www.czasopisma.pan.pl PAN POLSKA AKADEMIA NAUK www.journals.pan.pl

JOURNAL OF PLANT PROTECTION RESEARCH Vol. 52, No. 4 (2012)

DOI: 10.2478/v10045-012-0063-y

EFFECTS OF FARMING SYSTEM, CHEMICAL CONTROL, FERTILIZER AND SOWING DENSITY ON SHARP EYESPOT AND *RHIZOCTONIA* SPP. IN WINTER WHEAT

*Grzegorz Lemańczyk**

University of Technology and Life Sciences
Department of Phytopathology and Molecular Mycology
Kordeckiego 20, 85-225 Bydgoszcz, Poland

Received: March 13, 2012
Accepted: May 17, 2012

Abstract: Effects of agronomic practices on the occurrence of sharp eyespot and *Rhizoctonia* spp. in winter wheat were determined in two field experiments. In Experiment 1, in the village of Osiny, a comparison was made of disease in different farming systems. The farming systems were: organic, integrated, conventional, and monoculture. In Experiment 2, in the village of Mochełek, the effects of different chemical controls (no treatment, herbicide, herbicide + fungicide), mineral fertilizer doses (147 and 221 kg/ha NPK) and sowing densities (400 and 600 grains/m^2) on the occurrence of sharp eyespot were compared in wheat grown in short-term monoculture. There was considerably more sharp eyespot in 2007 (disease index 1.63–29.5%) than in other years. Significant effects of the treatments were mostly noted at the milk ripe growth stage. The fewest sharp eyespot symptoms were seen in the integrated farming system. The most sharp eyespot symptoms were seen in the conventional and organic systems. There was a tendency for an increased intensity of symptoms in successive wheat-growing years of short-term monoculture. The application of pesticides showed no clear effect on the occurrence of sharp eyespot. The herbicide resulted in increased or decreased disease intensity depending on the cultivation year and the date of observation. Fungicide application did not decrease infection. Without chemical control, more symptoms were observed at the lower NPK rate. There were more symptoms at the higher sowing density. Stems with sharp eyespot symptoms were mostly infected by *Rhizoctonia cerealis*, and less frequently by *R. solani*. Binucleate *Rhizoctonia* spp., which could not be identified to species using polymerase chain reaction (PCR) techniques, were also recorded. Two *R. zeae* isolates were also obtained from stems with disease symptoms in Mochełek. *R. solani* was more often isolated from roots or stems with symptoms of true eyespot or fusarium foot rot. Most isolates of *Rhizoctonia* spp. were obtained at the milk ripe stage. A wheat-growing system and chemical control did not greatly affect the frequency of *Rhizoctonia* spp.

Key words: winter wheat, sharp eyespot, *Rhizoctonia cerealis*, *R. solani*, fungicide, herbicide, monoculture, integrated farming, organic farming, fertilizer, sowing density

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the world's second most important cereal after maize, and the most important in Poland. In 2010, the global wheat acreage was 216,8 million ha, and in Poland 2,406 million ha, whereas the production output was 651,4 and 9,488 million tonnes, respectively (http://faostat.fao.org/default.aspx).

One of the diseases affecting wheat is sharp eyespot. This disease is caused by the soil-borne fungus *Rhizoctonia cerealis* van der Hoeven (teleomorph: *Ceratobasidium cereale* D. Murray & L.L. Burpee), which has a wide host range. The pathogen attacks numerous *Poaceae* species, including rye, triticale, barley and oats, but wheat appears most susceptible. The pathogen does not produce asexual spores and survives as sclerotia or mycelium in the soil and on host plant residues. Germinating sclerotia or mycelium growing from plant residue, infect host plant roots or shoots. The attacked leaf sheath of young seedlings are directly penetrated by mycelium developing from the soil. With time the mycelium grows over the leaf sheaths and onto the stems.

In cereals some role is also played by *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* [(A.B. Frank) Donk] which, before 1977, was described as the agent of sharp eyespot. This species can also be isolated from plants with symptoms typical of sharp eyespot. Unlike *R. cerealis*, *R. solani* does not lead to production of a clear brown border around the spots found on infected stems (Boerema and Verhoeven 1977; Mazzola *et al*. 1996).

Sharp eyespot is a cereal disease that occurs throughout most of the world where there are moderate temperature conditions. It does not usually occur in great intensities nor does it generate considerable economic losses. Slight infection does not cause much yield loss. With se-

*Corresponding address:
Grzegorz.Lemanczyk@utp.edu.pl

vere infection, losses can be significant and reach up to 26% (Clarkson and Cook 1983; Cromey *et al*. 2002). *R. cerealis* can result in the destruction of vascular bundles in the stems and leaf sheaths of the host plant, thus disturbing the transport of water and nutrients (Bockus *et al*. 2010).

Increased occurrence and intensity of sharp eyespot may be accounted for by the geographic spread of *R. cerealis* resulting from global environmental changes (Burpee 1980; Hamada *et al*. 2011a). Another cause may be the increased cultivation of plants (or their cultivars), including wheat, which are susceptible to infection. Earlier sowing encourages infection. In general, earlier sowing prolongs the time in which the plants can become infected before winter (Clarkson and Cook 1983; Colbach *et al*. 1997). It is commonly believed that disease development is helped by continually growing cereal in the same field. Cultivating a susceptible plant is favourable to mycelium development on the plants over the growing period as well as on the plant residue after harvest (Mazzola *et al*. 1996; Colbach *et al*. 1997; Bockus *et al*. 2010).

An increase in the importance of sharp eyespot has also been associated with an increase in fungicide use, especially carbendazim-generating fungicides for protection from true eyespot (*Oculimacula yallundae* (Wallwork & Spooner) Crous & W. Gams, and *O. acuformis* (Boerema, R. Pieters & Hamers) Crous & W. Gams (Mazzola *et al*. 1996; Hamada *et al*. 2011a). To date, the effects of different fungicides are not clear. Some show effectiveness under conditions *in vitro* (Kataria and Gisi 1989; Kataria *et al*. 1991). Some inhibition of disease development has been observed following the application of fungicide seed treatment (Hamada *et al*. 2011b) as well as foliar spraying (Gołębniak *et al*. 1993; Bateman *et al*. 2000). Frequently, the effect of these treatments is not considerable (Ray *et al*. 2004). Bearing in mind the relatively low effectiveness towards *R. cerealis*, the fungicide seed treatment and sprayings are usually not economically justifiable (Bockus *et al*. 2010). Commonly applied herbicides may also affect soil organisms, and thus soil-borne pathogens (Lévesque and Rahe 1992; Wisler and Norris 2005; Lemańczyk 2012).

The aim of this research was to compare the effects of various farming systems, and of different combinations of sowing density, fertilizer dose, and crop protection chemicals (fungicide and herbicide) on the intensity of sharp eyespot and the colonization of roots and stem bases by fungi.

MATERIALS AND METHODS

Field experiment 1 – Effects of farming systems on the occurrence of sharp eyespot

The wheat crops were grown in Osiny, Poland (51°28'N; 22°04'E) in 2002–2007. Winter wheat cv. Sukces was grown in organic, integrated, and conventional farming systems and in many years of monoculture. The experimental site was established in 1994 on Luvisol produced from glacial till composed of particles the size of loamy sand. The 13 ha field included areas of 5, 4, 3 and 1 ha under organic, integrated and conventional systems, and monoculture, respectively. In the first three systems, winter wheat was grown in rotation. All tillage and other agronomic practices complied with the requirements of the respective management systems. Wheat was sown in the last third of September. Crop rotations, mineral fertilization and crop protection chemicals applied in the respective farming systems are listed in table 1. In the organic farming system, no crop protection chemicals or mineral fertilizers were applied. Excessive weeds were removed by harrowing and hand-weeding. Fertilization was limited to the use of potassium sulphate (41–66 kg/ha K) and organic fertilizer applied under potato (25–30 t/ha manure or compost). Weather conditions are shown in table 2.

Field experiment 2 – Effects of crop protection chemicals, sowing density and fertilizer dose on the occurrence of sharp eyespot on winter wheat grown in short-term monoculture

The wheat crops were grown in Mochełek, Poland (53°13'N, 17°51'E). The experimental plots were on light and heavy loamy-sand soil. The experimental plots of winter wheat, cv. Tonacja, were established after white mustard (grown mainly for seed), in two 3-year series. The first series began in autumn 2003, and the second in 2004. The experiment was set up in a split-plot and split-block design with four replicates. Winter wheat was sown in the last third of September. Grain was treated with Raxil 02 DS (2% tebuconazole). Weather conditions throughout the research period are shown in table 2.

The experiment included the following factors:

I) chemical crop protection:
- no treatments (the control),
- herbicide Huzar 05 WG (5% iodosulfuron-methyl-sodium) at 200 g/ha,
- herbicide Huzar 05 WG at 200 g/ha + fungicide Alert 375 SC (125 g/l flusilazole + 250 g/l carbendazim) at 1 l/ha.

II) sowing density:
- 400 grains/m^2,
- 600 grains/m^2.

III) fertilization:
- 147 kg/ha NPK (20 kg/ha N + 17 kg/ha P + 50 kg/ha K in autumn, pre-sowing; 40 kg/ha N in the spring at the onset of growth; 20 kg/ha N at the stem elongation stage),
- 221 kg/ha NPK (30 kg/ha N + 26 kg/ha P + 75 kg/ha K in the autumn, pre-sowing; 60 kg/ha N in the spring at the onset of growth; 30 kg/ha N at the stem elongation stage).

Herbicide was applied in spring after the start of winter wheat growth. Fungicide was applied once, at the end of stem elongation.

Samplings and measurements

Observations on the occurrence of sharp eyespot on stem bases of wheat were made at the seedling growth [(growth stage (GS) 13–14; Zadoks *et al*. 1974)], stem elongation (GS 35–37) and milk ripe stages (GS 75–77). Health status was assessed on 50 (60 in the first year only) stems

Table 1. Experiment 1: crop management procedures used in different systems of wheat farming in Osiny, in 2002–2007

Treatment	Organic system	Integrated system	Conventional system	Monoculture
1	2	3	4	5
Crop rotation	winter wheat potato spring barley red clover with grass red clover with grass	winter wheat potato spring barley faba bean	winter wheat spring barley/spring wheat from 2005 winter rape	winter wheat
Chemical fertilizers	2002 autumn – potassium sulphate (66.4 kg K/ha)	2002 autumn – polifoska 6 (15 kg N/ha, 22 kg P/ha + 62.3 kg K/ha); 2004 spring – ammonium nitrate with Mg (150 kg N/ha) in four doses	2002 autumn – polifoska 6 (18 kg N/ha, 26.4 kg P/ha + 74.7 kg K/ha); 2003 spring – ammonium nitrate with Mg (150 kg N/ha) in three doses	2002 autumn – ammonium nitrate with Mg (40 kg N/ha) + polifoska 8 (24 kg N/ha, 31.7 kg P/ha + 59.8 kg K/ha); 2003 spring – ammonium nitrate with Mg (151 kg N/ha) in three doses
	2003 autumn – potassium sulphate (41.5 kg K/ha)	2003 autumn – polifoska 6 (15 kg N/ha, 22 kg P/ha + 62.3 kg K/ha); 2004 spring – ammonium nitrate with Mg (119 kg N/ha) in four doses	2003 autumn – polifoska 8 (24 kg N/ha, 31.7 kg P/ha + 59.8 kg K/ha); 2004 spring – ammonium nitrate with Mg (140 kg N/ha) in four doses	2003 autumn – ammonium nitrate with Mg (40 kg N/ha) + polifoska 8 (24 kg N/ha, 31.7 kg P/ha + 59.8 kg K/ha); 2004 spring – ammonium nitrate with Mg (130 kg N/ha) in three doses
	2004 autumn – potassium sulphate (49.8 kg K/ha)	2004 autumn – polifoska 8 (15 kg N/ha, 22 kg P/ha + 62.3 kg K/ha) + dolomite lime (3 000 kg/ha); 2005 spring – ammonium nitrate with Mg (90 kg N/ha) in two doses	2004 autumn – polifoska 8 (20 kg N/ha, 29 kg P/ha + 82.2 kg K/ha); 2005 spring – ammonium nitrate with Mg (150 kg N/ha) in three doses	2004 autumn – ammonium nitrate with Mg (34 kg N/ha) + polifoska 8 (20 kg N/ha, 29 kg P/ha + 82.2 kg K/ha) + dolomite lime (3 000 kg/ha); 2005 spring – ammonium nitrate with Mg (140 kg N/ha) in three doses
	2005 autumn – potassium sulphate (41.5 kg K/ha)	2005 autumn – polifoska 8 (20 kg N/ha, 26.4 kg P/ha + 49.8 kg K/ha); 2006 spring – ammonium nitrate with Mg (95 kg N/ha) in three doses	2005 autumn – polifoska 8 (24 kg N/ha, 31.7 kg P/ha + 59.8 kg K/ha); 2006 spring – ammonium nitrate with Mg (140 kg N/ha) in three doses	2005 autumn – ammonium nitrate with Mg (40 kg N/ha) + polifoska 8 (24 kg N/ha, 31.7 kg P/ha + 59.8 kg K/ha); 2006 spring – ammonium nitrate with Mg (135 kg N/ha) in three doses
	2006 autumn – potassium sulphate (62.3 kg K/ha) + ground rock phosphate (15.5 kg P/ha)	2006 autumn – polifoska 4 (21 kg N/ha, 25.5 kg P/ha + 66.4 kg K/ha) + ammonium phosphate (7.2 kg N/ha + 7.9 kg P/ha); 2007 spring – ammonium nitrate with Mg (95 kg N/ha) in three doses	2006 autumn – polifoska 8 (23 kg N/ha, 18 kg P/ha + 79.7 kg K/ha) + ammonium phosphate (7.2 kg N/ha + 7.9 kg P/ha); 2007 spring – ammonium nitrate with Mg (150 kg N/ha) in three doses	2006 autumn – polifoska 8 (24 kg N/ha, 28.2 kg P/ha + 79.7 kg K/ha) + ammonium phosphate (7.2 kg N/ha + 7.9 kg P/ha); 2007 spring – ammonium nitrate with Mg (130 kg N/ha) in three doses
Chemical control (seed treatment)		triadimenol + imazalil + fuberidazole (Baytan Universal 19,5 FS) applied on 30.09.2002; 25.09.2003; 24.09.2004; 23.09.2005; 24.09.2006	triadimenol + imazalil + fuberidazole (Baytan Universal 19,5 FS) applied on 25.09.2002; 24.09.2003; 21.09.2004; 26.09.2005; 22.09.2006	triadimenol + imazalil + fuberidazole (Baytan Universal 19,5 FS) applied on 30.09.2002; 25.09.2003; 27.09.2004; 28.09.2005; 23.09.2006; silthiofam (Latitude 125 FS) 25.09.2003; 27.09.2004; 28.09.2005; 23.09.2006

www.czasopisma.pan.pl PAN www.journals.pan.pl

1	2	3	4	5
Chemical control (fungicide spray)		tebuconazole + triadimenol (Folicur Plus 375 EC) 06.06.2003; 04.06.2004; 08.06.2005; tebuconazole + spiroxamine + triadimenol (Falcon 460 EC) 18.06.2003; picoxystrobin (Acanto 250 SC) 22.06.2004; carbendazim (Bavistin 500 WG) 12.05.2005; flusilazole + carbendazim (Alert 375 SC) 12.05.2005; propiconazole + fenpropidin (Tilt Plus 400 EC) 23.06.2005; carbendazim (Sarfun 500 SC) 16.05.2006; propiconazole + cyproconazole (Artea 330 EC) 28.06.2006; spiroxamine (Impuls 500 EC) 12.04.2007; carbendazim (Karben 500 SC) 12.04.2007; prothioconazole + fluoxastrobin (Fandango 200 EC) 20.05.2007	tebuconazole + triadimenol (Folicur Plus 375 EC) 06.06.2003; 04.06.2004; 09.06.2005; tebuconazole + spiroxamine + triadimenol (Falcon 460 EC) 18.06.2003; 07.06.2004; carbendazim (Sarfun 500 SC) 10.05.2004; 16.05.2006; propiconazole + cyproconazole (Artea 330 EC) 19.05.2004; 16.05.2006; 02.06.2006; propiconazole + fenpropidin (Tilt Plus 400 EC) 19.05.2004; carbendazim (Bavistin 500 WG) 12.05.2005; flusilazole + carbendazim (Alert 375) 12.05.2005; 23.06.2006; spiroxamine (Impuls 500 EC) 12.04.2007; carbendazim (Karben 500 SC) 12.04.2007; prothioconazole + fluoxastrobin (Fandango 200 EC) 20.05.2007	carbendazim + triadimefon (Bayleton Total 37,5 WP) 26.05.2003; tebuconazole + triadimenol (Folicur Plus 375 EC) 06.06.2003; 06.06.2004; 08.06.2005; tebuconazole + spiroxamine + triadimenol (Falcon 460 EC) 18.06.2003; 07.06.2004; carbendazim (Sarfun 500 SC) 10.05.2004; 16.05.2006; picoxystrobin (Acanto 250 SC) 24.06.2004; carbendazim (Bavistin 500 WG) 12.05.2005; flusilazole + carbendazim (Alert 375 SC) 12.05.2005; 25.05.2005; 13.05.2006; propiconazole + cyproconazole (Artea 330 EC) 02.06.2006; 21.06.2006; spiroxamine (Impuls 500 EC) 12.04.2007; carbendazim (Karben 500 SC) 12.04.2007; flusilazole + famoxadone (Charisma 207 EC) 25.05.2007; prothioconazole + fluoxastrobin (Fandango 200 EC) 10.06.2007
Chemical control (herbicide)		pendimethalin + isoproturon (Maraton 375 SC) 24.10.2002; 20.10.2005; MCPA + dicamba (Chwastox Turbo 340 SL) 13.05.2003; iodosulfuron-methyl-sodium (Huzar 100 OD) 17.10.2003; diflufenican + isoproturon (Cougar 600 SC) 19.10.2004; 2,4-D + dicamba (Aminopielik D 450 SL) 11.05.2006; chlorsulfuron (Glean 75 WG) 11.10.2006 ; fluroxypyr (Starane 250 EC) 16.04.2007; fenoxaprop-P-ethyl (Puma Uniwersal 069 EW) 28.04.2007	pendimethalin + isoproturon (Maraton 375 SC) 24.10.2002; 20.10.2004; 20.10.2005; 12.10.2006; MCPA + dicamba (Chwastox Turbo 340 SL) 13.05.2003; fenoxaprop-P-ethyl (Puma Uniwersal 069 EW) 28.05.2003; 11.05.2005; 24.04.2006; 28.04.2007; diflufenican + isoproturon (Cougar 600 SC) 17.10.2003; fluroxypyr (Starane 250 EC) 26.04.2006; 16.04.2007	pendimethalin + isoproturon (Maraton 375 SC) 24.10.2002; 12.10.2006; MCPA + dicamba (Chwastox Turbo 340 SL) 13.05.2003; diflufenican + isoproturon (Cougar 600 SC) 17.10.2003; 19.10.2004; glyphosate (Rofosat 360 SL) 26.06.2004; fenoxaprop-P-ethyl (Puma Uniwersal 069 EW) 10.05.2005; 28.04.2007; iodosulfuron-methyl-sodium + mesosulfuron-methyl (Atlantis 04 WG) 11.10.2005; sulfosulfuron (Apyros 75 WG) 05.05.2006; fluroxypyr (Starane 250 EC) 15.05.2007
Chemical control (insecticide)		cypermethrin (Cyperkil Super 25 EC) 08.10.2006	cypermethrin (Cyperkil Super 25 EC) 08.10.2006	cypermethrin (Cyperkil Super 25 EC) 08.10.2006
Chemical control (growth regulator)		chlormequat chloride (Cycocel 750 SL) 06.05.2004; chlormequat chloride + ethephon (Terpal C 460 SL) 19.05.2004; trinexapac-etil (Moddus 250 EC) 11.05.2006; 10.05.2007	chlormequat chloride (Cycocel 750 SL) 06.05.2004; chlormequat chloride + ethephon (Terpal C 460 SL) 18.05.2004; trinexapac-etil (Moddus 250 EC) 11.05.2005; 09.05.2006; 23.05.2006; 27.04.2007	chlormequat chloride (Cycocel 750 SL) 06.05.2004; chlormequat chloride + ethephon (Terpal C 460 SL) 18.05.2004; trinexapac-etil (Moddus 250 EC) 11.05.2005; 19.05.2006; 27.04.2007

Table 2. Weather conditions during Experiment 1 (Osiny) and Experiment 2 (Mochełek), in 2002–2007

Month	Temperature [°C]							Rainfall [mm]						
	2002	2003	2004	2005	2006	2007	mean[a]	2002	2003	2004	2005	2006	2007	mean[a]
Experiment 1														
January	–1.1	–3.2	–5.0	0.5	–8.0	2.9	–3.4	26.3	26.2	20.7	37.8	12.1	59.3	31.0
February	3.5	–5.8	–0.3	–3.6	–3.8	–0.1	–2.4	47.7	8.1	43.6	17.7	19.3	25.8	29.0
March	4.6	2.0	3.3	0.2	–0.7	6.7	1.5	39.7	12.9	34.1	27.8	40.3	28.0	30.0
April	8.9	7.5	8.6	9.0	9.2	9.2	7.7	13.0	19.3	38.9	16.3	27.1	13.4	40.0
May	17.6	16.6	12.5	13.9	13.9	15.7	13.4	10.1	51.6	19.0	66.9	58.0	79.8	57.0
June	18.0	18.2	16.5	16.4	17.7	19.1	16.7	88.4	46.4	52.1	31.7	19.2	62.8	70.0
July	21.6	20.4	18.5	20.2	22.5	19.3	18.4	78.8	54.2	93.0	106.5	20.7	49.0	83.0
August	20.5	18.9	18.9	17.2	17.7	19.2	17.3	26.3	45.6	62.3	55.9	239.7	26.6	75.0
September	13.2	13.4	13.1	14.7	15.5	13.1	13.2	34.5	42.0	32.7	24.0	8.1	86.2	51.0
October	7.3	5.4	10.1	9.2	10.4	7.8	7.9	92.9	49.3	30.8	3.6	29.7	7.3	44.0
November	4.8	5.0	3.6	3.3	5.8	1.4	2.7	24.8	20.1	54.5	27.4	35.7	36.2	39.0
December	–6.4	0.7	1.9	–0.4	3.3	0.7	–1.4	6.8	30.1	12.7	64.4	19.0	6.1	38.0
Experiment 2														
January		–3.1	–5.4	0.4	–8.1	2.7	–2.3		18.7	20.4	38.1	2.8	75.9	24.0
February		–4.9	–0.3	–2.9	–2.9	–1.0	–1.5		6.4	60.9	28.7	19.1	28.0	19.2
March		1.5	2.9	–0.4	–1.5	5.0	1.8		11.9	35.8	22.5	27.4	47.9	23.4
April		6.4	7.5	7.4	7.1	8.5	7.3		18.5	32.1	34.8	77.0	17.6	27.8
May		14.4	11.3	12.2	12.5	13.8	12.8		18.1	54.4	82.6	59.9	73.1	42.2
June		17.6	14.7	14.9	16.8	18.2	16.2		30.4	39.6	30.5	21.8	105.5	54.1
July		19.2	16.4	19.4	22.4	18.0	18.0		106.2	53.5	33.6	24.2	104.7	71.0
August		18.4	17.9	16.3	16.6	17.8	17.4		17.7	138.7	43.4	129.0	42.1	51.2
September		13.6	12.7	14.8	15.2	12.4	13.2		16.7	40.0	17.8	40.6	37.6	41.4
October		4.7	8.8	8.7	9.6	6.9	8.2		34.0	63.8	15.1	12.1	19.9	31.9
November		4.2	2.8	2.7	5.2	1.3	3.0		22.8	36.2	20.7	33.9	22.3	31.8
December		0.8	1.1	–0.3	3.7	0.3	–0.5		25.5	49.8	71.5	31.4	36.0	31.7

[a] long-term means:1871–1996 in Osiny; 1949–2007 in Mochełek

randomly selected from each replicate at each sampling in Experiment 1, and on 25 stems in Experiment 2. Disease was assessed using a 0–4 scale (Lemańczyk 2012). A disease index (DI) was calculated on a % scale using the Townsend and Heuberger formula (Wenzel 1948).

In Experiment 2, monocotyledonous and dicotyledonous weeds were taken when wheat was at the dough development growth stages from a 1 m^2 area in each plot, and counted, and dry-weighed.

Isolation and identification of fungi

Evaluation of plant health was facilitated by mycological analysis. The composition of the fungal communities on wheat stems with sharp eyespot symptoms was determined at GS 13–14 and GS 75–77. The occurrence of *R. cerealis*, *R. solani*, *R. zeae* Voorhees (teleomorph: *Waitea circinata Warcup* & P.H.B. Talbot) and binucleate *Rhizoctonia* spp. (BNR) was determined as a percentage of all fungal isolates from healthy and infected stem bases and roots. Isolations from roots were made at GS 13–14 and GS 35–37, and from the stem bases at GS 13–14 and GS 75–77. Fungi were isolated from 30 fragments of healthy stems and roots, and from 100 fragments of diseased roots, from each treatment. Separate isolations were made from stems with symptoms of sharp eyespot, true eyespot, and fusarium foot rot, since these may obscure sharp eyespot symptoms.

The root and stem pieces were rinsed for 45 min in running water, disinfected in 1% $AgNO_3$ solution for 15 s, rinsed three times for 1 min in sterile distilled water, and placed on potato dextrose agar (PDA; 40 g filtered white potatoes, 20 g agar, 1 l distilled water, pH = 7) with 50 mg of streptomycin per 1 l, in Petri dishes. Using available literature, 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). Hyphal staining was used to help identify *Rhizoctonia* fungi to species (Bandoni 1979).

To confirm the species classification of the *Rhizoctonia* isolates, polymerase chain reaction (PCR) was performed using specific primers in Sequence Characterized Amplified Region (SCAR), *i.e.* Rc2 F/R for *R. cerealis* (Nicholson and Parry 1996) and ITS1/GMRS-3 for *R. solani* (Johanson *et al*. 1998). This procedure was done for isolates that had been identified as *Rhizoctonia* spp., using conventional methods. Total DNA was isolated using the modified Doyle and Doyle method (1990). The PCR reaction was performed with the *Taq* PCR Core Kit (QIAGEN Inc., USA).

Statistical analysis

The results were tested statistically by analysis of variance (ANOVA), assuming significance at $p \leq 0.05$. Coefficients of Pearson's correlation were calculated to compare the relationship between the intensity of sharp eyespot and weed infestation in Experiment 2. Relationships between yield and disease intensity were determined using the statistical calculation package Statistica v. 10, (StatSoft Poland).

RESULTS

Experiment 1

Sharp eyespot intensity in wheat was significantly affected by the farming system only at GS 35–37 and GS 75–77 (Table 3). At GS 35–37, most symptoms were found in wheat grown in monoculture, in 2007. At GS 75–77, least sharp eyespot was recorded in the integrated system. There was considerably more disease in the conventional and organic systems than in the other systems in 2007 when DI values were highest: 16.5 and 11.0%, respectively. In the other years, the mean DI values were much less, from 0.21 to 1.78%. In 2004, there was the least amount of symptoms in the conventional farming system and in monoculture. In 2006, there was the least amount of symptoms in the integrated and conventional systems. In both these years, the most sharp eyespot was in the organic farming system and in 2006 also in monoculture. In the other years the differences were not significant.

Experiment 2

At GS 13–14, no sharp eyespot was found in the first year of monoculture. In the second and third years, DI, averaged over the two series, tended to be least when herbicide was applied, and most when herbicide + fungicide were used (Table 4).

Table 3. Experiment 1: disease index (%) of sharp eyespot on winter wheat in different farming systems in Osiny, in 2002–2007

Farming system	Growing season					Mean
	2002/2003	2003/2004	2004/2005	2005/2006	2006/2007	
	GS 13–14 (seedling growth stage)					
Organic	0	0	0	0.13	0	0.03
Integrated	0	0.13	0	0	0.13	0.05
Conventional	0	0	0	0	0	0
Monoculture	0	0	0	0	0	0
Mean	0	0.03	0	0.03	0.03	0.02
	GS 35–37 (stem elongation stage)					
Organic	0.21	0.25	0.13	0.38	0.88 a	0.37 a
Integrated	0	0.88	0	0.38	0.75 a	0.40 a
Conventional	0.31	0	0	0.25	1.13 a	0.34 a
Monoculture	0	0	0	0	3.38 b	0.68 b
Mean	0.13	0.28	0.03	0.25	1.53	0.45
	GS 75–77 (milk ripe stage)					
Organic	0.31	3.52 b	0.38	3.00 b	11.00 bc	3.64 bc
Integrated	0	1.53 ab	0.38	0.38 a	1.63 a	0.78 a
Conventional	0.52	0.94 a	0.38	0.88 a	16.50 c	3.84 c
Monoculture	0	1.02 a	0.13	2.88 b	8.75 b	2.55 b
Mean	0.21	1.75	0.31	1.78	9.47	2.71

Different letters indicate statistically significant differences between farming systems at $p \leq 0.05$

Table 4. Experiment 2: disease index (%, mean of two series) of sharp eyespot on winter wheat in monoculture with different chemical crop protection, sowing density and fertilizer dose, at GS 13–14 (seedling growth stage), in Mochełek, in 2003–2006

Chemical crop protection (I)	Sowing density grains/m^2 (II)	NPK fertilizer kg/ha (III)					
		2nd year monoculture			3rd year monoculture		
		147	221	mean	147	221	mean
None	400	0.13	0.13	0.13	0	0.25	0.13
	600	0.13	0	0.06	0.13	0.13	0.13
	mean	0.13	0.06	0.09	0.06	0.19	0.13
Herbicide	400	0	0.25	0.13	0	0	0
	600	0.13	0.13	0.13	0	0	0
	mean	0.06	0.19	0.13	0	0	0
Herbicide + fungicide	400	0.50	0.13	0.31	0.13	0.63	0.38
	600	0.13	0.13	0.13	0	0	0
	mean	0.31	0.13	0.22	0.06	0.31	0.19
Mean	400	0.21	0.17	0.19	0.04	0.29	0.17
	600	0.13	0.08	0.10	0.04	0.04	0.04
	mean	0.17	0.13	0.15	0.04	0.17	0.10
LSD 0.05		I×III – 0.227; others – ns			all – ns		

Factor I (chemical crop protection); factor II (sowing density); factor III (fertilizer dose); ns – no significant differences

Table 5. Experiment 2: disease index (%, mean of two series) of sharp eyespot on winter wheat in monoculture with different chemical crop protection, sowing density and fertilizer dose, at GS 35–37 (stem elongation stage), in Mochełek, in 2004–2007

Chemical crop protection (I)	Sowing density grains/m² (II)	Fertilizer NPK kg/ha (III)								
		1st year of monoculture			2nd year of monoculture			3rd year of monoculture		
		147	221	mean	147	221	mean	147	221	mean
None	400	1.89	0.91	1.40	3.29	1.50	2.39	8.25	6.98	7.61
	600	1.76	1.50	1.63	1.50	2.63	2.06	8.75	6.55	7.65
	mean	1.83	1.21	1.52	2.39	2.06	2.23	8.50	6.76	7.63
Herbicide	400	1.05	1.55	1.30	4.00	4.41	4.21	5.88	6.46	6.17
	600	1.76	1.01	1.39	4.35	5.58	4.96	8.25	6.63	7.44
	mean	1.41	1.28	1.34	4.18	4.99	4.58	7.06	6.54	6.80
Herbicide + fungicide	400	1.05	1.54	1.29	4.68	2.69	3.68	5.63	4.38	5.00
	600	1.99	1.46	1.73	2.91	2.45	2.68	8.00	5.13	6.56
	mean	1.52	1.50	1.51	3.79	2.57	3.18	6.81	4.75	5.78
Mean	400	1.33	1.33	1.33	3.99	2.87	3.43	6.58	5.94	6.26
	600	1.84	1.33	1.58	2.92	3.55	3.24	8.33	6.10	7.22
	mean	1.58	1.33	1.46	3.45	3.21	3.33	7.46	6.02	6.74
LSD 0.05		III×II – 0.41; others – ns			all – ns			III – 1.28; others – ns		

Factor I (chemical crop protection); factor II (sowing density); factor III (fertilizer dose); ns – no significant differences

Table 6. Experiment 2: disease index (%, mean of two series) of sharp eyespot on winter wheat in monoculture with different chemical crop protection, sowing densities and fertilizer doses, at GS 75–77 (milk ripe stage), in Mochełek, in 2004–2007

Chemical crop protection (I)	Sowing density grains/m² (II)	Fertilizer NPK kg/ha (III)								
		1st year of monoculture			2nd year of monoculture			3rd year of monoculture		
		147	221	mean	147	221	mean	147	221	mean
None	400	4.4	3.1	3.7	5.4	4.0	4.7	20.5	11.0	15.8
	600	3.9	4.6	4.3	6.0	3.6	4.8	15.3	15.6	15.4
	mean	4.1	3.8	4.0	5.7	3.8	4.8	17.9	13.3	15.6
Herbicide	400	5.3	9.0	7.1	5.8	8.6	7.2	12.1	19.8	15.9
	600	11.0	12.5	11.8	5.4	7.0	6.2	12.8	15.0	13.9
	mean	8.1	10.8	9.4	5.6	7.8	6.7	12.4	17.4	14.9
Herbicide + fungicide	400	7.9	6.1	7.0	6.9	4.3	5.6	14.5	13.8	14.1
	600	10.1	10.9	10.5	10.0	6.4	8.2	17.4	13.8	15.6
	mean	9.0	8.5	8.8	8.4	5.3	6.9	15.9	13.8	14.8
Mean	400	5.8	6.1	6.0	6.0	5.6	5.8	15.7	14.8	15.3
	600	8.3	9.3	8.8	7.1	5.7	6.4	15.1	14.8	15.0
	mean	7.1	7.7	7.4	6.6	5.6	6.1	15.4	14.8	15.1
LSD 0.05		I – 3.80; II – 2.20; IIIxI – 2.05; IxIII – 3.79; IIxIII – 2.41; others – ns			I – 2.09; IIxI – 1.28; IxII – 2.23; IxIII – 2.76; IIIxI – 2.58; others – ns			IIIxI – 3.42; IxIII – 4.829; others – ns		

Factor I (chemical crop protection); factor II (sowing density); factor III (fertilizer dose); ns – no significant differences

At GS 35–37, DI tended to be greatest at the higher sowing density and lower fertilizer dose (Table 5).

At GS 75–77, in the first and second years of monoculture there was less disease with no chemical control than where herbicide or herbicide + fungicide was applied (Table 6). The negative effect of herbicide was the greatest at the higher fertilizer dose. In the first and second years of monoculture there was usually more disease at the higher sowing rate, especially where herbicide or herbicide + fungicide was used.

In the first-year wheat crops, sharp eyespot intensity was significantly correlated with weed infestation. An increase in the abundance and dry weight of weeds, both monocotyledonous (*Apera spica-venti* dominant) and dicotyledonous (including *Viola arvensis*, *Capsella bursa-pastoris*, *Matricaria inodora* and *Veronica arvensis*), was associated with a decrease in disease intensity (Table 7). Weed infestation showed an increasingly non-significant correlation with disease intensity in successive monoculture wheat crops. A significant, positive relationship between sharp eyespot and wheat yield was recorded only in the first year. More disease in subsequent years did not result in a yield decrease.

Isolation and identification of fungi

Experiment 1 (Osiny)

Mostly *R. cerealis* was isolated from stems with sharp eyespot symptoms at the GS 75–77, accounting for, on average, 28.7% of the isolates (Table 8). Identification of

Table 7. Experiment 2: correlation coefficients between disease index of sharp eyespot and number of weeds, air-dry matter of weeds and grain yield of wheat, in Mochełek, in 2004–2007

		Year of monoculture		
		1st year	2nd year	3rd year
Number of weeds	monocotyledonous weeds	–0.845**	–0.524	0.133
	dicotyledonous weeds	–0.873**	–0.430	0.109
Air-dry weight of weeds	monocotyledonous weeds	–0.758*	–0.539	0.198
	dicotyledonous weeds	–0.808*	–0.497	0.080
Wheat grain yield		0.762*	0.232	–0.171

Significant at: * $p \leq 0.05$, ** $p \leq 0.001$, respectively

Table 8. Experiment 1: percentage of fungi occurring on winter wheat stem bases with sharp eyespot symptoms, grown in various farming systems, at GS 75–77 (milk ripe stage), in Osiny

Taxon	Farming system				Years					Mean
	O	I	C	M	2003	2004	2005	2006	2007	
Rhizoctonia cerealis van der Hoeven	47.1	40.9	19.5	12.5	16.6	64.0	38.4	27.4	15.4	28.7
R. solani Kühn	0	0	3.6	4.2	0	4.0	7.7	0	1.9	2.2
Binucleate *Rhizoctonia* spp.	8.8	4.5	3.6	4.2	4.2	16.0	0	4.5	1.9	5.2
Alternaria alternata (Fr.) Keissl.	0	4.6	1.8	4.2	4.2	0	0	0	3.9	2.2
Aspergillus fumigatus Fresen.	0	4.6	0	0	0	0	0	0	1.9	0.7
A. niger van Tieghen	0	9.1	0	0	0	0	15.4	0	0	1.5
Chaetomium funicola Cooke	0	4.5	0	8.3	0	0	0	0	5.8	2.2
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams	0	0	1.8	0	4.2	0	0	0	0	0.7
Epicoccum nigrum Link	8.8	0	5.4	0	0	0	0	0	11.5	4.5
Fusarium culmorum (W.G. Sm.) Sacc.	8.8	0	12.5	0	0	0	0	4.5	17.3	7.4
F. oxysporum Schltdl.	5.9	0	1.8	24.9	0	0	0	22.7	7.7	6.6
Gibberella avenacea R.J. Cook	17.7	0	8.9	12.5	0	0	0	31.9	13.5	10.3
G. intricans Wollenw.	0	0	1.8	0	0	0	0	0	1.9	0.7
G. tricincta El-Gholl, McRitchie, Schoult. & Ridings	0	4.6	0	0	0	0	0	4.5	0	0.7
Gaeumannomyces graminis var. *tritici* J. Walker	2.9	0	1.8	0	0	4.0	0	0	1.9	1.5
Khuskia oryzae H.J. Huds.	0	0	0	12.5	0	0	0	0	5.8	2.2
Microdochium bolleyi (R. Sprague) de Hoog & Herm.-Nijh.	0	0	7.1	0	16.6	0	0	0	0	2.9
Mucor spp.	0	0	14.2	4.2	33.3	0	0	0	1.9	6.6
Penicillium spp.	0	13.6	5.4	12.5	4.2	0	30.8	4.5	5.8	6.6
Sarocladium strictum (W. Gams) Summerb.	0	0	1.8	0	0	0	0	0	1.9	0.7
Trichoderma spp.	0	0	5.4	0	12.5	0	0	0	0	2.2
Non-sporulating mycelia	0	13.6	3.6	0	4.2	12.0	7.7	0	0	3.7
Total number of isolates	34	22	56	24	24	25	13	22	52	136
Total number of analysed piece of stem tissues	55	24	71	36	20	29	11	30	96	186

O – organic; I – integrated; C – conventional; M – monoculture

isolates as *R. cerealis* was confirmed by PCR using specific SCAR primers. The expected amplification product of 800 bp was generated by Rc2 F/R primers. *R. cerealis* had an especially high frequency among the isolates from wheat grown in the organic (47.1%) and integrated (40.9%) farming systems. Its greatest frequency was in 2004 (64%). Isolates of binucleate *Rhizoctonia* spp. were also produced. An attempt at classifying this species using PCR was unsuccessful, therefore that group was classified separately. The binucleate *Rhizoctonia* species accounted for, on average, 5.2% of all isolates produced, and were most frequent in 2004 (16%). They were found in all farming systems, and most often in the organic system (8.8%). PCR using the specific SCAR primers ITS1/GMRS-3 confirmed the multinucleate *Rhizoctonia* isolates as *R. solani*, producing the expected amplification product of 550 bp. Its frequency was, on average, 2.2% and it was recorded only in the conventional farming system and in monoculture. It was isolated most frequently in 2005. Among the other fungal isolates from wheat tissues with sharp eyespot symptoms, there were many which anamorphs represented the genus *Fusarium* as well as fungi considered saprotrophic on wheat. Those most often isolated were *Gibberella avenacea* (anamorph *F. avenaceum*), *F. culmorum* and *F. oxysporum*, which were isolated only in 2006 and 2007.

Fungi in the genus *Rhizoctonia* were also isolated from stem bases showing disease symptoms typical of infection by *Oculimacula* spp. and *Fusarium* spp. (Table 9). Mostly *R. solani*, and much less frequently *R. cerealis*, was isolated from such tissues. *R. cerealis* was isolated more often (3.5%) from healthy stem bases. These fungi were also identified in both healthy roots and roots with disease symptoms, but only at GS 35–37. *R. solani* was isolated from them much more often, accounting for 3.3 and 1.3% of isolates, respectively.

Experiment 2 (Mochełek)

Fungi in the genus *Rhizoctonia* were isolated from wheat shoots with sharp eyespot symptoms as early as GS 13–14, but only 22 isolates were recorded over the 4 years of research. Isolates of *R. cerealis* were, on average, 40.9% of fungi, while *R. solani* and binucleate *Rhizoctonia* spp. were each 9.1%. *Penicillium* spp. accounted for 27.3% (details not shown).

More *Rhizoctonia* isolates were obtained from stems with sharp eyespot symptoms at GS 75–77, when *R. cerealis* was predominant (38.9%), accounting for 56.1% of isolates in 2007 (Table 10). On average, 5.2% isolates

Table 9. Experiment 1: frequency (% of total number of fungi) of *R. cerealis*, *R. solani* and binucleate *Rhizoctonia* spp. isolated from healthy and diseased stem bases and roots of winter wheat, in Osiny, in 2002–2007

Farming system	Fungi	GS 13–14						GS 35–37		GS 75–77			
		DR	HR	R	O	F	HSB	DR	HR	R	O	F	HSB
Organic	*R. cerealis*	0	0	0	0	0	0	0.4	1.4	47.1	1.9	2.0	1.6
	R. solani	0	0	0	0	0	0	3.0	2.8	0	9.3	2.0	3.2
	binucleate *Rhizoctonia* spp.	0	0	0	0	0	0	0	0	8.8	0	0	0
	total number of isolates	36	17	0	7	51	56	233	72	34	54	51	62
Integrated	*R. cerealis*	0	0	0	0	0	0	0	0	40.9	0.6	0	7.7
	R. solani	0	0	0	0	0	0	1.0	3.2	0	1.3	1.9	0
	binucleate *Rhizoctonia* spp.	0	0	0	0	0	0	0	0	4.5	0	0	0
	total number of isolates	33	45	0	6	14	50	195	62	22	159	53	39
Conventional	*R. cerealis*	0	0	0	0	0	0	0	0	19.5	0.7	0	4.3
	R. solani	0	0	0	0	0	0	0.9	6.3	3.6	4.0	0	0
	binucleate *Rhizoctonia* spp.	0	0	0	0	0	0	0	0	3.6	0	0	0
	total number of isolates	29	39	0	17	32	12	227	32	56	151	91	46
Monoculture	*R. cerealis*	0	0	0	0	0	0	0	0	12.5	0.4	1.2	1.9
	R. solani	0	0	0	0	0	0	0	2.1	4.2	0.8	0	0
	binucleate *Rhizoctonia* spp.	0	0	0	0	0	0	0	0	4.2	0	0	0
	total number of isolates	75	16	0	8	41	43	201	47	24	248	81	52
Total	*R. cerealis*	0	0	0	0	0	0	0.1	0.5	28.7	0.7	0.7	3.5
	R. solani	0	0	0	0	0	0	1.3	3.3	2.2	2.5	0.7	1.0
	binucleate *Rhizoctonia* spp.	0	0	0	0	0	0	0	0	5.1	0	0	0
	total number of isolates	173	117	0	38	138	161	856	213	136	612	276	199

DR – diseased roots; HR – healthy roots; R – stems with sharp eyespot symptoms; O – stems with eyespot symptoms; F – stems with fusarium foot rot symptoms; HSB – healthy stem base

Table 10. Experiment 2: percentage (%) of fungi on stem bases of winter wheat with sharp eyespot symptoms, in 3 years of monoculture, at GS 75–77 (milk ripe stages), in Mochełek, in 2004–2007

Taxon	Chemical crop protection									Year of monoculture			Chemical control			Years				Mean
	1st year			2nd year			3rd year													
	N	H	H+F	N	H	H+F	N	H	H+F	1st	2nd	3rd	N	H	H+F	2004	2005	2006	2007	
Rhizoctonia cerealis van der Hoeven	55.6	44.5	76.5	5.6	26.8	35.1	51.6	33.0	46.8	53.4	24.3	42.3	36.5	34.1	50.7	26.1	48.3	21.8	56.1	38.9
R. solani Kühn	0	5.6	5.9	0	4.5	5.4	4.8	1.0	5.2	5.3	3.8	3.4	2.8	3.6	5.4	4.3	5.4	3.1	3.7	3.9
R. zeae Voorhees	0	0	0	0	0	0	0	0	2.6	0	0	0.8	0	0	1.3	0	0	0	1.2	0.4
Binucleate *Rhizoctonia* spp.	11.1	7.8	14.7	2.8	8.0	2.7	1.6	0	5.2	9.8	6.0	2.1	2.8	5.3	6.8	4.3	8.2	5.8	1.9	5.2
Alternaria alternata (Fr.) Keissl.	0	0	0	0	0	0	4.8	4.0	1.3	0	0	3.4	2.8	1.3	0.7	0	0	0	4.9	1.4
Aspergillus fumigatus Fresen.	0	0	0	0	8.9	5.4	3.2	1.0	0	0	6.6	1.3	1.9	3.6	1.3	0	0	6.7	0	2.7
A. niger van Tieghen	0	1.1	0	11.0	1.8	5.4	1.6	0	0	0.7	4.3	0.4	4.7	1.0	1.3	0	3.4	2.2	0	1.8
Aureobasidium pullulans (de Bary) G. Arnaud	0	0	0	0	0.9	8.2	1.6	4.0	0	0	2.2	2.1	0.9	1.7	2.0	0	0	2.2	2.5	1.6
Clonostachys rosea f. *catenulata* (J.C. Gilman & E.V. Abbott) Schroers	0	0	0	0	2.7	5.4	1.6	0	0	0	2.7	0.4	0.9	1.0	1.3	0	0	2.7	0	1.1
Epicoccum nigrum Link	0	0	0	0	0.9	0	0	3.0	0	0	0.5	1.3	0	1.3	0	0	0	0.4	1.9	0.7
Fusarium culmorum (W.G. Sm.) Sacc.	0	11.1	0	2.8	5.3	0	1.6	3.0	5.2	7.5	3.8	3.4	1.9	6.3	2.8	17.4	6.8	3.6	1.9	4.5
F. oxysporum Schltdl.	0	0	0	0	0.9	0	0	4.0	2.6	0	0.5	2.5	0	1.7	1.3	0	0	2.7	0.6	1.2
F. poae (Peck.) Wollenw.	0	0	0	0	0.9	0	1.6	0	0	0	0.5	0.4	0.9	0.3	0	0	0	0.4	0.6	0.4
F. sporotrichioides Sherb.	0	0	0	0	0	0	0	0	1.3	0	0	0.4	0	0	0.7	0	0	0.4	0	0.2
Gibberella avenacea R.J. Cook	0	8.9	2.9	25.0	9.8	0	8.2	12.0	15.5	6.8	10.9	12.1	13.1	10.2	8.9	0	6.1	11.6	14.2	10.4
G. intricans Wollenw.	0	1.1	0	5.6	1.8	13.5	0	2.0	0	0.7	4.9	0.8	1.9	1.7	3.4	0	0.7	4.9	0	2.1
G. tricincta El-Gholl, McRitchie, Schoult. & Ridings	0	0	0	0	0	0	0	0	1.3	0	0	0.4	0	0	0.7	0	0	0	0.6	0.2
Gaeumannomyces graminis var. *tritici* J. Walker	0	0	0	0	0.9	0	0	0	1.3	0	0.5	0.4	0	0.3	0.7	0	0.7	0	0.6	0.4
Glomerella graminicola D.J. Politis	0	1.1	0	0	0	0	0	0	0	0.7	0	0	0	0.3	0	0	0.7	0	0	0.2
Haematonectria haematococca (Berk. & Broome) Samuels & Rossman	0	1.1	0	0	0.9	0	0	6.0	1.3	0.7	0.5	2.9	0	2.7	0.7	4.4	0.7	2.7	0.6	1.6
Mucor spp.	0	0	0	2.8	0	0	0	0	0	0	0.5	0	0.9	0	0	0	0	0.4	0	0.2
Oculimacula yallundae (Wallwork & Spooner) Crous & W. Gams	0	0	0	0	0	0	0	2.0	0	0	0	0.8	0	0.7	0	0	0	0	1.2	0.4
Penicillium spp.	0	0	0	41.6	13.4	13.5	6.5	6.0	0	0	18.9	4.2	17.8	7.0	3.4	0	3.4	17.9	0	8.1
Phoma sp.	0	1.1	0	0	0	0	0	3.0	2.6	0.7	0	2.1	0	1.3	1.3	0	0.7	0	3.1	1.1
Trichoderma koningii Oudem.	0	2.2	0	0	1.8	0	0	0	0	1.5	1.1	0	0	1.3	0	8.7	0.7	0.4	0	0.7
T. polysporum (Link) Rifai	11.1	2.2	0	0	2.7	2.7	6.5	8.0	2.6	2.3	2.2	5.9	4.7	4.3	2.0	13.0	2.7	4.9	1.9	3.7
T. viride Pers.	0	2.2	0	0	1.8	2.7	1.6	0	1.3	1.5	1.6	0.8	0.9	1.3	1.3	4.4	2.7	0.4	0.6	1.3
Zygorhynchus sp.	0	0	0	2.8	0	0	0	0	0	0	0.5	0	0.9	0	0	0	0	0.4	0	0.2
Non-sporulating mycelia	22.2	10.0	0	0	5.3	0	3.2	8.0	3.9	8.4	3.2	5.4	3.7	7.7	2.0	17.4	8.8	4.4	1.9	5.4
Total number of isolates	9	90	34	36	112	37	62	100	77	133	185	239	107	302	148	23	147	225	162	557
Total number of analysed piece of stem tissues	18	98	54	43	111	63	101	102	96	170	217	299	162	311	213	40	168	298	180	686

N – without chemical plant protection; H – herbicide; H+ F – herbicide + fungicide

Table 11. Experiment 2: frequency (% of total number of fungi, mean of two series) of *R. cerealis*, *R. solani*, *R. zeae* and binucleate *Rhizoctonia* spp. isolated from healthy and diseased stem bases and roots of winter wheat, in Mochełek, in 2003–2007

Year of monoculture/ Chemical control		Fungi	GS 13–14						GS 35–37		GS 75–77			
			DR	HR	R	O	F	HSB	DR	HR	R	O	F	HSB
Year of monoculture	1st year	*R. cerealis*	0	0	0	0	8.3	0	0.4	0	53.4	6.7	22.8	5.9
		R. solani	0	0	0	0	0	0	0.8	0	5.3	8.5	12.7	5.9
		R. zeae	0	0	0	0	0	0	0	0	0	0	0	0
		binucleate *Rhizoctonia* spp.	0	0	0	0	0	0	0	0	9.8	3.0	12.7	11.8
		total number of all isolates	5	15	0	0	12	19	250	78	133	165	79	34
	2nd year	*R. cerealis*	0	1.4	27.3	0	11.8	3.2	0	0	24.3	0	0	0
		R. solani	3.8	1.4	9.1	0	11.8	1.6	0	0	3.8	1.1	0	0
		R. zeae	0	0	0	0	0	0	0	0	0	0	0	0
		binucleate *Rhizoctonia* spp.	0	0	9.1	0	2.0	0	0	0	6.0	0	0	0
		total number of all isolates	52	73	11	5	51	63	357	78	185	270	94	40
	3rd year	*R. cerealis*	0	1.2	54.5	0	12.3	4.4	0	0	42.3	2.8	2.6	1.8
		R. solani	0	0	9.1	0	2.7	4.4	0	0	3.4	7.5	1.3	0
		R. zeae	0	0	0	0	0	0	0	0	0.8	0	0	0
		binucleate *Rhizoctonia* spp.	0	0	9.1	0	0	0	0	0	2.1	0	0	0
		total number of all isolates	168	83	11	76	73	45	512	98	239	253	77	57
Chemical plant protection	none	*R. cerealis*	0	1.1	37.5	0	11.1	2.9	0.3	0	36.5	1.5	3.6	2.6
		R. solani	0	0	0	0	4.4	2.9	0.3	0	2.8	3.8	2.4	0
		R. zeae	0	0	0	0	0	0	0	0	0	0	0	0
		binucleate *Rhizoctonia* spp.	0	0	25.0	0	2.2	0	0	0	2.8	0.4	1.2	0
		total number of all isolates	64	90	8	24	45	70	377	72	107	263	83	38
	herbicide	*R. cerealis*	0	2.5	25.0	0	19.5	0	0	0	34.1	3.1	1.8	0
		R. solani	2.0	2.5	0	0	9.8	0	0.3	0	3.6	4.6	1.8	0
		R. zeae	0	0	0	0	0	0	0	0	0	0	0	0
		binucleate *Rhizoctonia* spp.	0	0	0	0	0	0	0	0	5.3	1.5	0	6.5
		total number of all isolates	102	40	4	31	41	24	396	96	302	195	56	31
	herbicide + fungicide	*R. cerealis*	0	0	50.0	0	6.0	6.1	0	0	50.7	3.5	14.4	3.2
		R. solani	0	0	20.0	0	4.0	3.0	0	0	5.4	7.4	7.2	3.2
		R. zeae	0	0	0	0	0	0	0	0	1.3	0	0	0
		binucleate *Rhizoctonia* spp.	0	0	0	0	0	0	0	0	6.8	0.4	8.1	3.2
		total number of all isolates	59	41	10	26	50	33	346	86	148	230	111	62
	Total	*R. cerealis*	0	1.2	40.9	0	11.8	3.1	0.1	0	38.9	2.6	8.0	2.3
		R. solani	0.9	0.6	9.1	0	5.9	2.4	0.2	0	3.9	5.2	4.4	1.5
		R. zeae	0	0	9.1	0	0	0	0	0	0.4	0	0	0
		binucleate *Rhizoctonia* spp.	0	0	0	0	0.7	0	0	0	5.2	0.7	4.0	3.1
		total number of all isolates	225	171	22	81	136	127	1119	254	557	688	250	131

DR – diseased roots; HR – healthy roots; R – stems with sharp eyespot symptoms; O – stems with eyespot symptoms; F – stems with fusarium foot rot symptoms; HSB – healthy stem base

were binucleate *Rhizoctonia* spp. and 3.9% were *R. solani*. In 2007, two isolates of *R. zeae* were also obtained, accounting for an average of 0.4%. The share of *Rhizoctonia* isolates produced from diseased tissues was, on average, most when exposed to full chemical control (50.7% for *R. cerealis*). They were isolated equally often, from stems of plants subjected to no chemical treatment, only in the third year of monoculture. Among the other fungi, *G. avenacea* (10.4%) and *F. culmorum* (4.5%) dominated.

Rhizoctonia spp., mostly *R. cerealis* and *R. solani*, were also isolated from shoots that were healthy or had symptoms of other diseases (Table 11). At the milk ripe stage (GS 75–77), *R. cerealis* was isolated more frequently from stems that had fusarium foot rot symptoms and *R. solani* more frequently from stems with eyespot symptoms. Both *R. cerealis* and *R. solani* were also isolated from diseased roots as well as from healthy roots at GS 13–14.

DISCUSSION

Sharp eyespot symptoms were relatively frequent in these experiments. Symptoms were already apparent in autumn and became much more common at the stem elongation and at milk ripe stages. Other authors have indicated the increasing importance of this disease in Poland (Żółtańska 2005; Kurowski and Adamiak 2007; Lemańczyk 2012). The importance of sharp eyespot has also been reported in New Zealand (Cromey *et al*. 2002), Turkey (Tunali *et al*. 2008) and China (Chen *et al*. 2010).

More symptoms were noted in the 2006/2007 growing season at both locations. This increase was probably because of the higher mean temperature and total rainfall over that period, and particularly the warm and wet autumn and winter, followed by a cool and wet spring (Table 2). Such conditions are favourable to infection and plant tissue infestation by *R. cerealis* and result in a more

intensive occurrence of sharp eyespot (Clarkson and Cook 1983; Polley and Thomas 1991; Bockus *et al*. 2010).

Sharp eyespot was more frequent in Mochełek (Experiment 2), especially in the second series (details not shown), than in Osiny (Experiment 1). The soil, which is the key source of infection, may have affected the between- location differences. A lighter dry, not very compact, sandy soil, rather poor in organic matter generally helps the persistence and colonization of plant tissues by *R. cerealis*, and thus increases disease intensity (Daamen and Stol 1990; Cromey *et al*. 2002; Bockus *et al*. 2010). This explains the greater infection in Mochełek, where the soil is slightly lighter than in Osiny. Hamada *et al*. (2011a) suggested that in addition to the environmental factors of soil type, temperature, and humidity, the occurrence of sharp eyespot is also affected by soil pH, sowing date, nutrient availability, mineral and organic fertilization, and tillage treatments.

Sharp eyespot symptoms were already present in the first wheat crop in Experiment 2 (Mochełek). White mustard used as a fore-crop (grown after winter wheat) probably did not contribute to limiting disease intensity, despite the presence of thioglycosides produced as a result of its decomposition (Larkin *et al*. 2011). According to Bockus *et al*. (2010), the occurrence of sharp eyespot depends very much on crop rotation. Their results are in agreement with our present results. An increase in infection by *R. cerealis* is usually observed when cereals are grown after a potential host (Colbach *et al*. 1997). This was not clearly confirmed, however, by the research on long-term monoculture by Kurowski and Adamiak (2007). Even though they isolated more *R. cerealis* from wheat grown in monoculture than from wheat in rotation, disease intensity did not differ significantly. The effect of crop rotation can be limited by the fact that *Rhizoctonia* spp., especially *R. solani*, can attack various plant species, not only cereals. It should be noted, however, that there is considerable variation within that species and not all anastomosis groups of *R. solani* infect cereals (Sneh *et al*. 1991).

The comparison of farming systems (Experiment 1) also did not definitely indicate a negative effect of long-term monoculture on the occurrence of sharp eyespot. In Experiment 1, the most disease was in wheat grown in monoculture and only at the stem elongation stage. At the later ripening stage, most disease was in the conventional and organic systems, which is in agreement with reports by Łukanowski (2009). That author isolated *Rhizoctonia* spp. most often from wheat grown in monoculture at the stem elongation stage, while at the milk maturity stage the farming system no longer had an effect. As in the present research, *Rhizoctonia* spp. were isolated the least from the integrated system. The least amount of infection occurred in the integrated system and may have resulted from adequately balanced fertilization. Well-fertilized plants show more resistance to pathogens. Adequate fertilization results in, for example, development of micro-organisms in soil that may limit the development of pathogens (MacNish 1988). The lack of significant differences between the conventional and the organic systems is in agreement with Matusinsky *et al*. (2008) whose research involved the PCR technique.

The higher dose of fertilizer (Experiment 2) sometimes resulted in significantly less sharp eyespot. A considerable decrease in infection by *R. cerealis* has been observed with a higher K content in soil (Hamada *et al*. 2011a). Increasing nitrogen rates, at a fixed level of phosphorus and potassium fertilization, has most often led to an increase in infection (Bremner 1969; Colbach *et al*. 1997). The effect of nitrogen, though, depends to a great extent on its form. The nitrate form of nitrogen most often results in a decrease in sharp eyespot intensity, and the ammonium form results in an increase (MacNish 1988). In the present research the application of a higher NPK rate generated an increase in infection in the third growing year, where herbicide only was applied annually. Such application may have directly or indirectly helped the contamination of soil with *Rhizoctonia* spp.

There was usually more sharp eyespot assessed at the stem elongation and ripening stage, in a higher sowing density of the first year of monoculture where herbicide was applied. Growing wheat at the same site in the following years would have helped inoculum accumulation in soil. In successive growing years, sowing density would no longer be important. Colbach *et al*. (1997) observed an increase in sharp eyespot intensity at a higher sowing density, only at early development stages. Higher plant density means a shorter distance between the inoculum and the host plant which considerably increases the probability of the pathogen reaching the plant. Glynne (1951), however, observed less disease intensity with higher plant density.

The active fungicidal ingredients used in all but the organic system in Experiment 1, did not clearly influence sharp eyespot. The active ingredients included flusilazole, fuberidazole, imazalil, picoxystrobin, propiconazole and triadimenol, which can inhibit the development and infection of cereals by *R. cerealis* (Kataria *et al*. 1991). Cyproconazole, which has some growth regulating properties, can also inhibit plant growth, and hence infection (Köller 1987).

In the short-term monoculture of Experiment 2, fungicides had no clear effect on sharp eyespot in wheat. Similarly, Kurowski and Adamiak (2007) did not observe a decrease in the intensity of sharp eyespot in wheat grown over many years of monoculture as a result of the application of fungicides, or fungicides and herbicides. Active ingredients of fungicides can, however, have different effects on *Rhizoctonia* spp. According to Kataria *et al*. (1991), flusilazole inhibits to a small extent, the development and infection by *R. cerealis* and *R. solani* on wheat. A clearly fungistatic effect of carbendazim towards *R. cerealis* is observed under conditions *in vitro*. Activity *in vitro* is not always reflected as effectiveness in the field, in fact, the opposite may be the case (Gisi 1996). In field crops, carbendazim sometimes results in a decrease in infection and sometimes an increase (Clarkson and Cook 1983). Carbendazim shows properties similar to cytokinins and it can affect the chemical processes of the host plant cells. Carbendazim can also influence the equilibrium of soil micro-organisms and thus pathogen development, including *Rhizoctonia* spp., or secondary pathogens (Cook 1981). This probably results mainly from reduced

competition. Increases in sharp eyespot, where true eyespot is controlled by fungicides, are well-known (Prew and McIntosh 1975). Increased infection can result from the effects of the pesticides applied in a given field in the preceding years (Daamen and Stol 1990).

Sharp eyespot was sometimes less intense where herbicide was applied. Decreased infection as a result of herbicide use may occur because herbicides may have some activity against micro-organisms, including pathogens, in the field. More often, herbicide, applied with or without fungicide, tended to increase sharp eyespot in Experiment 2. This effect may have resulted from an increased density of the main host plant (wheat). Increased infection with greater host-plant density was reported earlier (Lemańczyk 2012). Gisi (1996) reported on active ingredients of pesticides affecting *Rhizoctonia* spp. differently, depending on whether they were applied individually or in mixtures. Sometimes fungicides and herbicides applied together, inhibit the development of *R. cerealis*, while when applied separately do not show any response. Herbicides can enhance the effectiveness of fungicides considerably (Kataria and Gisi 1989). Any favourable effect of herbicide may not have been identified in the first year, since, as mentioned above, survival of *R. cerealis* may be strongly influenced by pesticides applied in previous years (Daamen and Stol 1990). The application of various pesticides can stimulate the development of *Rhizoctonia* in the soil (Scholte 1987).

The mechanisms by which herbicides affect plant pathogens are very complex. Herbicides can affect the pathogen itself directly as well as indirectly by affecting the crop, weeds, mycorrhizae, antagonists and the effectiveness of fungicides (Lévesque and Rahe 1992; Wisler and Norris 2005). There are four mechanisms by which disease intensity can be increased, namely a direct effect of herbicides on growth or virulence, or on host sensitivity, and/or changes in the relationships between the pathogen and other soil organisms. Herbicides can stimulate the processes of plant resistance to pathogens (Lévesque and Rahe 1992). Herbicides can also trigger pathogen development, including *Rhizoctonia* spp. in the soil (Altman and Rovira 1989; Lévesque and Rahe 1992; Velini *et al*. 2010). According to Eshel and Katan (1972), an observed initial increased *Rhizoctonia* spp. infection does not come from greater susceptibility of the plant host after the application of herbicides but from the initial inhibition of antagonistic organism developments in the soil.

Many authors report that herbicides enhance the development of soil micro-organisms (Altman and Rovira 1989). Increased development of micro-organisms in soil after the application of herbicides can be due to a more intensive production of substances by plant roots which stimulate the development of the micro-organisms (Lévesque and Rahe 1992). Foliar application of mecoprop helps to significantly increase the population of fluorescent *Pseudomonas* spp. in soil, and hence, less infection by pathogens (Lévesque and Rahe 1992). According to Busse *et al*. (2004), the application of herbicides on sandy loam soil, as in Mochełek (Experiment 2), can result in a decrease in the abundance of micro-organisms, which may have resulted in no increase in the population of antagonistic micro-organisms initially in the present research, in which there was also no improvement in the health status. The literature does not, however, provide information on direct or indirect effects of iodosulfuron-methyl-sodium on *Rhizoctonia* spp.

No reports on direct effects of the other pesticides applied in the present research on the infection by *R. cerealis* have been found. There is only information on an effect of herbicide on the colonization of wheat with sharp eyespot symptoms by *R. solani*. The application of herbicide 2,4-D (Rai *et al*. 2000) and glyphosate (Smiley et al. 1992) inhibits plant infection by *R. solani*, whereas chlorsulfuron can increase infection (Rovira and McDonald 1986). Vroumsia *et al.* (1996), on the other hand, reported that *R. solani* was able to cause the biodegradation of isoproturon in soil. Application of cypermethrin, an insecticide, could modify infection since Tu (1982) observed its inhibition of the growth of *R. solani in vitro*. The application of retardants in wheat also probably have an effect. Arora and Bajaj (1985) suggested that application of ethephon induced plant resistance to *R. solani* infection. Burpee (1998) reported that trinexapacethyl, a growth regulator, applied with the fungicide propiconazole, could increase plant infection by *R. solani*, while trinexapacethyl applied alone did not affect infection.

In Experiment 2, weeds were most abundant in the control (details not shown), less abundant after herbicide and fungicide, and least abundant where herbicide was used exclusively. Host plants of *R. solani*, which may also be associated with sharp eyespot, include numerous weed species representing various families (Black *et al*. 1996), while those of *R. cerealis* are mostly *Poaceae* (Bockus *et al*. 2010). Piekarczyk (2010b) reported how the occurrence of weeds can depend on crop protection. The dominant weed species were the grass *Apera spica-venti*, a potential host plant of *R. cerealis* (Bockus *et al*. 2010), followed by *Viola arvensis*, *Capsella bursa-pastoris*, *Matricaria inodora* and *Veronica arvensis*, which can be a host plant of *R. solani* (Peltier 1916). According to Black *et al*. (1996), the removal of weeds that are host plants of *R. solani* does not always result in decreased cereal infection by that fungus, as in the present research.

A decrease in yield was associated with weed infestation, even in diseased wheat. This indicates that weeds had a much greater effect than disease on yield, as reported by Piekarczyk (2010a). Sharp eyespot usually does not cause considerable yield losses (Clarkson and Cook 1983; Cromey *et al*. 2002).

Conforming to the guidelines for growing plants in organic farming, neither pesticides nor mineral fertilizers were used in the organic farming system. Only organic fertilization was applied, which considerably helped the development of micro-organisms in the soil, providing natural protection from pathogens (Grünwald *et al*. 2000). Development of fungi in the genera *Trichoderma*, *Gliocladium* and *Penicillium* is reported to be better in such soils. Knudsen *et al*. (1999), Lenc *et al*. (2011) found that high biological activity of the soil in organic and integrated systems is not always correlated with a high capacity for inhibiting pathogen development. In the organic system, the use of grass as well as cereals considerably increased

the proportion of host plants of *R. cerealis*, which would have helped its survival in soil (Colbach *et al*. 1997).

Mostly *R. cerealis* was isolated from stems with sharp eyespot symptoms; *R. solani* was much less frequent. *R. cerealis* is considered the main agent of that disease in Poland (Żółtanska 2005; Kurowski and Adamiak 2007; Lemańczyk 2012). *R. solani* also infects cereal stems, but symptoms are usually not typical of sharp eyespot, and it usually infests tissues already infected (Mazzola *et al*. 1996). *R. cerealis* is detected more at later wheat growth stages (Matusinsky *et al*. 2008). Nicholson *et al*. (2002) found that the amount of *R. cerealis* DNA relative to total DNA derived from the plant increased at successive wheat growth stages. Similarly, in the present research, *R. cerealis* and *R. solani* isolates were found more frequently at the end of plant growth than at the seedling stage.

R. cerealis was also isolated from plants not showing typical symptoms of sharp eyespot, as Lemańczyk (2012) also noted. Nicholson and Parry (1996) and Matusinsky *et al*. (2008) reported that there was only a little correlation between visual assessments and the PCR results, which were compared as methods for evaluating the occurrence of sharp eyespot, especially at early growth stages in wheat. Plants with symptoms typical of other diseases were most often infected by *R. solani*, confirming its capacity for saprotrophic development (Sneh *et al*. 1991).

Only two multinucleate *Rhizoctonia* isolates were not classified as *R. solani* using PCR. They were identified as *R. zeae*, a species that can also infect wheat, but usually does not result in disease symptoms (Mazzola *et al*. 1996). This is probably the first report on the occurrence of *R. zeae* in winter wheat in Poland. So far, the species was identified only in grasses, including seed grasses (Prończuk 2000). The development of *R. zeae* on wheat stems was helped by relatively high temperature in 2007, as noted by Mazzola *et al*. (1996), who added that this species is much less virulent than *R. cerealis* or *R. solani* on wheat.

Despite clear symptoms of sharp eyespot, fungi commonly isolated from tissues represented genera considered saprotrophic on cereals: *Aspergillus*, *Penicillium* and *Trichoderma* as well as *Fusarium* spp., especially *F. culmorum* and *G. avenacea*. A few species of fungi, usually not *Rhizoctonia* spp., were sometimes isolated from a single stem showing typical sharp eyespot symptoms, cut into smaller fragments and placed onto PDA. Only PCR enabled confirmation of the presence of *R. cerealis* or *R. solani* in such tissues (Lemańczyk 2011). Species that infest the infected tissues secondarily, or participate in mixed infections, including *Fusarium* spp., were often isolated. *Rhizoctonia cerealis* is a pathogen specialized for cereal infection; it grows relatively slowly on artificial media and is often overgrown by *Fusarium* spp. and saprotrophic fungi (Bateman and Kwaśna 1999; Kwaśna *et al*. 2010; Lemańczyk and Sadowski 2002). Frequently the aboveground plant tissues infected by *Rhizoctonia* are infested secondarily by less specialized pathogens, *e.g. Fusarium* spp. or saprotrophs. It is much easier to isolate *R. solani* than *R. cerealis*, which has a slower linear growth rate on PDA medium.

In conclusion, sharp eyespot and its pathogen, *R. cerealis*, at the levels recorded here, did not have any important effect on wheat yield. Potential risk may be lessened, however, by maintaining adequately balanced fertilization and optimum plant density, while avoiding growing wheat crops in succession.

ACKNOWLEDGEMENTS

This work was supported by Grant No PB 0842/P06/2005/28 from the Polish Ministry of Science and Higher Education. Special thanks to Prof. Jan Kuś of the Institute of Soil Science and Plant Cultivation, the State Institute Research in Puławy, for making it possible for the field observations to be performed in Osiny.

REFERENCES

Altman J., Rovira A.D. 1989. Herbicide-pathogen interactions in soil-borne root diseases. Can. J. Plant Pathol. 11 (2): 166–172.

Arora Y.K., Bajaj K.L. 1985. Peroxidase and polyphenol oxidase associated with, induced resistance of mung bean to *Rhizoctonia solani* Kühn. J. Phytopathol. 114 (4): 325–331.

Bandoni R.J. 1979. Safranin O as a rapid nuclear stain for fungi. Mycologia 71 (4): 873–874.

Bateman G.L., Edwards S.G., Marshall J., Morgan L.W., Nicholson P., Nuttall M., Parry D.W., Scrancher M., Turner A.S. 2000. Effects of cultivar and fungicides on stem-base pathogens, determined by PCR, and on diseases and yield of wheat. Ann. Appl. Biol. 137 (3): 213–221.

Bateman G.L., Kwaśna H. 1999. Effects of number of winter wheat crops grown successively on fungal communities on wheat roots. Appl. Soil Ecol. 13 (3): 271–282.

Black B.D., Griffin J.L., Russin J.S., Snow J.P. 1996. Herbicide effects on *Rhizoctonia solani in vitro* and *Rhizoctonia foliar* blight of soybean (*Glycine max*). Weed Sci. 44 (3): 711–716.

Bockus W.W., Bowden R.L., Hunger R.M., Morrill W.L., Murray T.D., Smiley R.W. (eds.) 2010. Compendium of Wheat Diseases and Pests. 3rd edition. APS Press, St. Paul, MN, 171 pp.

Boerema G.H., Verhoeven A.A. 1977. Check-list for scientific names of common parasitic fungi. Series 26: Fungi on field crops: Cereals and grasses. Neth. J. Plant Pathol. 83 (5): 165–204.

Bremner P.M. 1969. Effects of time and rate of nitrogen application on tillering, 'sharp eyespot' (*Rhizoctonia solani*) and yield in winter wheat. J. Agric. Sci. (Cambridge) 72 (2): 273–280.

Burpee L. 1980. *Rhizoctonia cerealis* causes yellow patch of turfgrasses. Plant Dis. 64 (12): 1114–1116.

Burpee L.L. 1998. Effects of plant growth regulators and fungicides on Rhizoctonia blight of tall fescue. Crop Prot. 17 (6): 503–507.

Busse M.D., Fiddler G.O., Ratcliff A.W. 2004. Ectomycorrhizal formation in herbicide-treated soils of differing clay and organic matter content. Water Air Soil Poll. 152 (1–4): 23–34.

Chen H.G., Cao Q.G., Xiong G.L., Li W., Zhang A.X., Yu H.S., Wang J.S. 2010. Composition of wheat rhizosphere antagonistic bacteria and wheat sharp eyespot as affected by rice straw mulching. Pedosphere 20 (4): 505–514.

Clarkson J.D.S., Cook R.J. 1983. Effects of sharp eyespot on yield loss in winter wheat. Plant Pathol. 32 (4): 421–428.

Colbach H., Lucas P., Cavelier N., Cavelier A. 1997. Influence of cropping system on sharp eyespot in winter wheat. Crop Prot. 16 (5): 415–422.

Cook R. 1981. Unexpected effects of fungicides on cereal yields. Bull. OEPP/EPPO Bull. 11 (3): 277–285.

Cromey M.G., Butler R.C., Boddington H.J., Moorhead A.R. 2002. Effects of sharp eyespot on yield of wheat (*Triticum aestivum*) in New Zealand. N.Z. J. Crop Hort. 30 (1): 9–17.

Daamen R.A., Stol W. 1990. Surveys of cereal diseases and pests in the Netherlands. 2. Stem-base diseases of winter wheat. Neth. J. P1ant Pathol. 96 (5): 251–260.

Doyle J.J., Doyle J.L. 1990. Isolation of plant DNA from fresh tissue. Focus 12 (1): 13–15.

Eshel Y., Katan J. 1972. Effect of time of application of diphenamid on pepper, weeds and disease. Weed Sci. 20 (5): 468–471.

Gisi U. 1996. Synergistic interaction of fungicides in mixtures. Phytopathology 86 (11): 1273–1279.

Glynne M.D. 1951. Effect of cultural treatments on wheat and on the incidence of eyespot, lodging, take-all and weeds. Ann. Appl. Biol. 38 (3): 665–688.

Gołębniak B., Weber Z., Smith C.M. 1993. Chemical control of *Rhizoctonia* spp. on potato and wheat. Phytopathol. Polonica 18: 45–54.

Grünwald N.J., Hu S., Bruggen A.H.C. van 2000. Short-term cover crop decomposition in organic and conventional soils: Characterization of soil C, N, microbial and plant pathogen dynamics. Eur. J. Plant Pathol. 106 (1): 37–50.

Hamada M.S., Yin Y., Chen H., Ma Z. 2011a. The escalating threat of *Rhizoctonia cerealis*, the causal agent of sharp eyespot in wheat. Pest Manag. Sci. 67 (11): 1411–1419.

Hamada M.S., Yin Y., Ma Z. 2011b. Crop protection sensitivity to iprodione, difenoconazole and fludioxonil of *Rhizoctonia cerealis* isolates collected from wheat in China. Crop Prot. 30 (8): 1028–1033.

Johanson A., Turner H.C., McKay G.J., Brown A.E. 1998. A PCR-based method to distinguish fungi of the rice sheath-blight complex, *Rhizoctonia solani, R. oryzae* and *R. oryzae-sativae*. FEMS Microbiol. Lett. 162 (2): 289–294.

Kataria H.R., Gisi U. 1989. Recovery from soil and sensitivity to fungicides of *Rhizoctonia cerealis* and *R. solani*. Mycol. Res. 92 (4): 458–462.

Kataria H.R., Hugelshofer U., Gisi U. 1991. Sensitivity of *Rhizoctonia* species to different fungicides. Plant Pathol. 40 (2): 203–211.

Knudsen I.M.B., Debosz K., Hockenhull J., Jensen D.F., Elmholt S. 1999. Suppressiveness of organically and conventionally managed soils towards brown foot rot of barley. Appl. Soil Ecol. 12 (1): 61–72.

Köller W. 1987. Isomers of sterol synthesis inhibitors: Fungicidal effects and plant growth regulator activities. Pestic. Sci. 18 (2): 129–147.

Kurowski T.P., Adamiak E. 2007. Occurrence of stem base diseases of four cereal species grown in long-term monocultures. Pol. J. Natur. Sc. 22 (4): 574–583.

Kwaśna H., Bateman G.L., Ward E. 2010. Microbiota in wheat roots evaluated by cloning of ITS1/2 rDNA and sequencing. J. Phytopathol. 158 (4): 278–287.

Larkin R.P., Honeycutt C.W., Olanya O.M. 2011. Management of Verticillium wilt of potato with disease-suppressive green manures and as affected by previous cropping history. Plant Dis. 95 (5): 568–576.

Lemańczyk G. 2011. Use of SCAR-PCR in diagnostics of stem base pathogens of the *Rhizoctonia* and *Oculimacula* genus. Acta Agrobot. 64 (4): 197–206.

Lemańczyk G. 2012. The role of the preceding crop and weed control in the transmission of *Rhizoctonia cerealis* and *R. solani* to winter cereals. J. Plant Prot. Res. 52 (1): 93–105.

Lemańczyk G., Sadowski Cz. 2002. Fungal communities and health status of roots of winter wheat cultivated after oats and oats mixed with other crops. BioControl 47 (3): 349–361.

Lenc L., Kwaśna H., Sadowski Cz. 2011. Dynamics of the root/soil pathogens and antagonists in organic and integrated production of potato. Eur. J. Plant Pathol. 131 (4): 603–620.

Lévesque C.A., Rahe J.E. 1992. Herbicide interactions with fungal root pathogens, with special reference to glyphosate. Annu. Rev. Phytopathol. 30: 579–602.

Łukanowski A. 2009. Winter wheat stem base infestation and fungal communities occurring on stems in dependence on cropping system. Phytopathologia 53: 43–55.

MacNish G.C. 1988. Changes in take-all (*Gaeumannomyces graminis* var. *tritici*), Rhizoctonia root rot (*Rhizoctonia solani*) and soil pH in continuous wheat with annual applications of nitrogenous fertilizer in Western Australia. Aust. J. Exp. Agric. 28 (3): 333–341.

Matusinsky P., Mikolasova R., Klem K., Spitzer T., Urban T. 2008. The role of organic vs. conventional farming practice, soil management and preceding crop on the incidence of stem-base pathogens on wheat. J. Plant Dis. Prot. 115 (1): 17–22.

Mazzola M., Smiley R.W., Rovira A.D., Cook R.J. 1996. Characterization of *Rhizoctonia* isolates, disease occurrence and management in cereals. p. 259–267. In: "*Rhizoctonia* Species: Taxonomy, Molecular, Biological, Ecological, Pathology, and Disease Control" (B. Sneh, S. Jabaji-Hare, S. Neate, G. Dijst, eds.). Kluwer Academic Publishers, Dordrecht, The Netherlands, 584 pp.

Nicholson P., Parry D.W. 1996. Development and use of a PCR assay to detect *Rhizoctonia cerealis,* the cause of sharp eyespot in wheat. Plant Pathol. 45 (5): 872–883.

Nicholson P., Turner A.S., Edwards S.G., Bateman G.L., Morgan L.W., Parry D.W., Marshall J., Nuttall M. 2002. Development of stem-base pathogens on different cultivars of winter wheat determined by quantitative PCR. Eur. J. Plant Pathol. 108 (2): 163–177.

Peltier G.L. 1916. Parasitic *Rhizoctonias* in America. Urbana, University of Illinois, Bull. 189, 108 pp.

Piekarczyk M. 2010a. Effect of fertilisation dose, plant protection input, and seeding density on the yield and grain technological quality of winter wheat grown in short-time monoculture on light soil. Acta Sci. Pol., Agricultura 9 (2): 15–23.

Piekarczyk M. 2010b. Wpływ poziomu nawożenia, ochrony roślin i gęstości siewu na zachwaszczenie pszenicy ozimej uprawianej w krótkotrwałej monokulturze. Ann. UMCS, Sec. E, Agricultura 65 (2): 48–57.

Polley R.W., Thomas M.R. 1991. Surveys of diseases of winter wheat in England and Wales, 1976–1988. Ann. Appl. Biol. 119 (1): 1–20.

Prew R.D., Mclntosh A.H. 1975. Effects of benomyl and other fungicides on take-all, eyespot and sharp eyespot diseases of winter wheat. Plant Pathol. 24 (2): 67–71.

Prończuk M. 2000. Choroby traw – występowanie i szkodliwość w uprawie na nasiona i użytkowaniu trawnikowym. [Grass diseases – occurrence and harmfulness in seed production and turf maintenance]. Monografie i Rozpr. Nauk. IHAR Radzików 4, 183 pp.

Rai J.P., Dubey K.S., Sinha A. 2000. Effect of some herbicides on the growth of *Rhizoctonia solani* Kühn in vitro. Indian J. Weed Sci. 32 (1/2): 112–113.

Ray R.V., Jenkinson P., Edwards S.G. 2004. Effects of fungicides on eyespot, caused predominantly by *Oculimacula acuformis*, and yield of early-drilled winter wheat. Crop Prot. 23 (12): 1199–1207.

Rovira A.D., McDonald A.J. 1986. Effects of the herbicide chlorsulfuron on Rhizoctonia bare patch and take-all of barley and wheat. Plant Dis. 70 (9): 879–882.

Scholte K. 1987. The effect of crop rotation and granular nematicides on the incidences of *Rhizoctonia solani* in potato. Potato Res. 30 (2): 187–199.

Smiley R.W., Ogg A.G.Jr., Cook R.J. 1992. Influence of glyphosate on Rhizoctonia root rot, growth, and yield of barley. Plant Dis. 76 (9): 937–942.

Sneh B., Burpee L., Ogoshi A. 1991. Identification of *Rhizoctonia* Species. APS Press, St. Paul, MN, USA, 133 pp.

Tu C.M. 1982. Effect of pyrethroid insecticides on soybean and its pathogen *Rhizoctonia solani* Kühn. J. Environ. Sci. Heal. B. 17 (1): 43–50.

Tunali B., Nicol J.M., Hodson D., Uçkun Z., Büyük O., Erdurmuş D., Hekimhan H., Aktaş H., Akbudak M.A., Bağcı S.A. 2008. Root and crown rot fungi associated with spring, facultative, and winter wheat in Turkey. Plant Dis. 92 (9): 1299–1306.

Velini E.D., Trindade M.L.B., Barberis L.R.M., Duke S.O. 2010. Growth regulation and other secondary effects of herbicides. Weed Sci. 58 (3): 351–354.

Vroumsia T., Steiman R., Seigle-Murandi F., Benoit-Guyod J.-L., Khadrani A. 1996. Biodegradation of three substituted phenylurea herbicides (chlortoluron, diuron, and isoproturon) by soft fungi. A comparative study. Chemosphere 33 (10): 2045–2056.

Wenzel H. 1948. Zur Erfassung des Schadenausmasses in Pflanzenschutzversuchen. Pflanzenschutz-Ber. 15: 81–84.

Wisler G.C., Norris R.F. 2005. Interactions between weeds and cultivated plants as related to management of plant pathogens. Weed Sci. 53 (6): 914–917.

Zadoks J.C., Chang T.T., Konzak C.F. 1974. A decimal code for the growth stages of cereals. Weed Res. 14 (6): 415–421.

Żółtańska E. 2005. The effect of previous crop and weather conditions on the incidence of stem base diseases in winter wheat. J. Plant Prot. Res. 45 (1): 37–40.