Acta Sci. Pol.
Silv. Colendar. Ratio Ind. Lignar. 16(4) 2017, 233–242
FORESTRY AND WOOD TECHNOLOGY
www.forestry.actapol.net pISSN 1644-0722 eISSN 2450-7997 http://dx.doi.org/10.17306/J.AFW.2017.4.24

ORIGINAL PAPER

Received: 29.10.2017
Accepted: 23.11.2017

DIVERSITY OF MULTINUCLEATE *RHIZOCTONIA* SPP. IN SOIL OF TWO FOREST NURSERIES GARNCARSKIBRÓD AND LIPKA

Marta Bełka✉, Małgorzata Mańka

Department of Forest Phytopathology, Poznań University of Life Sciences
Wojska Polskiego 71C, 60-628 Poznań, **Poland**

ABSTRACT

Damping-off disease is the most important of all diseases in forest nurseries with the most common pathogens causing the disease in forest nurseries worldwide include species from genera: *Fusarium*, *Rhizoctonia*, *Phytophthora*, *Pythium*. In the study two forest nurseries were screened for multinucleate *Rhizoctonia* isolates. Of all the soil samples collected, 132 isolates classified as *Rhizoctonia* were obtained, 89 isolates from Garncarskibród forest nursery and 43 from Lipka. All the isolates were multinucleate, that is representing *R. solani* anamorph. Isolates belonged to seven different anastomosis groups. In Lipka AG-5, AG1-IC, AG2-1, and AG2-2 were present while the diversity of AGs was greater in Grancarskibród, where AG1-IB, AG1-IC, AG2-1, AG2-2, AG2-3 and AG4-HG2 were isolated.

Keywords: *Rhizoctonia solani*, anastomosis group, forest nursery, damping-off

INTRODUCTION

Trees both in forest nurseries and in natural habitats are at risk of attacks by various pathogens (Hietala et al., 2005; Mańka, 2005; Sierota, 1998). Damping-off disease is the most common and the most important of all diseases at an early stage of their lives, the more frequently occurring, the longer a nursery is being operated, which is associated with accumulation of inoculum in soil (Hietala et al., 2005; Mańka, 2005). The most common pathogens causing damping-off in forest nurseries worldwide include species from genera: *Fusarium*, *Rhizoctonia*, *Phytophthora*, *Pythium* (Huang and Kuhlman, 1990; Lilja et al., 1995; 2010; Mohanan et al., 2005; Orlikowski and Oszako, 2009; Perrin and Sampagni, 1986; Sutherland and Davies, 1991; Vaartaja and Cram, 1956).

Fungi belonging to the *Rhizoctonia* genus represent a wide range of pathogenic, non-pathogenic and mutualistic species (Cubeta et al., 1995). Most studies on this group of fungi focus on agricultural plants, and only few of them were conducted in forest nurseries (Hietala and Sen, 1996).

Until recently, classifying an organism into the *Rhizoctonia* genus was based on biochemical, ecological and morphological criteria as well as the ability of an individual isolate to anastomosing with known testers. The later use of molecular techniques in phytopathology resulted in a serious rearrangement within the *Rhizctonia* genus (Sneh et al., 1998; 2008).

Species classified as *Rhizoctonia* spp. are a collection of fungi, in which the differences relate to the stage of anamorph, teleomorph, size and shape of sclerotia, number of nuclei per cell and colour of the mycelium (Sneh et al., 1998; Tu and Kimbrough, 1975). *Rhizoctonia solani* is the best-known plant pathogen within the genus of *Rhizoctonia*. In 1929 Wiant was the first to associate the occurrence of

✉marta.belka@up.poznan.pl

conifer seedlings damping-off with *R. solani.* Within the species *R. solani* (and other *Rhizoctonia* spp.) there are numerous anastomosis groups (AG) which are of great importance from the point of view of pathogenicity to plants. The share of various AGs in *Rhizoctonia* population in soil may affect the disease threat of plants.

MATERIAL AND METHODS

Soil samples were harvested from the top 15 cm of the soil profile from nurseries of Forest Districts Lipka and Oborniki (Fig. 1). The soil was collected in early spring, before any chemicals were used. Samples were placed in sterile cotton bags and proceeded immediately after reaching the laboratory.

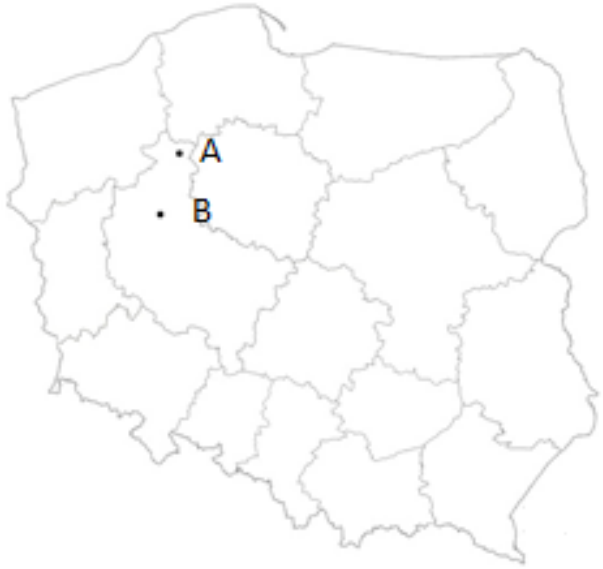

Fig. 1. Location of forest nurseries Lipka (A) and Garncarskibród (B) in Poland (R. Witkowski)
Rys. 1. Lokalizacja szkółek leśnych Lipka (A) i Garncarskibród (B) w Polsce (R. Witkowski)

Rhizoctonia isolates were obtained from soil samples using two trapping methods. The first method was based on the use of wooden toothpicks (Paulitz and Schroeder, 2005; modified). Toothpicks (10 per 700 ml of soil in pot) were inserted into the soil to a depth of 5 cm, evenly spaced in the pot. After 48 hours, toothpicks were removed and placed on Petri plates with PDA, supplemented with antibiotics (Streptomycin 0.1 g/l and Penicillin-G sodium salt 0.1 g/l). The plates with two toothpicks per plate were incubated for 24 hours at room temperature. All mycelia growing from toothpicks were transferred onto potato dextrose agar (PDA) plates to identify *Rhizoctonia* isolates.

In the second method pine seeds, which were sown (25 seeds per pot) to a volume of 700 ml of unified soil from a nursery, were used. Then, after emergence of the first seedlings observation was carried out to capture the very first symptoms of damping-off (Fig. 2). The symptomatic seedlings were disinfected with 0.5% sodium hypochlorite for 5 minutes, 70% ethanol (1 min) and washed three times in distilled sterilized water, for five minutes jointly. Next, the symptomatic parts of seedlings were transferred onto PDA, supplemented with antibiotics (Streptomycin 0.1 g/l and Penicillin-G sodium salt 0.1 g/l) to prevent the growth of bacteria. All isolates growing out of the inocula were transferred onto PDA.

Fig. 2. *Pinus sylvestris* seedlings with damping-off symptoms (Photo M. Bełka)
Rys. 2. Siewki *Pinus sylvestris* z objawami zgorzeli siewek (fot. M. Bełka)

The number of nuclei in the cells of obtained *Rhizoctonia* isolates was determined with the method described by Bandoni (1979) The average number of nuclei was calculated for 50 cells of each isolate (Table 1).

Table 1. Mean values and standard deviations of the average number of nuclei in a cell
Tabela 1. Wartości średnie oraz odchylenia standardowe średniej liczby jąder w komórce

Anastomosis group Grupa anastomozowa	Lipka		Garncarskibród	
	mean średnia	standard deviation odchylenie standardowe	mean średnia	standard deviation odchylenie standardowe
AG-5	11.917	2.341		
AG1-IB			10.362	2.063
AG1-IC	10.702	2.336	10.885	2.977
AG2-1	13.440	2.421	9.850	0.952
AG2-2	10.625	0.946	11.409	0.750
AG2-3			7.304	2.272
AG4-HG2			9.153	1.078
$NIR_{0.05}$	2.3		2.91	
$P > F$	<0.001		<0.001	

One-factor analysis of variance was conducted for both nurseries.
Jednoczynnikowa analiza wariancji została przeprowadzona dla obu szkółek.

To isolate DNA, isolates were grown on liquid broth for 7 days. After that time, the mycelium was placed on sterile filter paper and the excessive medium was removed. The dried mycelium was put into Eppendorf tubes (1.2 ml), and mashed with sterile metal rods. DNA extraction was carried out using a DNeasy Plant Mini Kit (Qiagen) according to manufacturer's recommendations. Isolates obtained in the study have been first separated based on the number of nuclei per cell. To assign isolates to the anastomosis subgroups the restriction enzymes digestion of RFLP of rDNA--ITS regions was performed. Primers ITS4 (TCCTCCGCTTATTGATATGC) and ITS5 (GGAAGTAAAAGTCGTAACAAGG) were used for amplification of the nuclear rDNA-ITS region (White et al., 1990). The PCR amplification reactions were conducted with PCR Mix (A&A Biotechnology). The denaturation step at 94°C for 10 min was followed by 35 cycles of 1 min at 94°C, 1 min at 60°C and 1 min at 72°C. Cycling ended with a final extension step at 72°C for 10 min (Biometra, T-Gradient Thermoblock). After the DNA extraction and restriction enzymes digestion of RFLP of rDNA-ITS regions the results of electrophoresis were checked under the UV light, and the resulting patterns were compared with those presented in the work of Guillemaut et al. (2003). Four restriction enzymes (MseI, AvaII, HincII, and MunI) were used in the study to assign isolates to a certain anastomosis group (AG; Table 2), it has also been verified by comparing the ITS-rDNA sequences of all isolates with sequences deposited in GenBank (http:// www.ncbi.nlm.nih.gov/GenBank). Sequencing of ITS-rDNA region was done by an outside company.

RESULTS

Of all the soil samples collected from the surveyed nurseries 132 isolates classified as *Rhizoctonia* were obtained, 89 isolates from Garncarskibród forest nursery and 43 from Lipka. All the isolates were multinucleate, that is representing *R. solani* anamorph. Isolates belonged to seven different anastomosis groups. In Lipka AG-5, AG1-IC, AG2-1, and AG2-2 were present while the diversity of AGs was greater in Grancarskibród, where AG1-IB, AG1-IC, AG2-1, AG2-2, AG2-3 and AG4-HG2 were isolated.

The lowest average number of nuclei was observed in AG2-3 isolates obtained from Garncarskibród

Table 2. Assignment of *Rhizoctonia* isolates to anastomosis groups and their percentage similarity with isolates deposited in GenBank

Tabela 2. Przynależność izolatów do poszczególnych grup anastomozowych i procent ich podobieństwa do izolatów zdeponowanych w GenBank

Isolate's code Kod izolatu	Anamorph Anamorfa	Anastomosis group Grupa anastomozowa	Teleomorph Teleomorfa	GenBank accession number Kod dostępu w bazie GenBank	Percentage of identity Procent podobieństwa
1	2	3	4	5	6
			Garncarskibród		
MB110460	*R. solani*	AG1-IC	*T. cucumeris*	AY881008.1	99
MB110424	*R. solani*	AG1-IC	*T. cucumeris*	AY881008.1	99
MB110511	*R. solani*	AG1-IC	*T. cucumeris*	AB122141.1	99
MB110508	*R. solani*	AG1-IC	*T. cucumeris*	AB122141.1	99
MB110534	*R. solani*	AG1-IC	*T. cucumeris*	AB122141.1	99
MB110649	*R. solani*	AG1-IC	*T. cucumeris*	AB122141.1	99
MB110629	*R. solani*	AG1-IC	*T. cucumeris*	AY154300.1	99
MB110672	*R. solani*	AG1-IC	*T. cucumeris*	EU591808.1	99
MB110684	*R. solani*	AG1-IC	*T. cucumeris*	EU591808.1	99
MB110627	*R. solani*	AG1-IC	*T. cucumeris*	AY154300.1	99
MB110743	*R. solani*	AG1-IC	*T. cucumeris*	AB122141.1	99
MB110758	*R. solani*	AG1-IC	*T. cucumeris*	AB122141.1	99
MB110764	*R. solani*	AG1-IC	*T. cucumeris*	AB122141.1	99
MB110766	*R. solani*	AG1-IC	*T. cucumeris*	AY881008.1	99
MB110772	*R. solani*	AG1-IC	*T. cucumeris*	AY154300.1	99
MB110784	*R. solani*	AG1-IC	*T. cucumeris*	AY881008.1	99
MB110799	*R. solani*	AG1-IC	*T. cucumeris*	AY881008.1	99
MB110802	*R. solani*	AG1-IC	*T. cucumeris*	AY154300.1	99
MB110823	*R. solani*	AG1-IC	*T. cucumeris*	AY154300.1	99
MB110844	*R. solani*	AG1-IC	*T. cucumeris*	AY154300.1	99
MB110913	*R. solani*	AG1-IC	*T. cucumeris*	FJ746943.1	99
MB110923	*R. solani*	AG1-IC	*T. cucumeris*	FJ746943.1	99
MB110940	*R. solani*	AG1-IC	*T. cucumeris*	FJ746943.1	99
MB110402	*R. solani*	AG1-IB	*T. cucumeris*	EU730824.1	99
MB110429	*R. solani*	AG1-IB	*T. cucumeris*	EU730824.1	99
MB110517	*R. solani*	AG1-IB	*T. cucumeris*	AB122138.1	99
MB110589	*R. solani*	AG1-IB	*T. cucumeris*	AB122137.1	99
MB110612	*R. solani*	AG1-IB	*T. cucumeris*	AB122137.1	99
MB110607	*R. solani*	AG1-IB	*T. cucumeris*	FJ440191.1	99
MB110613	*R. solani*	AG1-IB	*T. cucumeris*	FJ440191.1	99
MB110604	*R. solani*	AG1-IB	*T. cucumeris*	GU596491.1	99
MB110605	*R. solani*	AG1-IB	*T. cucumeris*	HQ185364.1	99

Table 2 – cont. \ Tabela 2 – cd.

1	2	3	4	5	6
MB110630	*R. solani*	AG1-IB	*T. cucumeris*	HQ185364.1	99
MB110624	*R. solani*	AG1-IB	*T. cucumeris*	FJ435105.1	100
MB110628	*R. solani*	AG1-IB	*T. cucumeris*	HQ185364.1	99
MB110711	*R. solani*	AG1-IB	*T. cucumeris*	AB122137.1	99
MB110707	*R. solani*	AG1-IB	*T. cucumeris*	FJ435105.1	100
MB110718	*R. solani*	AG1-IB	*T. cucumeris*	AB122137.1	99
MB110744	*R. solani*	AG1-IB	*T. cucumeris*	FJ435105.1	100
MB110817	*R. solani*	AG1-IB	*T. cucumeris*	GU585667.1	99
MB110809	*R. solani*	AG1-IB	*T. cucumeris*	FJ440191.1	99
MB110811	*R. solani*	AG1-IB	*T. cucumeris*	HQ185364.1	99
MB110807	*R. solani*	AG1-IB	*T. cucumeris*	GU596491.1	99
MB110810	*R. solani*	AG1-IB	*T. cucumeris*	EU730824.1	99
MB110914	*R. solani*	AG1-IB	*T. cucumeris*	AB122137.1	99
MB110905	*R. solani*	AG1-IB	*T. cucumeris*	EU730824.1	99
MB110917	*R. solani*	AG1-IB	*T. cucumeris*	FJ435105.1	100
MB110908	*R. solani*	AG1-IB	*T. cucumeris*	FJ435105.1	100
MB110904	*R. solani*	AG1-IB	*T. cucumeris*	FJ435105.1	100
MB110911	*R. solani*	AG1-IB	*T. cucumeris*	FJ435105.1	100
MB110910	*R. solani*	AG1-IB	*T. cucumeris*	EU730824.1	99
MB110411	*R. solani*	AG2-2	*T. cucumeris*	AB054856.1	99
MB110407	*R. solani*	AG2-2	*T. cucumeris*	AB054856.1	99
MB110404	*R. solani*	AG2-2	*T. cucumeris*	FJ492165.3	99
MB110603	*R. solani*	AG2-2	*T. cucumeris*	FJ492123.3	99
MB110710	*R. solani*	AG2-2	*T. cucumeris*	AB054856.1	99
MB110708	*R. solani*	AG2-2	*T. cucumeris*	FJ492165.3	99
MB110801	*R. solani*	AG2-2	*T. cucumeris*	FJ492123.3	99
MB110907	*R. solani*	AG2-2	*T. cucumeris*	FJ492123.3	99
MB110918	*R. solani*	AG2-2	*T. cucumeris*	FJ492123.3	99
MB110403	*R. solani*	AG2-1	*T. cucumeris*	JF792354.1	99
MB110611	*R. solani*	AG2-1	*T. cucumeris*	AB054853.1	99
MB110608	*R. solani*	AG2-1	*T. cucumeris*	JF792354.1	99
MB110614	*R. solani*	AG2-1	*T. cucumeris*	FJ492102.3	99
MB110414	*R. solani*	AG2-3	*T. cucumeris*	U57743.1	99
MB110440	*R. solani*	AG2-3	*T. cucumeris*	U57743.1	99
MB110502	*R. solani*	AG2-3	*T. cucumeris*	U57741.1	99
MB110516	*R. solani*	AG2-3	*T. cucumeris*	U57741.1	99
MB110640	*R. solani*	AG2-3	*T. cucumeris*	FJ435099.1	99
MB110623	*R. solani*	AG2-3	*T. cucumeris*	U57741.1	99

Table 2 – cont. \ Tabela 2 – cd.

1	2	3	4	5	6
MB110622	*R. solani*	AG2-3	*T. cucumeris*	U57743.1	99
MB110618	*R. solani*	AG2-3	*T. cucumeris*	U57743.1	99
MB110601	*R. solani*	AG2-3	*T. cucumeris*	U57740.1	99
MB110720	*R. solani*	AG2-3	*T. cucumeris*	AY154312.1	99
MB110711	*R. solani*	AG2-3	*T. cucumeris*	AY154312.1	99
MB110702	*R. solani*	AG2-3	*T. cucumeris*	AY154312.1	99
MB110712	*R. solani*	AG2-3	*T. cucumeris*	AY154312.1	99
MB110709	*R. solani*	AG2-3	*T. cucumeris*	U57743.1	99
MB110721	*R. solani*	AG2-3	*T. cucumeris*	U57743.1	99
MB110704	*R. solani*	AG2-3	*T. cucumeris*	U57743.1	99
MB110804	*R. solani*	AG2-3	*T. cucumeris*	FJ435099.1	99
MB110806	*R. solani*	AG2-3	*T. cucumeris*	U57740.1	99
MB110815	*R. solani*	AG2-3	*T. cucumeris*	AY154312.1	99
MB110912	*R. solani*	AG2-3	*T. cucumeris*	AY154312.1	99
MB110929	*R. solani*	AG2-3	*T. cucumeris*	AY154312.1	99
MB110930	*R. solani*	AG2-3	*T. cucumeris*	AY154312.1	99
MB110819	*R. solani*	AG4-HG2	*T. practicola*	HQ629864.1	99
MB110820	*R. solani*	AG4-HG2	*T. practicola*	HQ629864.1	99
MB110808	*R. solani*	AG4-HG2	*T. practicola*	HQ629864.1	99
			Lipka		
MB120411	*R. solani*	AG1-IC	*T. cucumeris*	FJ435098.1	99
MB120434	*R. solani*	AG1-IC	*T. cucumeris*	FJ435098.1	99
MB120574	*R. solani*	AG1-IC	*T. cucumeris*	FJ435106.1	99
MB120518	*R. solani*	AG1-IC	*T. cucumeris*	HQ185375.1	99
MB120519	*R. solani*	AG1-IC	*T. cucumeris*	FJ435098.1	99
MB120501	*R. solani*	AG1-IC	*T. cucumeris*	HQ185375.1	99
MB120513	*R. solani*	AG1-IC	*T. cucumeris*	FJ746943.1	99
MB120529	*R. solani*	AG1-IC	*T. cucumeris*	FJ746943.1	99
MB120551	*R. solani*	AG1-IC	*T. cucumeris*	EU591808.1	99
MB120516	*R. solani*	AG1-IC	*T. cucumeris*	EU591808.1	99
MB120604	*R. solani*	AG1-IC	*T. cucumeris*	AB122141.1	99
MB120605	*R. solani*	AG1-IC	*T. cucumeris*	FJ435106.1	99
MB120612	*R. solani*	AG1-IC	*T. cucumeris*	HQ185375.1	99
MB120727	*R. solani*	AG1-IC	*T. cucumeris*	FJ435098.1	99
MB120712	*R. solani*	AG1-IC	*T. cucumeris*	FJ435098.1	99
MB120702	*R. solani*	AG1-IC	*T. cucumeris*	FJ746943.1	99
MB120708	*R. solani*	AG1-IC	*T. cucumeris*	HQ185375.1	99
MB120714	*R. solani*	AG1-IC	*T. cucumeris*	FJ435098.1	99

Table 2 – cont. \ Tabela 2 – cd.

1	2	3	4	5	6
MB120813	*R. solani*	AG1-IC	*T. cucumeris*	FJ435106.1	99
MB120904	*R. solani*	AG1-IC	*T. cucumeris*	FJ435106.1	99
MB120546	*R. solani*	AG-5	*T. cucumeris*	AY586186.1	99
MB120528	*R. solani*	AG-5	*T. cucumeris*	AY586186.1	99
MB120503	*R. solani*	AG-5	*T. cucumeris*	AY586186.1	99
MB120640	*R. solani*	AG-5	*T. cucumeris*	AY586166.1	99
MB120637	*R. solani*	AG-5	*T. cucumeris*	AY586166.1	99
MB120764	*R. solani*	AG-5	*T. cucumeris*	AY586186.1	99
MB120753	*R. solani*	AG-5	*T. cucumeris*	AY586186.1	99
MB120728	*R. solani*	AG-5	*T. cucumeris*	HQ185377.1	99
MB120717	*R. solani*	AG-5	*T. cucumeris*	HQ185373.1	99
MB120749	*R. solani*	AG-5	*T. cucumeris*	HQ185373.1	99
MB120934	*R. solani*	AG-5	*T. cucumeris*	HQ185377.1	99
MB120948	*R. solani*	AG-5	*T. cucumeris*	AY586166.1	99
MB120939	*R. solani*	AG-5	*T. cucumeris*	EU244843.1	99
MB120954	*R. solani*	AG-5	*T. cucumeris*	HQ629863.1	99
MB120972	*R. solani*	AG-5	*T. cucumeris*	HQ629863.1	99
MB120511	*R. solani*	AG2-2	*T. cucumeris*	FJ492124.3	99
MB120504	*R. solani*	AG2-2	*T. cucumeris*	FJ492124.3	99
MB120803	*R. solani*	AG2-2	*T. cucumeris*	FJ492124.3	99
MB120912	*R. solani*	AG2-2	*T. cucumeris*	FJ492124.3	99
MB120817	*R. solani*	AG2-1	*T. cucumeris*	EU730857.1	99
MB120844	*R. solani*	AG2-1	*T. cucumeris*	EU730857.1	99
MB120819	*R. solani*	AG2-1	*T. cucumeris*	EU730857.1	99
MB120808	*R. solani*	AG2-1	*T. cucumeris*	EU730857.1	99

(Table 1). The highest number of nuclei per cell was observed in AG2-1 isolates obtained from Lipka, with the average number of 13.44 nuclei per cell (AG2-1 isolates from Garncarskibród with the average of 9.85 nuclei per cell). The most common AG found in both nurseries, AG 1-IC, was characterized by the average number of 10.702 and 10.885 nuclei per cell Lipka and Garncarskibród, accordingly.

In Lipka forest nursery the biggest number of isolates belonged to AG1-IC (20 isolates), AG-5 (15 isolates), and 4 isolates of AG2-1 and AG2-2. In Garncarskibród the biggest number of isolates assigned to AG1-IB (28 isolates) followed by AG1-IC (23 isolates), and AG2-3 (22 isolates) were found. There were 9 isolates AG2-2, 4 isolates AG2-1 and three AG4-HG2 isolates obtained from Garncarskibród.

DISCUSSION

From among the 132 *Rhizoctonia* isolates obtained all represented *R. solani*, a species widely distributed in Polish forest nurseries. The lower number of isolates in Lipka forest nursery is also shown on the site, where damping-off is not as severe as in Garncarskibród.

The knowledge of the occurrence of *Rhizoctonia* anastomosis groups in forest nurseries is insufficient.

As different anastomosis groups have different sensitivity to fungicides (Carling et al., 1999; Campion et al., 2003) and are characterized by different pathogenicity (Bełka and Mańka, 2014), the knowledge of their occurrence in soil could have a big impact on seedling protection approach. The results of some studies (Herr, 1995; Sharon et al., 2011) suggest the possibility of using non-pathogenic *Rhizoctonia* strains as biological control agents against those highly pathogenic ones. In their research Camporota and Perrin (1998) demonstrated the predominant role of *R. solani* as the primary pathogen causing damping-off of pine seedlings. In Polish nurseries, so far only the research by Stępniewska-Jarosz et al. (2006), and Bełka and Mańka (2014) described the differences between anastomosis groups in Polish forest nurseries. Stępniewska-Jarosz et al. (2006) found five, and Bełka and Mańka (2014) isolated four different anastomosis groups from Polish forest nurseries soil. The research by Stępniewska-Jarosz et al. (2006) and Bełka and Mańka (2014) were partially conducted in the same area giving different results. In the later work (Bełka and Mańka, 2014) isolates AG4-HG2 were observed. The difference may have resulted from possible bridging of some isolates belonging to certain AGs with isolates belonging to the other (Ogoshi, 1972) or from the fact that different techniques were used to assign isolates to certain AGs. While Stępniewska-Jarosz et al. (2006) used classic techniques, with culture of isolates *in vitro* and subsequent observation of their growth under microscope, Bełka and Mańka (2014) based their study fully on molecular methods. As most of the research on *Rhizoctonia* not only in Poland, but worldwide has been conducted on agricultural plants, it is highly desirable to further screen forest nurseries for the pathogen and its anastomosis groups. Better recognition of the problem would help in saving considerable amounts of money spent on chemicals used for damping-off prevention and in approaching the principles of integrated pest management (IPM).

Many AGs and subgroups of *R. solani* and binucleate *Rhizoctonia* spp. have been reported as causal agents of *Rhizoctonia* diseases on a wide range of host species (Sneh et al., 2008). For example, AG1-IA possesses the ability to cause the disease in such plants as rice (*Oryza sativa*), corn (*Zea mays*), barley (*Hordeum vulgare*), potato (*Solanum tuberosum*), and many other agriculturally important species but cannot attack trees (Sneh et al., 2008). In contrast, *Rhizoctonia* species belonging to the same anastomosis group – AG1 subgroup IB cause diseases in *Larix* spp., *Acacia* spp., *Eucalyptus* spp., *Pinus* spp. and *Cupressus* spp. Not only pathogenicity of different anastomosis groups differs between species. The study carried out on uninucleate isolates confirmed that they were the causing agents of damping-off in many Finnish nurseries (Grönberg et al., 2006; Sen, 2001). At the same time from Norwegian and Finnish forest nurseries uninucleate and binucleate strains of *Rhizoctonia* have been isolated from healthy seedlings of Scots pine and Norway spruce (Hietala, 1995; Lilja et al., 1992; 1994).

In conclusion, more research on *Rhizoctonia* spp. in forest nurseries is needed. The knowledge of AGs present in soil and their pathogenicity would be helpful in choosing the right protection method.

REFERENCES

Bandoni, R. J. (1979). Safranin O as a rapid nuclear stain for fungi. Mycologia, 11(4), 873–874.

Bełka, M., Mańka, M. (2014). Characteristics and diversity of *Rhizoctonia* spp. population in soil of selected forest bare-root nurseries in Poland. Acta Mycol., 49(2), 279–290.

Campion, C., Chatot, C., Perratoon, B., Andrivon, D. (2003). Anastomosis groups, pathogenicity and sensitivity to fungicides of *Rhizoctonia solani* isolates collected on potato crops in France. Eur. J. Plant Pathol., 109, 983–992.

Camporota, P., Perrin, R. (1998). Characterization of *Rhizoctonia* species involved in tree seedling damping-off in French forest nurseries. Appl. Soil Ecol., 10, 65–71.

Carling, D. E., Pope, E. J., Brainard, K. A., Carter, D. A. (1999). Characterization of mycorrhizal isolates of *Rhizoctonia solani* from an orchid, including AG-12, a new anastomosis group. Ecol. Populat. Biol., 89(10), 942–946.

Cubeta, M. A., Vilgalys, R., Gonzales, D. (1995). Molecular approaches for examining species concepts in *Rhizoctonia*. In: International Symposium on *Rhizoctonia*, ISR'95, Leeuwenhorst Congres Centrum. Noordwijkerhout, Netherlands, June 27–30, 1995.

Grönberg, H., Kaparakis, G., Sen, R. (2006). Binucleate *Rhizoctonia* (*Ceratorhiza* spp.) as non-mycorrhizal endophytes alter *Pinus sylvestris* L. seedling root architecture

and affect growth of rooted cuttings. Scand. J. For. Res., 21, 450–457.

Guillemaut, C., Edel-Hermann, V., Camporota, P., Alabouvette, C., Richard-Molard, M., Steinberg, C. (2003). Typing of anastomosis groups of *Rhizoctonia solani* by restriction analysis of ribosomal DNA. Can. J. Microbiol., 49, 556–558.

Herr, L. J. (1995). Biological control of *Rhizoctonia solani* by binucleate *Rhizoctonia* spp. and hypovirulent *R. solani* agents. Crop Prot., 14, 179–186.

Hietala, A. M. (1995). Uni- and binucleate *Rhizoctonia* spp. co-existing on the roots of Norway spruce seedlings suffering from root dieback. Eur. J. For. Pathol., 25, 136–144.

Hietala, A. M., Mehli, L., Nagy, N. E., Kvaalen, H., La Porta, N. (2005). *Rhizoctonia solani* AG 2-1 as a causative agent of cotyledon rot on European beech (*Fagus sylvatica*). For. Pathol., 35, 397–410.

Hietala, A. M., Sen, R. (1996). *Rhizoctonia* associated with forest trees. In: B. Sneh, S. Jabaji-Hare, S. Neate, G. Dijst (Eds.), *Rhizoctonia* species: Taxonomy, molecular biology, ecology, pathology and disease control (pp. 351–358). Dordrecht: Kluwer Acad. Publ.

Huang, J. W., Kuhlman, E. G. (1990). Fungi associated with damping-off of Slash pine seedlings in Georgia. Plant Disease, 74, 27–30.

Lilja, A. (1994). The occurence and pathogenicity of uni- and binucleate *Rhizoctonia* and *Pythiaceae* fungi among cornifer seedlings in Finnish forest nurseries. Eur. J. For. Pathol., 24, 181–192.

Lilja, A., Hallaksela, A.M., Heionen, R. (1995). Fungi colonizing Scots pine cones scales and seed and their pathogenicity. Eur. J. For. Pathol., 25, 38–46.

Lilja, A., Lilja, S., Poteri, M., Ziren, L. (1992). Conifer seedling root fungi and root dieback in Finnish nurseries. Scand. J. For. Res., 7, 547–556.

Lilja, A., Poteri, M., Petäistö, R.-L., Rikala, R., Kurkela, T., Kasanen, R. (2010). Fungal diseases in forest nurseries in Finland. Silva Fenn., 44(3), 525–545.

Mańka, K. (2005). Fitopatologia leśna [Forest phytopathology]. Warszawa: PWRiL [in Polish].

Mohanan, C., Retheesh, N., Nair, L. P., Rajesh Kumar, K. C. (2005). Disease problems in root trainer forest nurseries in Kerala State and their management. Working Papers of the Finnish Forest Research Institute 11.

Ogoshi, A. (1972). Grouping of *Rhizoctonia solani* Kuehn with hyphal anastomosis. Ann. Phytopathol. Soc. Japan, 38, 117–122.

Orlikowski, L., Oszako, T. (red., 2009). Fytoftorozy w szkółkach i drzewostanach leśnych: Klucz do oznaczania *Phytophthora*: atlas fytoftoroz siewek i drzew leśnych [Phytophthora species in forest nurseries and forest stands: The key to *Phytophthora*: seedlings and forest trees phytophthorosis atlas]. Warszawa: Centr. Inform. LP [in Polish].

Paulitz, T. C., Schroeder, K. L. (2005). A new metod for the quantification of *Rhizoctonia solani* and *R. oryzae* from soil. Plant Disease, 89, 767–72.

Perrin, R., Sampagni, R. (1986). La fonte des semis en pepiniere forestiere. Eur. J. For. Pathol., 16, 309–321.

Sen, R. (2001). Multitrophic interactions between a *Rhizoctonia* sp. and mycorrhizal fungi affect Scots pine seedling performance in nursery soil. New Phytol., 152, 543–553.

Sharon, M., Freeman, S., Sneh, B. (2011). Assessment of resistance pathways induced in *Arabidopsis thaliana* by hypovirulent *Rhizoctonia* spp. isolates. Genet. Resist., 101(7), 828–838.

Sierota, Z. (1998). Choroby infekcyjne w szkółkach leśnych [Infectious diseases in forest nurseries]. In: Z. Sierota, M. Małecka (Eds.), Profilaktyka i terapia w szkółkach leśnych zagrożonych przez choroby infekcyjne. Materiały konferencji naukowo-technicznej, 24–25 III 1998. Warszawa–Sękocin (pp. 5–9). Warszawa: Inst. Bad. Leśn. [in Polish].

Sneh, B., Burpee, L., Ogoshi, A. (1998). Identification of *Rhizoctonia* species. USA: APS Press.

Sneh, B., Sharon, M., Kuninaga, S. (2008). Comprehensive classification of *Rhizoctonia* spp. using rDNA+ITS sequence analysis complemented by percent sequence similarity. In: 4th International Symposium on Rhizoctonia, 20–22 August 2008. The International Society of Plant Pathology, Rhizoctonia Subject Matter Committee, Berlin, Germany.

Stępniewska, S., Mańka, M., Asiegbu, O. (2006). Studies on anastomosis groups of *Rhizoctonia solani* isolates causing disease in two forest nurseries in Poland. For. Pathol., 36, 97–109.

Sutherland, J. R., Davis, C. (1991). Diseases and insects in forest nurseries in Canada. In: J. R. Sutherland, S. G. Glover (Eds.), Proceedings of the first meeting of UFRO Working Party S2.07-09 Diseases and insects in forest nurseries (pp. 25–32). Victoria, British Columbia, Canada, August 23–30, 1990. Forestry Canada, Pacific and Yukon Region, Pacific Forestry Centre, BC-X-331.

Tu, C. C., Kimbrough, J. W. (1975). A modified soil-over culture method for inducing basidia in *Thanatephorus cucumeris*. Phytopathology, 65, 730–731.

Vaartaja, O., Cram, W. H. (1956). Damping-off of conifers and of caragana in Saskatchewan. Phytopathology, 46, 505–507.

White, T. J., Bruns, T. D., Lee, S., Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA for phylogenetics. In: M. A. Innis, D. H. Gelfland, J. J. Sninsky, T. J. White (Eds.), PCR protocols: A guide to methods and applications (pp. 315–322). San Diego: Academic Press.

Wiant, J. S. (1929). The *Rhizoctonia* damping-off of cornifers and its control by chemical treatment of the soil. New York State Agricultural Experiment Station – Cornell University 124.

RÓŻNORODNOŚĆ WIELOJĄDROWYCH *RHIZOCTONIA* SPP. W GLEBIE DWÓCH SZKÓŁEK LEŚNYCH GARNCARSKIBRÓD I LIPKA

ABSTRAKT

Zgorzel siewek jest najpoważniejszą spośród wszystkich chorób występujących w szkółkach leśnych. Choroba ta występuje na siewkach drzew na całym świecie, a powodują ją między innymi patogeny z rodzajów: *Fusarium*, *Rhizoctonia*, *Phytophthora* i *Pythium*. W pracy przedstawiono wyniki badań przeprowadzonych w dwóch szkółkach leśnych przebadanych pod kątem izolatów wielojądrowych *Rhizoctonia*. Ze wszystkich zebranych próbek gleby uzyskano 132 izolaty sklasyfikowane jako Rhizoctonia, 89 izolatów ze szkółki leśnej Garncarskibród i 43 z Lipki. Izolaty należały do siedmiu różnych grup anastomozowych. W Lipce opisano grupy AG-5, AG1-IC, AG2-1 i AG2-2. Zróżnicowanie grup anastomozowych w szkółce leśnej Grancarskibród było większe – wyizolowano AG1-IB, AG1-IC, AG2-1, AG2-2, AG2-3 oraz AG4-HG2.

Słowa kluczowe: *Rhizoctonia solani*, grupa anastomozowe, szkółka leśna, zgorzel siewek