PYTHIUM INTERMEDIUM, A NEWLY RECOGNIZED PATHOGEN OF CONIFEROUS SEEDLINGS IN CANADA

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Abstract

Pythium intermedium de Bary caused up to 50% mortality in 2- to 3-week-old container-grown seedlings of Pinus contorta Dougl. var. latifolia Engelm., Picea glauca (Moench) Voss var. albertiana (S. Brown) Sarg., Picea engelmannii Parry, and Pseudotsuga menziesii (Mirb.) Franco. It killed 3-week-old aseptic seedlings of all species in vitro within 3 days. This is the first report of this fungus as a pathogen of conifers in North America.

Introduction

Damping-off is a continuing problem in the raising of container seedlings for reforestation. Unusual devastation occurred during April 1969 in the greenhouses at the Provincial Tree Nursery, R.R. 6, Edmonton, Alberta. Mortality was widespread in all coniferous species being grown, reaching 40 to 50% by the 3rd week after emergence. This note reports on the pathogenicity of the organism principally responsible and previously unreported as a pathogen of conifers in North America.

Materials and methods

Seedlings of Pinus contorta Dougl. var. latifolia Engelm., Picea glauca (Moench) Voss var. albertiana (S. Brown) Sarg., Picea engelmannii Parry, and Pseudotsuga menziesii (Mirb.) Franco., were grown in the greenhouse on non-sterilized, locally dug sphagnum peat in split plastic tubes (3/4 inch diam. by 3 1/2 inches long), packed in plastic flats holding 220 tubes. Seeds were sown by a vacuum seeder, which deposited 2 to 8 seeds per tube. Irrigation for about 10 sec every half hour was by automatic mister, which had the additional function of reducing temperature during sunny periods and therefore tended to water the seedlings to excess. Temperature controls were set at 70F (21C), upon reaching which an extraction fan would cut in; however temperatures exceeding 100F (38C) occurred for periods up to 1 hour or so almost daily. Thus, many conditions predisposing the seedlings to epidemic damping-off existed: overcrowding, water-logged organic soil, high humidity, high and periodically extreme temperatures, and inoculum in non-sterile seeds or substratum.

Soon after emergence, about 2 weeks after sowing, damping-off was observed, and isolations were started. At the end of each week, for 3 weeks, mortality was assessed on two full flats of each species of tree (Table 1).

Results and discussion

Isolations.—Each week, 20 damped-off seedlings of each species were collected from various flats and placed individually in separate vials. They were individually washed in sterile water, surface-sterilized in a 1:1 mixture (v/v) of ethanol and aqueous-saturated $HgCl_2$, and plated on 2% agar in tap water. Isolations were made from hyphal tips or spores growing on or out of the seedling fragments.

Most isolates from samples taken in the 1st week were pure cultures of Pythium intermedium de Bary. In the 2nd and 3rd weeks, Pythium intermedium was still the most common isolate. Other fungi, mostly Fusarium spp., also occurred, but only irregularly and

Table 1. Mortality of coniferous seedlings from damping-off at different seedling ages (1-3 weeks from emergence)

Species	1 week		2 weeks		3 weeks	
	Number sampled	Mortality (%)	Number sampled	Mortality (%)	Number sampled	Mortality (%)
Pinus contorta	760	28	678	44	683	58
Picea glauca	598	6	635	27	667	41
Picea engelmannii	616	19	763	38	706	50
Pseudotsuga menziesii	410	2	470	21	588	27

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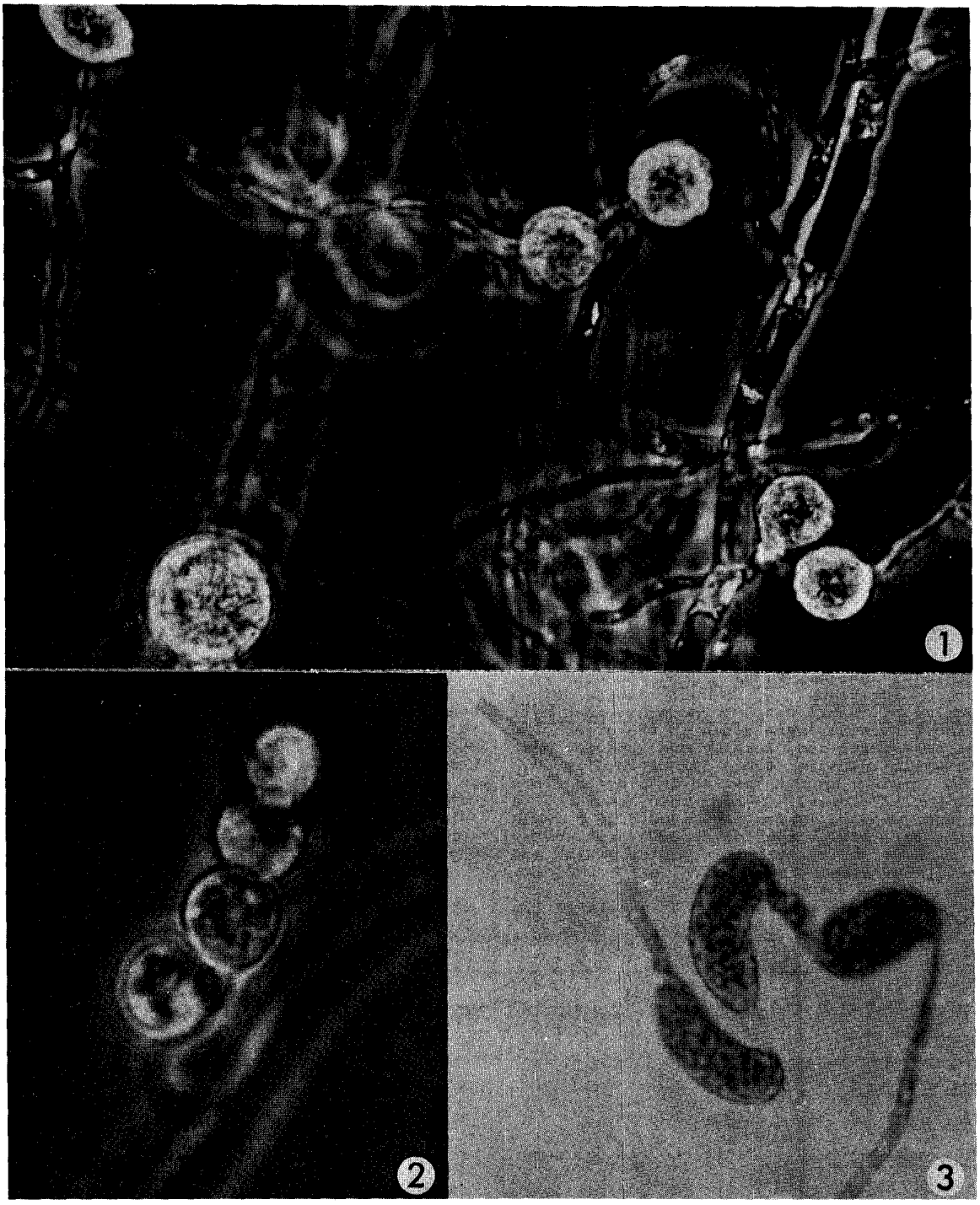

Figure 1–3. *Pythium intermedium.* 1) Single sporangia, Xco. 450; 2) Chain of sporangia, diagnostic of *P.* infermedium, Xca. *900;* 3) Repetitive appressoria formed *by P.* intermedium from *a* sporangium germinating on a glass surface, Xca. 450.

in the later stages. During the first 3 weeks Pythium intermedium consistently grew out of root and collar fragments of seedlings.

Most cultures also yielded moderate to large numbers of nematodes. These were subseauently identified as belonging to the genera Rhabditis Dujardin (1845) and Cephalobus Bastian (1865) by Dr. J.R. Sutherland, Canadian Forestry Service, Victoria, B.C., and Dr. E.J. Hawn, Canada Department of Agriculture, Lethbridge, Alta. These genera are non-stylet-bearing and hence are not considered to be pathogenic to plants.

Isolates of Pythium intermedium were identified by Dr. D.J. Stamps, Commonwealth Mycological Institute, Kew, England; and Dr. O. Vaartaja, Canadian Forestry Service, Ottawa. Isolates did not form oogonia, perhaps because complementary sexual strains were lacking. Other workers have found isolates from Holland to be heterothallic (9). Single sporangia were frequently formed (Fig. 1). Most isolates, however, formed deciduous chains of sporangia (Fig. 2), a diagnostic characteristic unique to P. intermedium (1,5). In cultures on glass slides, germinating sporangia commonly formed repetitive appressoria (Fig. 3).

Inoculations. - To test the pathogenicity of Pythium intermedium, I inoculated, in vitro, aseptic tree seedlings. They were grown for 3 weeks from pre-germinated, surface-sterilized seeds on 2% tap water agar in 18 X 150 mm test tubes, under fluorescent light. Additional test-seedlings were grown aseptically in the greenhouse in 250 ml conical flasks containing vermiculite and a mineral nutrient solution.

Ten seedlings of each species grown in test tubes and 10 grown in flasks were inoculated by dropping a 5 mm diam by 3 mm thick agar plug from 3-day-old cultures of Pythium intermedium into each tube or flask. The seedlings were then incubated in the light at 22C. All the inoculated seedlings were killed by the 3rd day. Reisolations on water agar yielded pure cultures of Pythium intermedium. Uninoculated controls remained healthy and yielded no microorganisms when plated out.

Pythium intermedium de Bary is a rarely isolated member of the genus. Middleton (5) records 24 reports dating from the original description by de Bary in 1881 (1). This species is missing from recent accounts of damping-off of coniferous tree seedlings in the southern United States (2), Britain (3), and Canada (8). It is recorded from nursery soil in Britain (10) and the United States (4) and from Pinus halepensis Mill. seedlings in Australia (6). In Canada, there is only one published report of its occurrence, and that is from nursery soil (7).

Dr. A.W. Henry, in a personal communication, reports that in a study of root disease of ornamental elders (Sambucus spp.) during 1968 and 1969, he isolated Pythium infermedium from .soil beneath diseased bushes from six widely separated areas in Alberta: Beaverlodge, Bowden, Ellerslie, Edmonton, Vulcan, and Devon.

Such widespread incidence suggests that earlier investigators may have overlooked the species, or simply relegated it to Pythium sp. The present report clearly establishes Pythium intermedium as a potentially dangerous pathogen to coniferous seedlings, especially when grown in containers in the greenhouse.

Literature cited

1. Bary, A. de. 1881. Zur Kenntniss der Peronosporeen. Bot. Z. 39:553-558.
2. Campbell, W.A., and F.F. Hendrix, Jr. 1967. Pythium and Phytophthora species in forest soils in the southeastern United States. Plant Dis. Rep. 51:929-932.
3. Griffin, D.M. 1965. A study of damping-off, root damage and related phenomena in coniferous seedlings in British forest nurseries, p. 212-227. In Blanche Benzian, Experiments on nutrition problems in forest nurseries, Vol. I. Gt. Brit. For. Comm. Bull. 37: 1-251.
4. Hendrix, Floyd F., Jr., and W.A. Campbell. 1969. Distribution of Phytophthora and Pythium species in soils of the continental United States. Can. J. Bot. 48:337-384.
5. Middleton, J.T. 1943. The taxonomy, host range and geographic distribution of the genus Pythium. Mem. Torrey Bot. Club 20:1-171.
6. Vaartaja, O. 1967. Damping-off pathogens in South Australia nurseries. Phytopathology 57:765-768.
7. Vaartaja, O. 1967. Pythium and Mortierella in soils of Ontario forest nurseries. Can. J. Microbiol. 14:265-269.
8. Vaartaja, O., and W.H. Cram. 1956. Damping-off pathogens of conifers and of Caragana in Saskatchewan. Phytopathology 46:391-397.
9. Van Der Plaats-Niterink. 1968. The occurrence of Pythium in the Netherlands 1. Heterothallic species. Acta Bot. Neer. 17:320.
10. Warcup, J.H. 1952. Effect of partial sterilization by steam or formalin on damping-off of Sitka spruce. Trans. Brit. Mycol. Soc. 35:248-262.