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FREQUENCY OF ISOLATION AND DIVERSITY OF *RHIZOCTONIA* SPP. FROM TREE SEEDLINGS WITH DAMPING-OFF SYMPTOMS

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Abstract: The occurrence and diversity of fungi from the genus *Rhizoctonia* in seedlings of 10 tree species with damping-off symptoms collected in seven forest nurseries of southern Poland were studied. The fungi were found in each nursery investigated, colonizing on average 38% of the seedlings studied. Binucleate and multinucleate isolates were found. The multinucleate isolates were highly diversified in respect of culture morphology, sclerotia morphology, and tendency of sclerotia to form. The binucleate isolates, relatively homogenous, were more frequent in seedlings of broadleaf species.

Key words: *Rhizoctonia*, diversity, broadleaf trees, conifers, forest nurseries

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INTRODUCTION

Damping-off of tree seedlings is caused by a group of fungi including *Rhizoctonia solani* J. G. Kühn. This fungus has been reported from forest nurseries in western and northern Poland many times, most often as a causal agent of pine (*Pinus sylvestris* L.) seedling damping-off (Kwaśna 1987; Mańka & Gierczak 1971; Przezbórski 1984). Studies in other countries have shown that other fungi of the same genus may occur in forest nurseries (Huang & Kuhlman 1989; Lilja 1994; Saksena & Vaartaja 1961). Various anastomosis groups (AGs) of *R. solani* have been found as well (Parmeter *et al.* 1969). They show some differences in morphology and pathogenicity (Sherwood 1969).

This study assesses the frequency of occurrence of *Rhizoctonia* spp. in tree seedlings with damping-off symptoms, collected in some forest nurseries of southern Poland, and makes a preliminary analysis of their diversity.

MATERIAL AND METHODS

This study used seedlings with damping-off symptoms, of the following tree species: *Abies alba* Mill., *Acer pseudoplatanus* L., *Alnus glutinosa* (L.) Gaertn., *A. incana* (L.) Moench, *Betula pendula* Roth, *Fagus sylvatica* L., *Larix decidua* Mill., *Picea abies* (L.) H. Karst., *Pinus nigra* J. F. Arnold, and *Pinus sylvestris* L. Twenty-two seedling samples (660 seedlings in total) were collected in 2000 and 2001 from seven forest nurseries of southern Poland: Cianowice – *F. sylvatica* (1), *P. abies* (2), *P. sylvestris* (3); Grodziec – *B. pendula* (4), *F. sylvatica* (5), *L. decidua* (6), *P. sylvestris* (7); Kłaj – *P. sylvestris* (8); Królówka – *F. sylvatica* (9), *P. sylvestris* (10); Pogórska Wola – *A. alba* (11), *A. glutinosa* (12), *P. nigra* (13), *P. sylvestris* (14); Pomorzany – *A. pseudoplatanus* (15), *A. incana* (16), *P. nigra* (17), *P. sylvestris* (hotbed) (18), *P. sylvestris* (greenhouse) (19); Salma – *A. glutinosa* (20), *B. pendula* (21), *P. sylvestris* (22). The seedlings were cultivated in the field or under cover (greenhouses, hotbeds, tunnels), on various organic substrata (Table 1).

The seedlings were surface-sterilized with 4% sodium hypochlorite (NaOCl). Fragments 3–5 mm long taken from roots or root collars of seedlings with decay symptoms were placed on standard PDA medium (Becton Dickinson, U.S.A.) with tetracycline hydrochloride added (2 mg/l) to stop development of bacteria. The cultures were incubated in the dark at room temperature.

Isolates of *Rhizoctonia* spp. obtained from the seedlings were grafted on PDA. After two weeks, disks 7 mm in diameter were cut from the edges of the cultures and placed (mycelia down) in Petri dishes 10 cm in diameter, with PDA just at the edge of the dish. The cultures were incubated at 22–24°C in the dark. The morphology of cultures (color, zonation) and sclerotia (color, structure) were studied after three weeks.

Table 1. Isolation frequency of *Rhizoctonia* spp. from seedlings with damping-off symptoms.

Forest District	Nursery	Tree species									
		Abies alba	*Acer pseudo-platanus*	*Alnus glutinosa*	*Alnus incana*	*Betula pendula*	*Fagus sylvatica*	*Larix decidua*	*Picea abies*	*Pinus nigra*	*Pinus sylvestris*
Miechów	Cianowice 2001, bed soil 2001, tunnel						(30)[a]20[b]:13[c]:7[d]		(30)[a]0[b]		(30)[a]33[b]:0[c]:33[d]
Siewierz	Grodziec 2000, bed soil 2000, tunnel					(30)[a]96[b]:13[c]:83[d]	(30)[a]83[b]:20[c]:63[d]	(30)[a]50[b]:3[c]:47[d]			(30)[a]63[b]:17[c]:46[d]
Niepołomice	Kłaj 2001, bed soil										(30)[a]67[b]:17[c]:50[d]
Kobiór	Królówka 2001, bed soil						(30)[a]7[b]:3[c]:7[d]				(30)[a]63[b]:0[c]:63[d]
Gromnik	Pogórska Wola 2000, bed soil 2000, hotbed	(30)[a]0[b]		(30)[a]0[b]						(30)[a]17[b]:7[c]:10[d]	(30)[a]10[b]:0[c]:10[d]
Olkusz	Pomorzany 2000, hotbed 2000, greenhouse 2001, hotbed		(30)[a]0[b]		(30)[a]7[b]:0[c]:7[d]					(30)[a]77[b]:0[c]:77[d]	(30)[a]10[b]:0[c]:10[d] (30)[a]80[b]:0[c]:80[d]
Złoty Potok	Salma 2001, bed soil 2001, tunnel			(30)[a]0[b]		(30)[a]60[b]:53[c]:7[d]					(30)[a]83[b]:7[c]:76[d]

[a] number of seedlings investigated (100%)
[b] percentage of seedlings colonized by *Rhizoctonia* spp.
[c] percentage of seedlings colonized by binucleate *Rhizoctonia* spp.
[d] percentage of seedlings colonized by multinucleate *Rhizoctonia* spp.

Disks 7 mm in diameter were taken from the edges of 2-week-old cultures grown on PDA and placed in the center of Petri dishes 10 cm in diameter, with 2% water agar. The cultures were incubated in the dark at 22–24°C. After 48 h the number of nuclei in hyphae and the width of the hyphae were determined. An alkaline solution of Safranin O was used for staining the nuclei (Bandoni 1979).

RESULTS

FREQUENCY OF ISOLATION OF *RHIZOCTONIA* SPP.

Fungi were isolated from 22 samples (660 seedlings in total), including 270 seedlings of broadleaf trees and 390 of conifers (Table 1). In only 5 of the 22 samples there were no fungi of the genus *Rhizoctonia.* On average, 49% of the seedlings of the remaining 17 samples were colonized by these fungi. *Rhizoctonia* spp. colonized an average 45.5% of the broadleaf seedlings. Most frequently these fungi were isolated from seedlings of *B. pendula* (average 78%), considerably less frequently from seedlings of *F. sylvatica* (37%), sporadically from seedlings of *A. incana* (7%), and not at all from seedlings of *A. glutinosa* and *A. pseudoplatanus*. Fungi of the genus *Rhizoctonia* were slightly more frequent in conifer seedlings (average 50%) than in the broadleaf seedlings. The majority (51%) of *P. sylvestris* seedlings were colonized by these fungi. The frequencies of *Rhizoctonia* spp. in *L. decidua* and *P. nigra* seedlings were similar, 50% and 47%, respectively. Seedlings of *A. alba* and *P. abies* were not colonized at all.

Fungi of the genus *Rhizoctonia* were found in each of the seven nurseries studied (Table 1). They occurred most frequently in seedlings taken from the Grodziec nursery; on average, 73% of the seedlings from the four samples tested from this nursery were colonized by these fungi. They were least frequent in seedlings from the nursery of Pogórska Wola: on average 7% for the four tested samples.

In this study the seedling production technologies affected the degree of seedling colonization by *Rhizoctonia* spp. Fungi were more frequent in seedlings cultivated in greenhouses and tunnels (average 52% for eight tested samples) than in those produced in the field (average 39% for ten tested samples) and in hotbeds (average 4% for four tested samples) (Table 1).

MORPHOLOGY OF CULTURES

The *Rhizoctonia* spp. isolates obtained were initially compared within each sample; 118 isolates were distinguished and investigated further. These isolates differed in culture morphology after 3 weeks of growing on PDA (Figs 1, 2). Cultures of cream to light cream color, with no or scant air mycelia and with distinct concentric zones dominated. The isolates produced the sclerotia of loose structure typical of this genus. The sclerotia were yellow to light brown, and were submerged in the medium or on the surface, and arranged radially or in concentric zones. In most of the isolates sclerotia occurred mainly in the oldest part of the colony, where they formed a compact crust on the medium surface. Infrequently there were isolates producing large sclerotia or clusters of them, brown in color, often covered with tan exudates. Also distinguished was a group of isolates forming very numerous, tiny (*ca* 0.5 mm in diameter), brown-black and hard sclerotia of structure different from those described above, on

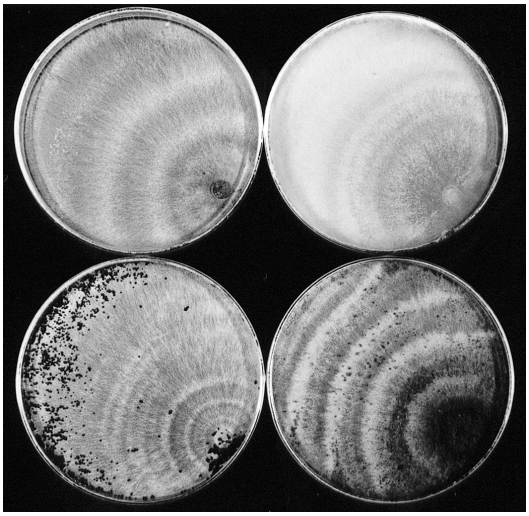

Fig. 1. Cultures of some multinucleate isolates grown on PDA for three weeks.

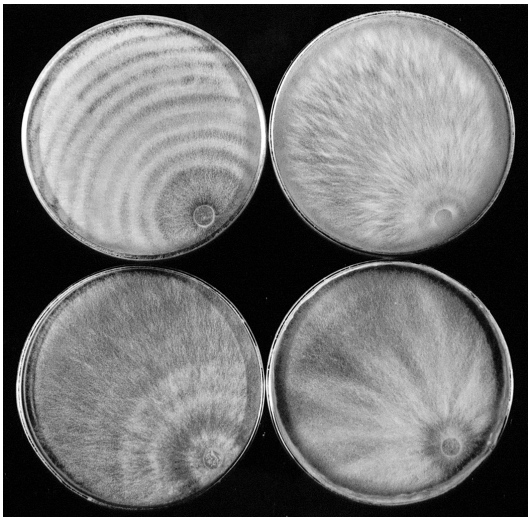

Fig. 2. Cultures of some binucleate isolates grown on PDA for three weeks.

the medium surface. Within the group of 118 isolates of *Rhizoctonia* spp. tested, there were also isolates of relatively ample air mycelium, white in color, with concentric zones or without zonation. Usually these isolates did not produce sclerotia. In some of them there were loose irregular conglomerations of monilioid cells on the surface.

CYTOMORPHOLOGY OF HYPHAE

Among the 118 isolates of *Rhizoctonia* spp. studied, there were 88 (75%) multinucleate and 30 (25%) binucleate isolates. There were 3–10 cell nuclei in the multinucleate isolates, most often 6–7 nuclei. The width of hyphae of the binucleate isolates varied from 3.1 to 6.2 μm, smaller than in the multinucleate isolates (5.1–9.3 μm). The cultures of the binucleate isolates were white and usually did not produce sclerotia (Fig. 2). The cultures of the multinucleate isolates varied in morphology in respect of color, structure, and number and morphology of sclerotia (Fig. 1). Binucleate isolates were more frequent in broadleaf seedlings. In the 29 isolates from seedlings of *A. incana, B. pendula* and *F. sylvatica* there were 15 binucleate isolates (52%). Such isolates were scarce in conifer seedlings. In the 89 isolates from seedlings of *L. decidua, P. nigra* and *P. sylvestris* there were 15 binucleate isolates (17%). Binucleate *Rhizoctonia* spp. were isolated from 31 (17%) tested seedlings of *A. incana, B. pendula* and *F. sylvatica*, and from only 17 (5%) *L. decidua, P. nigra* and *P. sylvestris* seedlings.

DISCUSSION

Fungi of the genus *Rhizoctonia* were found in seven forest nurseries situated in southern Poland. The degree of seedling colonization was low (7%) in only one nursery, and varied from 18 to 73% in the others. This indicates a wide distribution of these fungi in the nurseries tested. They were more abundant in seedlings taken from greenhouses and tunnels than in those from the field. This may be associated with the higher temperature and humidity connected with such production technologies, conditions favoring the development of *R. solani* (Kwaśna 1987; Kacprzak & Mańka 2001). This species is the only one from this genus described many times as a cause of infectious damping-off of seedlings in forest nurseries, mainly in western Poland (Kwaśna 1987; Mańka & Gierczak 1971; Przezbórski 1984). Usually the pathogen caused damping-off of conifer seedlings. In this study, fungi of the genus *Rhizoctonia* were also found in a considerable percentage of seedlings of *B. pendula* and *F. sylvatica.*

In papers dealing with the occurrence of *Rhizoctonia solani* in Polish nurseries there is no information on the morphology of cultures and sclerotia obtained from isolates, and very sparse information on the number of nuclei in hyphae cells and perfect stages (Mańka & Stępniewska 2001). Our present knowledge of the diversity of *Rhizoctonia* spp. suggests that isolates identified earlier as *R. solani* may have represented other species of this genus. Their presence has been reported from forest nurseries in Finland, Norway and North America (Hietala 1995; Hietala *et al.* 1994; Huang & Kuhlman 1989; Lilja 1994; Lilja *et al.* 1992; Saksena & Vaartaja 1961). The genus *Rhizoctonia* comprises mono- or binucleate isolates with a perfect stage of *Ceratobasidium* D. P. Rogers and *Tulasnella* J. Schroet. and multinu-

cleate isolates including *R. solani* with a perfect stage of *Thanatephorus* Donk and *Waitea* Warcup & P. H. B. Talbot (Sneh *et al.* 1994). Binucleate and multinucleate isolates show differences in morphology of cultures and sclerotia. Parmeter *et al.* (1967) found that binucleate isolates usually do not produce sclerotia, have thinner hyphae, slower growth, are lighter (sometimes almost white), have distinct zonation of colonies, and more ample air mycelium compared with cultures of multinucleate isolates. Among the 118 isolates analyzed in this study there were 30 (25%) binucleate isolates. The cultures of these isolates corresponded to the characteristics given by Parmeter *et al.* (1967), and the average width of hyphae was 4.7 μm, while the width of hyphae of the multinucleate isolates was greater, 7.2 μm on average. The multinucleate isolates were distinctly more numerous, comprising 75% of all those tested. They varied considerably in the morphology of cultures and in the production and morphology of sclerotia. This may indicate different species of *Rhizoctonia* (Andersen 1996; Tu & Kimbrough 1975) or great variation of *R. solani* isolates (Sherwood 1969; Sneh *et al.* 1994). The species is characterized by a high degree of diversity in respect of morphology and physiological characteristics, pathogenic ability and geographic distribution, resulting in the differentiation of 13 anastomosis groups (AGs) (Vilgalys & Cubeta 1994). *Rhizoctonia solani* AG1, AG2, and AG4 have been found in forest nurseries in other countries (Parmeter *et al.* 1969; Sneh *et al.* 1994). Anastomosis groups of *R. solani*, as well as other fungi of this genus, are unknown in forest nurseries in Poland. The results presented here indicate the great diversity of these fungi. Further studies will lead to their definitive designation (AGs, teleomorphs). It is important to learn about the relations between the fungi and their hosts, because not all fungi of the genus *Rhizoctonia* are pathogenic to tree seedlings (Hietala 1995; Lilja 1994; Sneh *et al.* 1994).

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