disease caused by *R. cerealis*, included 17, 10 and 3 cultivars, respectively (Table 8). Categories 4, 5 and 6, which indicate low, moderate and high levels of disease caused by *R. solani*, included 3, 12 and 15 cultivars, respectively (Table 9). Cultivars Baltiko and Zorro developed low levels of the disease after inoculation with each *Rhizoctonia* species, and cv. Cultivo developed high levels.

There was a significant positive correlation between disease on coleoptiles caused by *R. cerealis* and *R. solani*

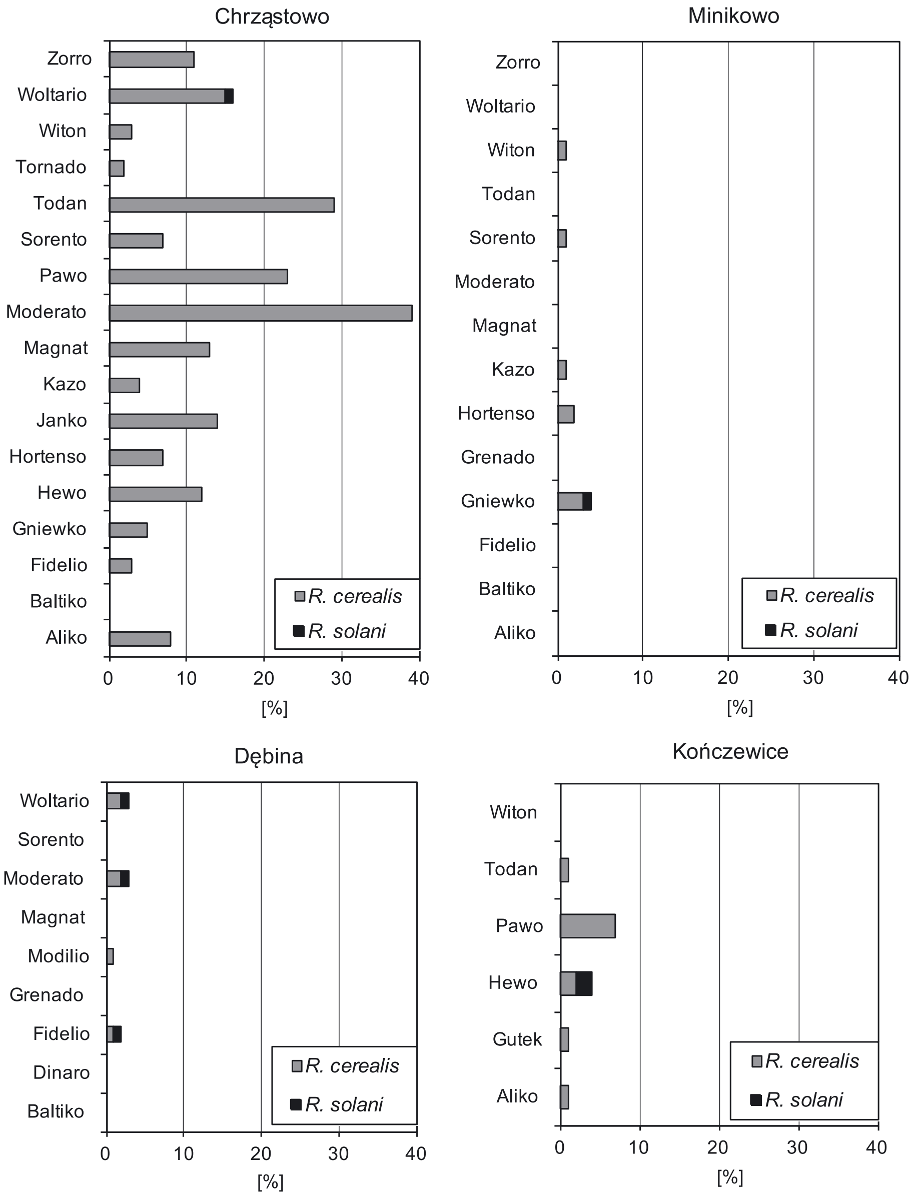

Fig. 3. Frequency of *R. cerealis* and *R. solani* in fungal communities in triticale stems with sharp eyespot symptoms, at Chrząstowo, Dębina, Kończewice, and Minikowo, in 2007

Table 6. Disease on triticale cultivars after inoculation of coleoptiles, leaves, and roots with *R. cerealis*

No.	Cultivar	Coleoptile		Leaf		Root	
		% seedlings infected	DI [%]	% seedlings infected	DI [%]	% seedlings infected	DI [%]
1	2	3	4	5	6	7	8
1.	Alekto	96.7	66.3	76.7	42.0	85.0	27.5
2.	Algoso	100.0	73.3	71.7	24.9	100.0	46.3
3.	Aliko	100.0	68.8	85.0	37.7	98.3	31.7
4.	Atletico	100.0	92.9	98.3	81.7	100.0	61.3
5.	Baltiko	100.0	54.2	60.0	15.8	100.0	38.8
6.	Borwo	100.0	53.8	68.3	28.5	98.3	29.2
7.	Cultivo	100.0	80.4	93.3	51.5	100.0	49.6
8.	Dinaro	100.0	66.3	58.3	33.8	98.3	39.2
9.	Fidelio	100.0	74.2	75.0	33.7	100.0	41.7
10.	Gniewko	100.0	65.4	90.0	28.7	100.0	45.0
11.	Grenado	100.0	52.9	71.7	26.1	98.3	29.6
12.	Hewo	100.0	72.9	66.7	24.7	100.0	35.8
13.	Hortenso	100.0	63.8	56.7	16.8	100.0	35.4
14.	Janko	100.0	63.8	58.3	21.3	100.0	41.7

1	2	3	4	5	6	7	8
15.	Kazo	100.0	48.3	81.7	20.6	91.7	33.8
16.	Leontino	98.3	74.2	83.3	46.8	98.3	30.0
17.	Magnat	100.0	62.9	93.3	27.3	96.7	32.9
18.	Moderato	100.0	62.9	78.3	33.3	91.7	30.4
19.	Pawo	100.0	59.2	85.0	28.9	98.3	28.8
20.	Pigmej	100.0	90.4	95.0	62.4	98.3	31.3
21.	Pizzaro	100.0	79.6	91.7	48.7	96.7	27.1
22.	Sorento	100.0	57.5	58.3	21.3	100.0	52.9
23.	Todan	98.3	53.8	78.3	29.4	96.7	32.9
24.	Tornado	100.0	64.2	58.3	21.3	91.7	33.8
25.	Trigold	98.3	79.6	76.7	39.4	95.0	28.3
26.	Trismart	100.0	55.8	43.3	17.7	100.0	41.7
27.	Tulus	100.0	75.0	100.0	52.1	71.7	22.1
28.	Witon	100.0	64.2	86.7	31.8	93.3	29.6
29.	Woltario	100.0	62.1	70.0	27.1	100.0	33.8
30.	Zorro	96.7	38.8	65.0	18.3	63.3	18.8
	Mean	99.6	65.9	75.8	33.1	95.4	35.4
	F-ratio	1.24	11.36*	6.34*	9.98*	4.66*	10.08*

*significant at $p < 0.001$; DI – disease index

Table 7. Disease on triticale cultivars after inoculation of coleoptiles, leaves, and roots with *R. solani*

No.	Cultivar	Coleoptile		Leaf		Root	
		% seedlings infected	DI [%]	% seedlings infected	DI [%]	% seedlings infected	DI [%]
1.	Alekto	96.7	59.2	88.3	39.0	41.7	10.4
2.	Algoso	98.3	82.1	93.3	76.7	33.3	9.2
3.	Aliko	98.3	65.4	66.7	44.3	18.3	4.6
4.	Atletico	100.0	71.3	66.7	38.8	36.7	9.2
5.	Baltiko	93.3	45.0	68.3	24.7	18.3	7.5
6.	Borwo	96.7	72.9	81.7	62.5	28.3	7.1
7.	Cultivo	100.0	85.4	95.0	71.8	5.0	1.3
8.	Dinaro	91.7	33.3	25.0	12.3	6.7	1.7
9.	Fidelio	98.3	68.8	75.0	46.8	16.7	4.2
10.	Gniewko	100.0	69.2	93.3	66.8	36.7	9.2
11.	Grenado	100.0	66.3	76.7	50.5	21.7	5.4
12.	Hewo	88.3	67.9	71.7	53.8	30.0	7.9
13.	Hortenso	100.0	75.0	75.0	53.5	8.3	2.1
14.	Janko	98.3	68.8	75.0	46.8	10.0	2.5
15.	Kazo	100.0	77.5	85.0	55.4	11.7	2.9
16.	Leontino	98.3	88.3	90.0	77.0	16.7	4.2
17.	Magnat	98.3	76.7	75.0	58.8	71.7	18.8
18.	Moderato	90.0	70.4	75.0	62.2	8.3	2.1
19.	Pawo	95.0	63.3	53.3	45.0	20.0	5.0
20.	Pigmej	100.0	67.9	75.0	41.8	11.7	2.9
21.	Pizzaro	100.0	79.6	80.0	56.3	28.3	7.1
22.	Sorento	95.0	65.4	61.7	38.8	10.0	2.5
23.	Todan	96.7	72.5	71.7	56.7	20.0	5.0
24.	Tornado	100.0	66.3	53.3	45.0	23.3	5.8
25.	Trigold	100.0	73.3	66.7	37.3	33.3	8.8
26.	Trismart	93.3	76.3	86.7	62.4	50.0	12.9
27.	Tulus	96.7	77.9	85.0	60.8	23.3	5.8
28.	Witon	100.0	75.8	90.0	62.2	23.3	5.8
29.	Woltario	93.3	72.5	85.0	64.3	46.7	11.7
30.	Zorro	88.3	30.8	56.7	33.8	6.7	1.7
	Mean	96.8	68.8	74.7	51.5	23.9	6.2
	F-ratio	2.41*	8.16*	3.88*	5.03*	5.98*	6.19*

*significant at $p < 0.001$; DI – disease index

Table 8. Categories of triticale cultivars based on the susceptibility to *R. cerealis*

	Category 1	Category 2	Category 3	F-ratio
	Low susceptibility	Moderate susceptibility	High susceptibility	
	Algoso, Baltiko, Borwo, Gniewko, Grenado, Hewo, Hortenso, Janko, Kazo, Magnat, Pawo, Sorento, Todan, Tornado, Trismart, Woltario, Zorro	Alekto, Aliko, Dinaro, Fidelio, Leontino, Moderato, Pizzaro, Trigold, Tulus, Witon	Atletico, Cultivo, Pigmej	
Coleoptile	58.9 ± 2.09 a*	71.1 ± 1.96 b	87.9 ± 3.82 c	21.2***
Leaf	23.5 ± 1.12 a	39.3 ± 2.28 b	65.2 ± 8.83 c	54.3***
Roots	39.5 ± 1.93 ab	30.8 ± 1.82 a	47.4 ± 8.73 b	4.97**

*different letters in the same line indicate statistically significant differences

**, ***significant at $p < 0.01$ and $p < 0.001$, respectively

Table 9. Categories of triticale cultivars based on the susceptibility to *R. solani*

	Category 4	Category 5	Category 6	F-ratio
	Low susceptibility	Moderate susceptibility	High susceptibility	
	Baltiko, Dinaro, Zorro	Alekto, Aliko, Atletico, Fidelio, Grenado, Hewo, Pawo, Pigmej, Sorento, Trigold, Janko, Tornado	Algoso, Borwo, Cultivo, Gniewko, Hortenso, Kazo, Leontino, Magnat, Moderato, Pizzaro, Todan, Trismart, Tulus, Witon, Woltario	
Coleoptile	36.4 ± 4.38 a*	66.9 ± 1.05 b	76.8 ± 1.38 c	84.88**
Leaf	23.6 ± 6.23 a	43.9 ± 1.47 b	63.2 ± 1.87 c	54.48**
Roots	3.63 ± 1.93	5.77 ± 0.78	7.01 ± 1.23	1.02

*different letters in the same line indicate statistically significant differences

**significant at $p < 0.001$

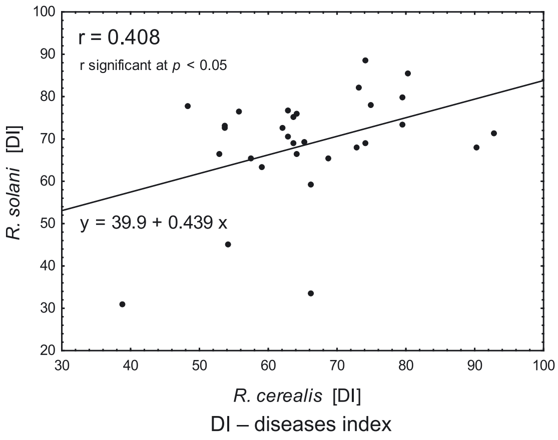

Fig. 4. Correlation between disease on coleoptiles caused by *R. cerealis,* and that caused by *R. solani*

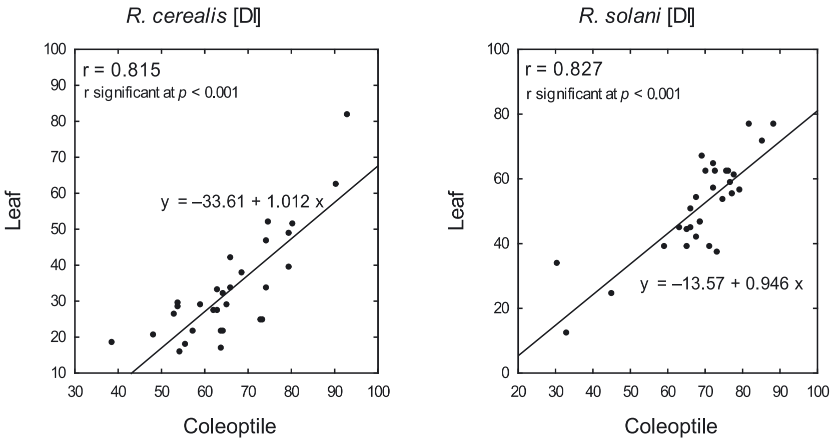

Fig. 5. Correlation between disease on coleoptiles and disease on leaves, caused by *R. cerealis* or *R. solani*

(Fig. 4). Disease on coleoptiles caused by *R. cerealis* or *R. solani* was significantly correlated with disease on the leaves (Fig. 5) but not with that on roots. Disease on the leaves was not correlated with that on the roots.

DISCUSSION

Infection of triticale by *R. cerealis* usually results in a less severe disease display than on wheat (Kurowski 2002; Lemańczyk 2012a). The disease may also occur only locally on triticale. Of the four locations surveyed here, disease was more frequent and severe at Chrząstowo than at the other locations. Geographical differences in the occurrence of sharp eyespot on winter triticale grown commercially were also previously noticed (Lemańczyk 2010a). This can result from the type of rotation, which seems to affect significantly the occurrence and intensity of disease (Colbach *et al*. 1997; Żółtańska 2005; Bockus *et al*. 2010; Lemańczyk 2012b). The lower incidence and severity of disease at Dębina, Kończewice, and Minikowo may have resulted from the use of winter oilseed rape as the preceding crop. Volatiles released from the breakdown of thioglycosides produced by oilseed rape have shown antagonistic activity towards soil-borne pathogens (Larkin *et al*. 2011). Yellow and narrow-leaved lupins, used as the preceding crop at Chrząstowo, do not produce compounds that could inhibit the growth and activity of *Rhizoctonia.* However, preceding crops of neither *Brassicaceae* (*e.g.* oilseed rape) nor *Fabaceae* (*e.g.* lupins) have been found to affect the intensity of sharp eyespot in commercially grown winter triticale (Lemańczyk 2010a).

The smaller amount of nitrogen fertilizer applied at Kończewice and Minikowo does not seem to have contributed to decreased infection by *Rhizoctonia*, as reported by Colbach *et al*. (1997). The amount of disease was similar or less at Dębina, where nitrogen fertilizer had been applied the most.

Differences in disease intensity could also have resulted from the strategies of applied chemical control. Azoxystrobin is reported to have some activity against sharp eyespot (Bateman *et al*. 2000). Amistar 250 SC (containing azoxystrobin) was used at Chrząstowo (2007–2010), Kończewice (2007, 2008) and Minikowo (2006–2008), but no controlling effect was observed (particularly at Chrząstowo and Kończewice). This may be because of the late application of the fungicide. Azoxystrobin applied after inflorescence emergence has little effect on infection by *R. cerealis* (Lemańczyk 2012b). Neither the type of fungicide nor time of its application affected the intensity of sharp eyespot in commercially grown winter triticale (Lemańczyk 2010a).

The higher incidence of sharp eyespot at Chrząstowo in 2009 may have resulted partly from the application of carbendazim (in Alert 375 SC). Its stimulatory effect has previously been noticed (Prew and McIntosh 1975; van der Hoeven and Bollen 1980). Application of Alert 375 SC, at Dębina (2006) and Kończewice (2006, 2007), however, seems to have had no effect. A long-term effect on disease intensity of pesticides applied in the past at the survey locations can not be excluded. Such an effect was observed by Daamen and Stol (1990).

Soil is the main reservoir of *Rhizoctonia*. Differences in soil type may contribute to differences in the scale of infection at different localities. The soil type was similar, however, at Chrząstowo, Dębina, Kończewice and Minikowo and unlikely to have had an effect on disease. The soil type was silty loam or loam, which usually favours the survival and activity of *R. cerealis* (Cromey *et al*. 2002; Bockus *et al*. 2010). Hamada *et al*. (2011) observed, however, that soil temperature, moisture and acidity may also affect the intensity of sharp eyespot. Crop management procedure, including time of sowing, nutrient availability, and cultivation practices are also important.

Considerable differences in disease intensity between years were observed. The most intensive occurrence of disease took place in 2007 and 2009 at Chrząstowo, in 2007 at Kończewice and Minikowo, and in 2008 at Dębina. Temporary increases in disease intensity seem to result from more rainfall during plant growth and favourable temperature conditions, *i.e.* warm and wet autumn and winter followed by cold and wet spring. Such conditions favour infection and colonization of plant tissues by *R. cerealis* and result in increased incidence of sharp eyespot (Polley and Thomas 1991; Colbach *et al*. 1997; Cromey *et al*. 2002; Bockus *et al*. 2010).

There were moderate differences between cultivars in the amount of colonization by *R. cerealis* and sharp eyespot development. These differences were also affected by year and locality. The available literature does not provide information on differences in susceptibility of triticale cultivars to *R. cerealis.* Studies have mostly concerned susceptibility of wheat or rice (Bateman *et al*. 2000; Li *et al*. 2011; Liu *et al*. 2011). Nicholson *et al*. (2002), Ray *et al*. (2004) and Matusinsky *et al*. (2008) found differences in DNA concentrations of *R. cerealis* which had colonized different cultivars of wheat. Since observations were not repeatable for different years and localities of experimentation, it was concluded that sharp eyespot intensity did not depend entirely on cultivar (Bateman *et al*. 2000; Nicholson et al. 2002; Ray *et al*. 2004). Cromey *et al*. (2005) also observed much variation in susceptibility of wheat to *Rhizoctonia*, concluding that sharp eyespot intensity was affected by environment and agronomic practices.

Symptoms observed on triticale plants in laboratory trials were consistent with those observed on plants in the field. Inoculation with either *R. cerealis* or *R. solani* resulted in most symptoms occurring on coleoptiles. There were fewer symptoms on the leaves, and the roots had the fewest symptoms. Such occurrences were as expected from the general preferences of both *Rhizoctonia* species. The symptoms produced by *R. solani* were not typical of sharp eyespot. Lesions did not have the sharply defined dark brown borders which are formed on plants infected by *R. cerealis* (Ogoshi *et al*. 1990; Mazzola *et al*. 1996; Demirci 1998; Wachowska 2000; Tewoldemedhin *et al*. 2006; Bockus *et al*. 2010). Different reactions to *R. cerealis* may occur in the field, however. Earlier studies suggested much variation in susceptibility of commercially grown winter triticale to *Rhizoctonia*, but differences were statistically insignificant (Lemańczyk 2010a).

R. cerealis used in the laboratory studies was from AG-D subgroup I. This group is strongly virulent on triticale plants, on which typical sharp eyespot symptoms form after infection (Boerema and Verhoeven 1977; Lipps and Herr 1982; Bockus *et al*. 2010). According to Toda *et al*. (1999), most isolates of *Rhizoctonia* from AG-D subgroup I are strongly virulent on many species of *Poaceae*, including cereals.

The anastomosis groups of *R. solani* that occur and are significant in cereals include AG-2, AG-4, AG-5 (Mazzola *et al*. 1996; Demirci 1998; Okubara *et al*. 2008) and AG-8 (Ogoshi *et al*. 1990; Mazzola *et al*. 1996; Bockus *et al*. 2010). Group AG-5 is currently the most pathogenic on cereals in Europe (Schade-Schütze pers. comm.). Its strong virulence on cereals (Mazzola *et al*. 1996; Demirci 1998; Okubara *et al*. 2008) and its common occurrence determined its use in the present study on triticale cultivars.

Plants inoculated with *R. solani* (Ww 11) produced symptoms 4 days earlier than those inoculated with *R. cerealis* (Ww 542). This prompted the earlier assessment of disease caused by *R. solani*. Lesions formed earlier after inoculation with *R. solani* because of its faster colonization of plant tissues resulting from faster linear growth (Boerema and Verhoeven 1977; Lipps and Herr 1982).

There was much variation in susceptibility to *R. cerealis* and *R. solani* among the studied winter triticale cultivars. Only two cultivars, Baltiko and Zorro, showed low susceptibility to both *Rhizoctonia* species at the seedling stage. Only cv. Cultivo showed similarly high susceptibility to both *Rhizoctonia* species. No cultivar was resistant to *Rhizoctonia.*

Breeding for resistance to *R. cerealis* may be a possible strategy for decreasing incidence of sharp eyespot, but is difficult in triticale because of its polyploidy. There are also difficulties resulting from the character of the pathogen. Mechanisms of resistance to *Rhizoctonia* are still not well recognized. A plant's resistance often results from production of enzyme inhibitors. Resistance to *R. cerealis* in wheat results mainly from the activity of peroxidase (Liu *et al*. 2011). Transgenic wheat lines resistant to *R. cerealis,* with resistance that is environmentally independent and inherited for at least four generations, were developed in China after successfully integrating genes *TiERF1, RsAFP2* and *TaPIEP1* into the wheat genome (Chen *et al*. 2008; Li *et al*. 2011; Liu *et al*. 2011). In Poland only eight wheat breeding lines completely resistant or highly resistant to infection by *Oculimacula acuformis* and *O. yallundae* causing true eyespot were bred. All these cultivars have Pch1 resistance gene (Kwiatek *et al.* 2012).

R. cerealis was more frequent in the fungal communities colonizing diseased stems than was *R. solani*, which occurred only sporadically. This agrees with the general understanding of the behaviour of these *Rhizoctonia* species. *R. cerealis* is the main cause of the sharp eyespot in cereals in Poland while *R. solani* can be considered only as a secondary pathogen (Mazzola *et al*. 1996).

The actual colonization rate of triticale stems by *R. cerealis* in the field was probably greater than detected. There are records of failure to isolate *R. cerealis* from typical symptoms of sharp eyespot. It was concluded that, in such cases, *R. cerealis* was probably suppressed by fast-growing saprotrophs present in rotting stems and dominating on agar isolation media (Lemańczyk 2012a). *R. cerealis* is a cereal-specific pathogen. It grows slowly on agar media, away from the host plant. It is often dominated by *Fusarium* spp. and numerous saprotrophs *in vitro* (Lemańczyk and Sadowski 2002; Kwaśna *et al*. 2010). *R. cerealis* can, however, also be isolated from asymptomatic plants (Lemańczyk 2012a). Nicholson and Parry (1996) and Matusinsky *et al*. (2008) reported the poor correlation of results obtained by two techniques applied for assessment of sharp eyespot on field plants, *i.e.* classical, based on visual identification of symptoms, and molecular, using DNA amplification by PCR to identify the pathogen. Plants with symptoms of other diseases are more often colonized by *R. solani* than by *R. cerealis*. This proves the saprotrophic abilities of the former (Sneh *et al.* 1991).

In conclusion, it should be emphasized that no definite resistance to *R. cerealis* or *R. solani* was identified among the 36 cultivars of winter triticale. Only two cultivars, Baltiko and Zorro, had low susceptibility at the seedling stage to both pathogens. These cultivars reacted with moderate stability at the various localities and years and never developed more than slight disease. They may, therefore, be considered for growing in regions with a high sharp eyespot risk. There was no positive correlation between intensity of sharp eyespot on triticale plants in the field and in laboratory-grown seedlings. Such results suggest that the laboratory response to *Rhizoctonia* may be temporary and changing as plants develop in the field. The positive correlation between infection by *R. cerealis* and *R. solani* indicates that breeding for resistance in triticale can be simultaneous for both *Rhizoctonia* species.

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