JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 43, No. 1 (2003)

OCCURRENCE OF *RHIZOCTONIA* ROT OF COMMON ALDER AND BIRCH SEEDLINGS IN FOREST NURSERIES*

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Accepted: February 18, 2003

Abstract: *Rhizoctonia solani* was isolated from 91% of alder and birch seedlings with stem rot symptoms and 2–3% of seeds. Sowing of seeds to substratum infested with *R. solani* resulted in pre-and postemergence damping off. On leaves and stem parts of alder and birch, inoculated with 3 isolates of *R. solani*, necrosis spread from 0.22 to 0.52 mm/hr.

Key words: seedling, seed, fungi, isolation, *Rhizoctonia*, occurrence, isolate, necrosis, spread

INTRODUCTION

One of the main problems in production of forest nursery plants are root and/or stem rot incited by soil-borne pathogens. A severe outbreak of Common alder (*Alnus glutinosa* L. Gaertn.) and Common birch (*Betula verrucosa* Ehrh.) have been observed since many years on seedlings grown under covering and in field. On 3–4-month-old seedlings, growing especially in plastic tunnels but also in open field, stem rot was observed in different points of nurseries. Infected stems turned brown and dark-brown near the base or sometimes to 10 cm above the substratum level. Invaded seedlings showed wilt symptoms and died a few days later due to disruption of translocation of water and nutrients. Pale-brown mycelia were often observed on affected plants, especially when they grown very dense. *Rhizoctonia solani* Kühn is often isolated from diseased roots and stems of plants (Anderson 1982). At present, *R. solani* is considered a species complex rather than single species (Anderson 1982). Isolates of *Rhizoctonia* vary greatly in their colony morphology, growth

*This work was supported by the State Committee for Scientific Research (KBN), grant 3PO6L 01622

characteristic and pathogenicity toward plants (Ogoshi 1987). Because of increasing size of effected area in forest nurseries, in 2002 studies were done to determine causal agents of seedlings mortality and possibility of their transmission by seeds.

MATERIALS AND METHODS

Seedling survey and isolation of fungi. Plants with disease symptoms were found in 6 surveyed nurseries in the middle of Poland but seedling mortality was mainly observed in 2 places, where alder and birch were grown under plastic. Diseased seedlings were collected from different points of tunnels on July and August, 2002. Plant samples (together 128 seedlings) were put into plastic bags and transferred to laboratory. The same or next day symptomatic stem parts were washed thoroughly in running tap water and dried on blotting paper. Five mm pieces, taken from the border of diseased and healthy tissues, after disinfection over a burner flame, were placed on Petri dishes containing potato-dextrose agar (Difco PDA). Tissue parts from each plant were placed on 2–3 Petri dishes (6 pieces/plate). After 2–4-day-incubation at 24°C colonies growing around tissue pieces were transferred to tube slants on PDA. After segregation and cleaning representative cultures were identified to genera and species.

Isolation of fungi from seeds. Two hundred of seeds from each species of plant were analysed. After 2 min disinfection in 0.5% solution of sodium hypochlorite, seeds were rinsed 3 times for 2 min. in sterile, distilled water, dried in sterilized blotting paper and placed on PDA in 90 mm diam Petri dishes (10 seeds/plate). Further procedure was similar like with isolation of fungi from diseased plant tissues.

Colonisation of seedlings and plant parts by *R. solani*. Seeds of alder and birch were sown to 1 dm^3 pots filled with peat artificially infested with isolates of *R. solani* from diseased seedlings. The species inocula were prepared on Quick oats (Orlikowski 1999). Control seeds were placed in pots filled with noninoculated peat. Pots were incubated on greenhouse bench at 17–22°C. Germination of seeds and number of seedlings with *Rhizoctonia* rot were observed after 9- and 12-day-incubation. Experimental design was completely randomised with 8 replications and 25 seeds in each rep. Trials were repeated twice at 3-week-interval.

Colonisation of leaf blades and alder stems were determined using procedure described by Orlikowski and Szkuta (2002). Mycelial disks were taken from 5-day-old *R. solani* cultures from diseased seedlings of alder (A10), birch (B3) and P7 from paulownia (*Paulownia tomentosa* Thunb. Zieb. and Zucc. ex Stend.). Length of necrosis was measured after 3 and 6-day-incubation of inoculated leaves and stem parts in moisture chambers at 20–22°C. Experimental design was completely randomised with 4 replications and 10 leaves or stem parts in each rep. Trials were repeated 3 times at 3-week-intervals.

RESULTS

Isolation and identification of fungi from diseased seedlings. Only seven genera and fungal species were isolated from 68 diseased stems of common alder seedlings (Tab. 1). *R. solani* was a dominant species in surveyed seedlings. It was isolated in both observation periods. Occurrence of *Botrytis cinerea* was observed

Table 1. Fungi isolated from diseased stems of alder seedlings grown in plastic tunnel; number of seedlings settled by fungi (a) and number of isolates obtained (b)
Isolation: I – 2002.07.09; II – 2002.08.09

Species of fungi	Nurseries			
	I (25 plants)		II (43 plants)	
	a	b	a	b
Botrytis cinerea Pers.	7	18	9	17
Fusarium equiseti (Cda) Sacc.	–	–	3	7
Fusarium solani (Mart.) Sny. et Hans.	1	6	2	4
Mucor spp.	3	11	1	3
Pythium sp.	–	–	2	5
Rhizoctonia solani Kühn	24	116	38	101
Trichoderma spp.	1	3	–	–

separately or together with *R. solani* and other species including *Fusarium*. *Pythium* sp. was isolated only sporadically (Tab. 1).

On diseased seedlings of birch *R. solani* dominated among 6 genera and species isolated from affected stems (Tab. 2). *Fusarium* species and *Trichoderma* spp. were found rarely or sporadically (Tab. 2).

Isolation of fungi from alder and birch seeds. On alder seeds *Penicillium notatum* and *Trichoderma* spp. were dominated microorganisms (Tab. 3). *Alternaria alternata, B. cinerea* and *F. culmorum* occurred only on 10% of analysed seeds whereas *R. solani* was isolated from 3% of them (Tab. 3).

On birch seeds *A. alternata* and *Trichoderma* spp. dominated among 7 isolated genera and species. *R. solani* was found on 2% of seeds (Tab. 3).

Colonisation of seedlings and plant parts by *Rhizoctonia solani*. Sowing of alder seeds to peat infested with *R. solani*, resulted in the occurrence of pre-and postemergence seedlings rot observed after 9- and 12-day-incubation. In control, noninfested peat, more than 50% of seeds germinated and the disease was not observed on seedlings whereas in the presence of the pathogen in the substratum only about 16% of plants survived (Tab. 4). There were no differences between pathogenicity of isolates from alder and birch (Tab. 4). Similar results were obtained with birch (Tab. 4). Inoculation of leaf blades with 3 isolates of *R. solani* resulted in the

Table 2. Fungi isolated from diseased stems of birch seedlings grown in plastic tunnel; number of plants settled by fungi (a) and number of isolates obtained (b)
Isolation: I – 2002.07.08; II – 2002.08.09

Species of fungi	Nurseries			
	I (28 plants)		II (32 plants)	
	a	b	a	b
Fusarium avenaceum (Fr.) Sacc.	2	6	–	–
Fusarium culmorum (W.G.Sm.) Sacc.	–	–	3	8
Fusarium solani (Mart.) Sny et Hans.	–	–	3	7
Mucor hiemalis Wehmer	4	11	7	18
Rhizoctonia solani Kühn	26	92	28	106
Trichoderma spp.	6	21	–	–

Table 3. Fungi isolated from 100 seeds of alder and birch; number of settled seeds (a) and isolates obtained (b)
Isolation: 2002.08.20

Fungal species	Alder		Birch	
	a	b	a	b
Alternaria alternata Nees.	6	7	14	22
Botrytis cinerea Pers.	2	2	3	5
Fusarium culmorum (W.G.Sm.) Sacc.	2	4	1	2
Fusarium equiseti (Cda) Sacc.	–	–	1	3
Penicillium notatum West.	13	20	–	–
Penicillium spp.	4	6	5	11
Rhizoctonia solani Kühn	3	3	2	3
Trichoderma spp.	16	21	16	31

fast spread of necrosis (Tab. 5). Three days after inoculation similar spread of disease symptoms was observed when alder and birch leaves were treated with isolates from those plants (A10 and B3). When isolate P7 from paulownia was used for inoculation of birch blades, browning of leaves spread 3 times faster than in case of their treatment with 2 other strains (Tab. 5). After 6-day-incubation isolates A10 and P7 were significantly more pathogenic to leaves than B3. Mean spread of necrosis on alder leaves was 0.22 mm/hr whereas on birch 0.35 mm/hr (Tab. 5).

Table 4. Germination of seeds of alder and birch in peat artificially infested with *R. solani*; 2002.09.25

Plant	Source of *R. solani*	% of germinated seeds (n=20) after days	
		9	12
Alder	Control	32.5 b	57.5 b
	Alder	12.5 a	17.5 a
	Birch	12.5 a	14.0 a
Birch	Control	30.0 b	42.5 b
	Alder	8.8 a	16.3 a
	Birch	10.0 a	12.5 a

Note: means in columns, followed by the same letter, do not differ with 5% of significance (Duncan's multiple range test)

Table 5. Spread of necrosis (in mm) on alder (a) and birch (b) leaves inoculated with *R. solani*
Inoculation: 2002.07.20

Isolates	Source of isolates	Days after inoculation			
		3		6	
		a	b	a	b
A 10	Alder	8.8 a	10.6 a	30.8 b	58.3 c
B 3	Birch	6.1 a	9.6 a	16.7 a	37.0 b
P 7	Paulownia	11.1 a	30.5 b	45.8 c	58.0 c

Note: see table 4

Table 6. Spread of necrosis (in mm) on alder (a) and birch (b) stems inoculated with *R. solani* Inoculation: 2002.07.27

Isolates	Source of isolates	Part of stem	Days after inoculation			
			3		6	
			a	b	a	b
A 10	Alder	Base	9.8 a	20.0 a-c	44.3 b	71.5 c
		Top	9.9 a	15.0 a	61.0 c	85.6 f
B 3	Birch	Base	15.3 a	31.3 cd	37.4 b	77.5 ef
		Tope	14.9 a	36.5 d	59.8 c	69.0 e
P 7	Paulownia	Base	40.9 b	29.9 b-d	69.8 c	71.5 e
		Top	42.8 b	18.4 ab	62.8 c	76.5 ef

Note: see table 4

Inoculation of base or top parts of stems of alder and birch seedlings resulted in the brown and later dark-brown discoloration of bark and wood. After 3-day-incubation of alder stem parts necrosis spread significantly faster (about 2–3 times) when isolate P7 from paulownia was used for inoculation. Within the next 3 days necrosis spread, in general, significantly faster on the top parts of stems. Mean spread of necrosis was 0.39 mm/hr (Tab. 6). Inoculation of birch stem parts resulted also in the development of necrosis. After 6-day-incubation (Tab. 6) length of necrosis was longer on birch than on alder stem parts (about 0.52 mm/hr).

DISCUSSION

R. solani dominated on diseased seedlings of alder and birch. The species was isolated from about 91% of analysed plants. Occurrence of the species in different points of nurseries and its spread from diseased plants on the surface of affected seedlings and soil resulted in the cumulation of inoculum and increase the possibility of infection of plants in the next season. Even when surface part of soil or substratum is changed or disinfected, the species may survived in soil near the tunnels construction and on pathways. Isolation of *R. solani* from 2–3% of seeds suggest that they may be also a potential source of the pathogen. The fast spread of *Rhizoctonia* stem rot during the summer time indicate that the pathogen develops quickly in higher temperature rates. Liu and Sinclair (1991) and Priyatmojo et al. (2001) found that *R. solani* isolates from miniature roses had optimum growth at 28°C and they still grown at 35°C. That is the reason why the disease develops much faster and on larger area on seedlings grown under plastic tunnels than in field nurseries. Our study shown lack of host specialisation of 3 tested isolates of *R. solani*. All of them caused brown or dark-brown rot of leaf blades and stem parts. Faster spread of necrosis on leaves and stems, inoculated with isolate from paulownia indicated that that strain was more pathogenic to alder and birch than those from 2 studied plant species. It indicates that even rotation system in nursery does not restrict or eliminate *R. solani* from soil. In such case chemical treatment of seeds, soil disinfection and plant spray during vegetation period are necessary to minimize *Rhizoctonia* seedling rot.

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POLISH SUMMARY

WYSTĘPOWANIE RIZOKTONIOZY SIEWEK OLSZY I BRZOZY W SZKÓŁKACH LEŚNYCH

Placowe występowanie zgnilizny pędu siewek olszy i brzozy obserwowano w szkółkach polowych ale szczególnie w produkcji roślin w tunelach foliowych. *Rhizoctonia solani* izolowano średnio z około 91% porażonych siewek oraz z 2–3% nasion. Po wysianiu nasion do podłoża sztucznie zakażonego izolatami grzyba z olszy i brzozy, obserwowano masowe zamieranie siewek przed ich ukazaniem się nad powierzchnią ziemi lub po kilku dniach po ich skiełkowaniu. Naniesienie krążków grzybni 3 izolatów *R. solani* na ogonki liściowe oraz części łodyg powodowało rozprzestrzenianie się objawów zgnilizny od 0,22 do 0,52 mm/godzinę.