Table 10. Share [in %] of *R. cerealis* and *R. solani* in total number of fungi isolated from healthy and diseased stem base and roots of winter wheat depending on the role played by the growth stage and preceding crop (2003–2005)

Preceding crop	Fungi	GS 13–14					GS 34–36		GS 75–77			
		DR	HR	O	F	HSB	DR	HR	R	O	F	HSB
Spring barley	Rc	0.0	0.0	0.0	0.0	0.0	1.1	0.0	22.2	2.4	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	4.3	0.0	11.1	3.5	0.0	0.0
	TNI	15	37	4	13	43	92	37	18	85	42	10
Oats	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	2.3	0.0	0.0	0.0	0.0	0.0
	TNI	22	14	6	9	44	129	36	18	131	39	13
Spring wheat	Rc	0.0	0.0	0.0	0.0	4.0	0.0	0.0	11.5	0.8	0.0	0.0
	Rs	3.2	0.0	0.0	0.0	0.0	0.9	0.0	1.6	3.3	0.0	0.0
	TNI	31	18	5	3	25	215	62	61	122	27	15
Spring triticale	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	31.6	0.8	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	0.8	0.0	5.3	3.3	0.0	0.0
	TNI	41	27	1	17	27	131	38	19	120	37	11
Fallow	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	29.4	2.8	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	1.6	0.0	11.8	5.5	0.0	0.0
	TNI	17	35	13	10	26	182	25	17	109	77	13
Mean	Rc	0.0	0.0	0.0	0.0	0.8	0.2	0.0	25.6	1.4	0.0	0.0
	Rs	0.6	0.0	0.0	0.0	0.0	2.0	0.0	6.0	3.1	0.0	0.0
	TNI	126	131	29	52	165	749	198	133	567	222	62

DR – diseased roots; HR – healthy roots; O – stems with eyespot symptoms; F – stems with Fusarium foot rot symptoms; HSB – healthy stem base; R – stems with sharp eyespot symptoms; Rc – *R. cerealis*; Rs – *R. solani*; TNI – total number of all isolates

Table 11. Share [in %] of *R. cerealis* and *R. solani* in total number of fungi isolated from healthy and diseased stem base and roots of winter triticale depending on the role played by the growth stage and preceding crop (2003–2005)

Preceding crop	Fungi	GS 13–14					GS 34–36		GS 75–77			
		DR	HR	O	F	HSB	DR	HR	R	O	F	HSB
Spring barley	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	60.0	0.5	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	2.2	0.0	0.0
	TNI	36	36	7	6	30	69	24	5	183	21	30
Oats	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0
	TNI	15	18	2	21	28	57	32	7	196	56	25
Spring wheat	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0
	TNI	22	20	6	15	28	154	23	14	136	50	36
Spring triticale	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.6	0.0	0.0
	TNI	21	33	4	18	27	139	28	2	84	47	29
Fallow	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	TNI	18	44	5	29	40	59	27	2	138	56	33
Mean	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.0	0.5	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	0.4	0.0	4.0	1.3	0.0	0.0
	TNI	112	151	24	89	153	478	134	30	737	230	153

DR – diseased roots; HR – healthy roots; O – stems with eyespot symptoms; F – stems with Fusarium foot rot symptoms; HSB – healthy stem base; R – stems with sharp eyespot symptoms; Rc – *R. cerealis*; Rs – *R. solani*; TNI – total number of all isolates

Table 12. Share [in %] of *R. cerealis* and *R. solani* in total number of fungi isolated from healthy and diseased stem base and roots of winter rye depending on the role played by the growth stage and preceding crop (2003–2005)

Preceding crop	Fungi	GS 13–14					GS 34–36		GS 75–77			
		DR	HR	O	F	HSB	DR	HR	R	O	F	HSB
Spring barley	Rc	0.0	0.0	0.0	0.0	5.4	0.0	0.0	7.1	0.0	0.0	9.1
	Rs	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	TNI	7	22	0	55	37	94	11	42	66	33	22
Oats	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.1	0.0	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	TNI	2	18	5	14	22	75	22	9	98	37	11
Spring wheat	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	26.7	1.0	2.4	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	1.0	0.0	6.7	2.1	0.0	0.0
	TNI	13	34	8	9	28	105	33	15	97	42	23
Spring triticale	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	0.0	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	TNI	14	15	0	19	22	99	23	10	74	44	16
Fallow	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	46.2	0.0	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.7	0.0	0.0	7.7
	TNI	1	21	1	11	28	82	22	13	127	54	13
Mean	Rc	0.0	0.0	0.0	0.0	1.1	0.0	0.0	22.2	0.2	0.5	1.8
	Rs	0.0	0.0	0.0	0.0	0.0	0.2	0.0	2.9	0.4	0.0	1.5
	TNI	37	110	14	108	137	455	111	89	462	210	85

DR – diseased roots; HR – healthy roots; O – stems with eyespot symptoms; F – stems with Fusarium foot rot symptoms; HSB – healthy stem base; R – stems with sharp eyespot symptoms; Rc – *R. cerealis*; Rs – *R. solani*; TNI – total number of all isolates

Table 13. Share [in %] of *R. cerealis* and *R. solani* in total number of fungi isolated from healthy and diseased stem base and roots of winter barley depending on the role played by the growth stage and preceding crop (2003–2005)

Preceding crop	Fungi	GS 13–14					GS 34–36		GS 75–77			
		DR	HR	O	F	HSB	DR	HR	R	O	F	HSB
Spring barley	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Rs	0.0	5.1	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0
	TNI	64	59	0	3	42	151	31	11	99	23	10
Oats	Rc	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	3.8	0.0	0.0	0.0	0.0	0.0
	TNI	14	16	5	16	20	104	25	9	108	33	5
Spring wheat	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	36.4	0.0	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.1	1.2	0.0	0.0
	TNI	37	31	6	26	29	215	24	11	85	33	0
Spring triticale	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0
	TNI	39	20	4	14	43	172	33	16	138	25	0
Fallow	Rc	0.0	0.0	0.0	0.0	0.0	0.0	0.0	20.0	0.9	0.0	0.0
	Rs	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.9	0.0	0.0
	TNI	6	82	0	13	36	149	29	5	111	24	9
Mean	Rc	0.0	0.0	0.0	0.0	0.0	0.2	0.0	11.3	0.2	0.0	0.0
	Rs	0.0	1.0	0.0	0.0	0.0	1.2	0.0	1.8	0.4	0.0	0.0
	TNI	160	208	15	72	170	791	142	52	541	138	24

DR – diseased roots; HR – healthy roots; O – stems with eyespot symptoms; F – stems with Fusarium foot rot symptoms; HSB – healthy stem base; R – stems with sharp eyespot symptoms; Rc – *R. cerealis*; *R. solani*; TNI – total number of all isolates

were considered sparotrophic for that group of plants as well as the fungi which at the conidial stage represent genus *Fusarium*. On average, the share of *R. cerealis* in wheat was 17.3%, in triticale – 5.8%, in rye – 13.3% and in barley – for 7.8% (Tables 6–9). The share of *R. solani* in respective cereal species accounted for: 2.4, 1.9, 1.3 and 1.5%, respectively. The share of those fungal species differed across the years. For all the cereals investigated, fungal species were the highest in 2004 when there were also the most symptoms of sharp eyespot observed. No clear effect of the preceding crop on the share of *R. cerealis* and *R. solani* of all the fungi isolated from the infected tissues, was reported. Among the other fungal species, *Haematonectria haematococca* (anamorph *Fusarium solani*) was isolated most frequently, especially in 2003, from the cereals tissues showing the symptoms of sharp eyespot. The infected tissues were often infested by *Gibberella avenacea* (anamorph *Fusarium avenaceum*), *G. intricans* (anamorph *F. equiseti*) and *F. culmorum. G. avenacea* was mostly isolated in 2005. *H. haematococca* dominated in the cereals grown after spring forms of the same species. In the case of rye, *H. haematococca* dominated in the cereals that were cultivated after triticale.

Fungi representing the genus *Rhizoctonia,* were also isolated from the stem base showing the disease symptoms typical for infection caused by *Oculimacula* spp. and *Fusarium* spp. (Tables 10–13). Mostly *R. solani* was obtained, and mostly at the end of the vegetation period. *R. solani* was often separated from the tissues, from the symptoms of true eyespot. From healthy stem bases, *R. cerealis* was isolated more often. The fungi were also isolated from both healthy roots and roots demonstrating disease symptoms. *R. cerealis* were much more often obtained when performing the second isolation though *R. solani* remained the dominant species. The amount of those species did not depend on the preceding crop.

The PCR reaction performed using starters Rc2 F/R facilitated the verification of the selected *R. cerealis* isolates, giving an expected product of amplification of the length of 800 pairs of bases (Fig. 1). Besides the PCR reaction, using starters ITS1/GMRS-3 confirmed the occurrence of *R. solani*, giving an expected product of amplification 550 bp (Fig. 2).

DISCUSSION

The applicable literature offers information about the considerable role played by the preceding crop, in the occurrence of sharp eyespot (Colbach *et al*. 1997; Bockus *et al*. 2010). Our own results partially confirm the information found in the literature since most disease symptoms in winter wheat were reported after spring triticale and the least symptoms – after spring triticale or oats. Żółtańska (2005) also found a essentially stronger infection of wheat grown after spring barley, as compared with winter rape. Her results, however, were recorded only in two of the five years of observation. Kurowski and Adamiak (2007) did not confirm that crop rotation in wheat and rye played a considerable role. No significant effect of the preceding crop on the incidence of sharp eyespot was also noted under winter triticale production conditions (Lemańczyk 2010a) and spring cereals (Lemańczyk 2010b). According to Colbach *et al*. (1997), more symptoms of infection with *R. cerealis* are observed when cereals are grown after the plants which are a potential host of that pathogen. They stated that cultivation after plants which are not a host of *R. cerealis,* limited the incidence of sharp eyespot considerably. What is also important, is that the amount of the plant residue from plants on which *R. cerealis* can develop, is in the soil. Growing cereals after a host plant means a greater amount of residue, thus, better conditions for pathogen development (Pitt 1966). In

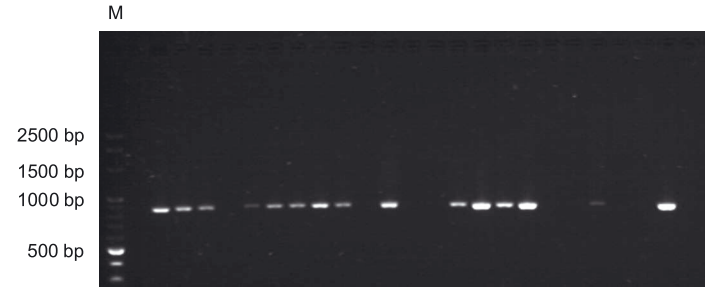

Fig. 1. Confirmation of *R. cerealis* with a PCR assay

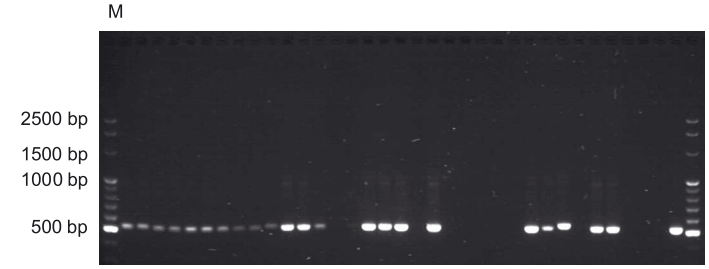

Fig. 2. Confirmation of *R. solani* with a PCR assay

the present research, winter cereals were grown after the various spring cereals which can be attacked by *R. cerealis* as well as by *R. solani*. The research performed on spring cereals commodity plantations, show that most symptoms of sharp eyespot were noted in wheat, followed by triticale, barley and oats (Lemańczyk 2010b), which could have the reason of the infection of wheat grown after oats. However, it is important that residues, on which saprotrophic pathogens in soil develop, are close to the plants. This closeness is especially important for *R. cerealis*, since *R. cerealis* shows a slow linear growth of mycelium. The closer to the inoculum of the host, the higher the probability of infection. The closeness means the mycelium has less distance to cover, to reach the crop and infect it (Colbach *et al*. 1997). A high amount of the preceding crop residue does not automatically mean high disease intensity. The role of the preceding crop can be limited by the fact that fungi representing the genus *Rhizoctonia*, especially *R. solani,* can attack various plant species, not only cereals. Interestingly, however, within that species there occurs a very high variation, and not all the anastomosis groups of that pathogen infect cereals (Sneh *et al*. 1991).

Growing cereals after fallow did not show a considerable effect on the incidence of sharp eyespot. According to Robertson (2002), maintaining fallow enhances the increase in biodiversity and abundance of soil microorganisms. The result is biological suppression of soil. A considerable part of soil microorganisms can also limit the development of *Rhizoctonia* spp. Excluding a field from cultivation for a year, was not sufficient to limit the population of *R. cerealis* and *R. solani*. Those fungi, thanks to the sclerotia they produce, can survive in soil for a few years (Sneh *et al*. 1991).

The hosts of *R. solani* can also be numerous weed species, representing various families (Black *et al*. 1996), whereas for *R. cerealis*, the host is mostly *Poaceae* (Bockus *et al*. 2010). In their earlier publication, Jaskulski and Piasecka (2009) report on the effect of respective spring cereal species as preceding crops, on the weed infestation of winter cereals. Their report was similar, although compared with the other species, oats as a preceding crop considerably limited the weed infestation of winter wheat, spring barley limited weed infestation of winter barley, and spring triticale limited weed infestation of rye. Of all the spring cereals, the lowest weed infestation was in oats. They found that dominant weed species in winter cereals in autumn at all the stands, were as follows: *Viola arvensis*, *Thlaspi arvense*, *Stellaria media*, which can be the hosts of *R. solani* (Peltier 1916), and *Apera spica-venti*, a potential host of *R. cerealis* (Bockus *et al*. 2010). *Elymus repens,* also appeared at the stand after fallow. *Elymus repens,* can be infected by *R. cerealis* (Bockus *et al*. 2010). At that stand, there were also other weeds which were more numerous; and here could be the cause of the limited role played by fallow in the present research. Black *et al*. (1996) found that removing weeds which are the hosts of *R. solani* AG-IA and AG-IB, does not always result in a decrease in plant infection.

The inter cereal most susceptible to weed infestation was wheat, which was earlier presented by Jaskulski and Piasecka (2009). It could be why wheat was the only one in which herbicides had a limited effect on the incidence of sharp eyespot. Fungi representing the genus *Rhizoctonia,* particularly infect weakened plants living under the highest stress. Wheat which was under heavy weed infestation, and which did not have herbicides applied, had to compete with weeds for mineral compounds and water. Thus, wheat was more susceptible to infection. The other cereals investigated show a greater competitiveness for nutrients and at the same time, when exposed to lower weed infestation, they were less susceptible to infection. Colbach *et al*. (1997) report on cereals infected by *R. cerealis.* They stated it is the plant density which is essential. The closer the stems, the greater the probability of infection since the expanding pathogen mycelium has to cover a shorter distance. Once the herbicide application was given up, the plants of rye, triticale and barley were further from one another, which was not favourable to infection.

In the present research, significantly more symptoms of sharp eyespot were found in the plots of triticale, rye and barley treated with herbicides. Other authors reported different results. Kurowski *et al*. (2010), applied herbicides in triticale and observed a limited incidence of sharp eyespot. According to Kurowski and Adamiak (2007), the application of herbicides in rye grown in adequate crop rotation limits the occurrence of sharp eyespot. They found that only in Warko rye grown in monoculture, did herbicide application increased the intensity of the disease. The authors also noted a different reaction across the wheat cultivars to the infection by *R. cerealis* treated with herbicides. In Elena wheat, the use of herbicides helped infection, while in Korweta herbicide application inhibited the disease development. In both cereals the best inhibiting effect was observed when herbicides and fungicides were applied. A different effect of herbicides on the occurrence of sharp eyespot in the present research, could have come from the fact that a varied protection from weeds was already provided in the preceding crops.

The effect of herbicides on plant pathogens is a very complex process. Herbicides can have a direct effect on the pathogen itself as well as an indirect effect by affecting the crop, weeds, mycorrhizae, antagonists, and the effectiveness of fungicides (Lévesque and Rahe 1992; Wisler *et al*. 2005). Herbicides can stimulate the processes of plant resistance to pathogens (Descalzo *et al*. 1990; Lévesque and Rahe 1992). It was also observed, that some herbicides can trigger a considerable increase in fungicide effectiveness. Such a reaction has been confirmed by Kataria and Gisi (1990) who observed that application of the fungicide (cyproconazole) combined with one of the herbicides [dicamba, 4,6-dinitro-o-cresol (DNOC), bromoxynil, ioxynil] inhibited *R. cerealis* infection of wheat seedlings much more effectively than the application of the fungicide alone. Interestingly, dicamba *in vitro*, inhibited the development of *R. cerealis* the least, while under field conditions it was most effective. Much more information on the effect of herbicides on pathogens can be found for *R. solani* than for *R. cerealis* (Altman and Campbell 1977; Lévesque and Rahe 1992).

Many authors report on herbicides enhancing the development of soil microorganisms (Altman and Rovira 1989). There can be a better development of microorganisms in soil after the use of herbicides. This result can be due to a greater secretion by the roots of plants treated with herbicides of various substances stimulating plant development (Lévesque and Rahe 1992).

For example, the foliar application of mecoprop, which was also applied in the present research, contributes to a significant increase in the population of fluorescent *Pseudomonas* spp. in soil. The result is a weaker infection by pathogens (Lévesque and Rahe 1992). According to Rai *et al*. (2000), 2,4-D under laboratory conditions inhibits the development of *R. solani*. According to Busse *et al*. (2004), the application of herbicides in sandy loam soil, found at the Mochełek Experiment Station, can decrease the biomass content of microorganisms. Maybe this is the reason there was no increase in the population of antagonistic organisms in the present research. Neither was there observed an unambiguous effect of those herbicides.

The use of herbicides does not always inhibit the development of pathogens in soil, it can also stimulate pathgenes (Lévesque and Rahe 1992; Smiley and Wilkins 1992; Velini *et al*. 2010). Altman and Rovira (1989) reported 25 herbicides recommended for plant production, which stimulated the growth of *R. solani in vitro*. According to Katan and Eshel (1973), there are four mechanisms which can increase the intensity of diseases, namely by a direct effect of herbicides on pathogen growth, pathogen virulence, susceptibility of the host, and/or changes in the dependences between the pathogen and other soil organisms. Eshel and Katan (1972) concluded that an increase in plant infection by *R. solani* is not a result of a greater susceptibility of the host after the application of herbicides but from the inhibition of the development of antagonistic organisms in soil.

From the stems with the symptoms of sharp eyespot, *R. cerealis* was isolated most often and *R. solani* – much more rarely, which coincides with the reports by Kurowski and Adamiak (2007). Boerema and Verhoeven (1977) consider *R. cerealis* to be the main cause of sharp eyespot, but note that sharp eyespot can also be triggered by *R. solani*. Despite clear symptoms of sharp eyespot, fungi commonly considered saprotrophic for cereals were isolated from tissues. These were fungi representing the genera *Penicillium*, *Trichoderma* and *Aspergillus*, as well as *Fusarium* spp., especially *F. culmorum* and *G. avenacea*. Sometimes, despite clear disease symptoms characteristic for a specific pathogen, other species are isolated which infest the infected tissues secondarily, or take part in mixed infection, including infection by *Fusarium* spp. The pathogen *R. cerealis* is specialized in cereal infection. It grows relatively slowly on artificial media and it is often overgrown with *Fusarium* spp. and saprotrophic fungi (Bateman and Kwaśna 1999). Fungi representing the *Rhizoctonia* genus were rarely isolated from tissues which had symptoms of other diseases. Such tissues were most often infested by *R. solani*, which confirms this pathogenes capacity for saprotrophic development (Sneh *et al*. 1991).

Kurowski and Adamiak (2007) isolated much more *R. cerealis* from the stems of wheat and rye when grown in monoculture as compared with crop rotation, which is not unambiguously confirmed by the present research. Matusinsky *et al*. (2008), applying the PCR technique, did not observe any variation in the intensity of the incidence of *R. cerealis* in wheat grown after various preceding crops. The use of the PCR technique with the application of specific primers type Sequence Characterized Amplified Region (SCAR), made it possible to also confirm the species representation of *R. cerealis* and *R. solani* in the present research. Nicholson *et al*. (2002) and Ray *et al*. (2006) found that the amount of DNA of *R. cerealis,* in relation to total DNA obtained from the plant, was increasing at successive development stages of wheat. Similarly in the present research, many more isolates of *R. cerealis* and *R. solani* were obtained at the end of the plant vegetation period than at the cereals seedling phase, which also coincides with the reports by Bateman (1993).

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