Relative Prevalence and Virulence of Phytophthora Species Involved in Rhododendron Root Rot

H. A. J. Hoitink and A. F. Schmitthenner

Associate Professor and Professor, respectively, Department of Plant Pathology, Ohio Agricultural Research and Development Center, Wooster 44691.

Supported by the Cooperative Agreement No. 12-14-100-10,640(34) from the United States Department of Agriculture.

Approved as Journal Article No. 20-74 of the Ohio Agricultural Research and Development Center, Wooster, Ohio.

Accepted for publication 14 May 1974.

ABSTRACT

Phytophthora cinnamomi was found to be the most important species involved in the rhododendron root rot syndrome in the United States. *P. cactorum* and *P. citricola* cause root rot, but occur more frequently as dieback pathogens. *P. cryptogea, P. lateralis, P. megasperma,* and *P. gonapodyides* are involved in the root rot syndrome, but are considered minor root pathogens. *P. citrophthora* causes stem dieback only. All isolates of *P. cinnamomi* from ericaceous plants appeared to be equally virulent in our tests; however, some strains of *P. cinnamomi* isolated from nonericaceous plants were significantly less virulent than others to the susceptible rhododendron hybrid cultivar Purple Splendour. The hybrid cultivar English Roseum was resistant to all eight *Phytophthora* spp. Roots were rotted by all species, but regenerated from the crown in the top 2- to 4-cm layer of soil.

Phytopathology 64:1371-1374.

Additional key words: Phytophthora root rot, *P. cinnamomi, P. cactorum, P. citricola.*

Phytophthora root rot of *Rhododendron* is a severe disease in many parts of the world. It was first described in 1927 in New Jersey (17), and has been reported several times since from various parts of the United States, Europe, and Australia (1, 7, 11, 12). In the past, these losses have been attributed to *Phytophthora cinnamomi* Rands (18). In Ohio, *P. cactorum* (Leb. & Cohn) Schroet., *P. citricola* Sawada, and *P. cryptogea* Pethyb. & Laff also are involved in the root rot syndrome (4, 5). In Europe, *P. cinnamomi* causes root rot (10), whereas *P. citricola* (9) and *P. cambivora* (Petri) Buisman (8) cause stem dieback.

During the 1970 and 1971 growing seasons, surveys were made of the rhododendron production centers in the Pacific northwest, northern California the southeastern United States and in the New England states. A number of *Phytophthora* spp., other than *P. cinnamomi,* were isolated from rhododendron. We undertook this study to determine the relative prevalence and virulence of these *Phytophthora* spp. involved in rhododendron root rot.

MATERIALS AND METHODS.—*Source of isolates.*—Most isolates were obtained from rhododendron, using the selective medium described earlier (4). Bark was sliced off lower stems and coarse roots. Chips of dark-pigmented woody vascular tissue were placed on the selective medium without surface sterilization. Agar was inverted over blocks of diseased tissue and incubated for 2-5 days at ambient temp. Isolates obtained were transferred to the selective medium and agar was inverted to ensure inhibition of bacteria. Bacteria-free cultures in dilute V-8 juice agar (VA) (4) were stored at 5 C in 15-ml medicine bottles. Cultures were identified from characteristics produced on VA and hemp seed (HS) cultures (13). For HS cultures, seed were boiled 1 h in distilled water, then transferred to petri plates, one per plate, containing 10 ml of a salt solution prepared by mixing two solutions (A and B) after autoclaving. Solution A: KH_2PO_4, 75 mg; K_2HPO_4, 75 mg; $MgSO_4 \cdot 7H_2O$, 50 mg; $ZnSO_4 \cdot 7H_2O$, 2.2 mg; $FeSO_4 \cdot 7H_2O$, 0.5 mg and $MnCl_2 \cdot 4H_2O$, 0.035 mg in 950 ml distilled water. Solution B: $Ca(NO_3)_2 \cdot 2H_2O$, 0.1 gm in 50 ml distilled water. A block of agar culture of the unknown Phytophthora isolate was placed adjacent to the hemp seed in the petri plate. Characteristic structures generally developed in 6 days. Identification was based on the description of Waterhouse (15, 16).

During the surveys, isolations were made from other woody ornamentals with root rot symptoms. A culture each of *P. cinnamomi, P. citricola,* and *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian from rhododendron was obtained from N. Jackson, Department of Plant Pathology and Entomology, University of Rhode Island, Kingston, Rhode Island. Two cultures of *P. cinnamomi* were obtained from H. Rattink, Instituut voor Plantenziektenkundig Onderzoek, Wageningen, The Netherlands and cultures of *P. cinnamomi* from rhododendron cultivar Hino Red (an evergreen azalea), a *Cornus* sp. and an *Ilex* sp. from R. C. Lambe, Department of Plant Pathology and Physiology, Virginia Polytechnic Institute, Blacksburg, Virginia.

Source of plants.—Cultivars English Roseum and Purple Splendour were used to compare virulence of the isolates. Cuttings of the resistant English Roseum were propagated in a 1:1 mixture of Canadian peat and Perlite (Grace, Zonolite Div., W. R. Grace Co., Chicago, Ill.). Rooted cuttings were potted in 1-liter containers, forced for 3 mo, repotted, and grown for 1 yr in 4-liter containers in a 2:2:1 mixture of Canadian peat, muck, and silica sand. Rooted cuttings of the susceptible cultivar Purple Splendour were received from Van Veen Nurseries, 428 S.E. Franklin Street, Portland, Oregon, and grown in identical 4-liter containers. The pH of the mix varied from 6.2 - 6.9. Plants were fertilized with soluble 20-20-20 fertilizer (100 μg N/ml) once weekly. All tests were performed in a greenhouse at 28-32 C.

Method of inoculation.—Inoculum was grown in hemp seed broth (13). Hemp seed (50 g/liter distilled water) was autoclaved for 30 min, filtered through cheesecloth, dispensed into 250-ml Erlenmeyer flasks (50 ml/flask), and autoclaved to sterilize. Sterile broth was

inoculated with an agar disk from a 5-day culture and incubated for 14 days without agitation at 25 C. Inoculum and substrate of one flask were poured on exposed feeder roots below the crown and covered with sterile peat (1 flask per plant). Care was taken to avoid pouring inoculum on the stem. Plants were watered with an automatic system and reisolations were made according to procedures described previously (4). Ten plants of the cultivar English Roseum and three of cultivar Purple Splendour were inoculated with each isolate. After 4 mo all plants were harvested and rated for root rot severity. A root rot index as follows was used: 1=healthy plants, 2 and 3 indicating increasing root rot severity, 4=crown rot and 5=dead plants (4). Ratings were analyzed for significant differences with a least squares analysis method.

RESULTS.—*Identification and occurrence of Phytophthora spp.*—1) *P. cinnamomi.*—During this study, more than 500 isolates of *P. cinnamomi* were obtained from roots and crowns of rhododendron. The pathogen was isolated from 52 different locations; 18 in Ohio, 17 from the West Coast (British Columbia, Pacific northwest and California), 9 from the southeast (Florida, Georgia, The Carolinas, Virginia and Maryland) and 8 from the northeast (Rhode Island, New Jersey, Pennsylvania, and Delaware). It was also isolated from the other ericaceous plants: azalea cultivar Hino Red (five isolates), *Calluna vulgaris* (L.) Salisb. (three isolates), *Kalmia latifolia* L. (one isolate), and *Arctostaphylos uva ursi* L. Spreng (one isolate). In addition it was isolated from a *Cryptomeria* sp. (one isolate) and juniper (two isolates from unidentified types).

2) *P. cactorum.*—Isolates could be distinguished readily by characteristic sporangia and oospores (16) produced on VA and in HS. It was the second most frequently isolated *Phytophthora* sp. from rhododendron and was primarily isolated from branches and crowns from all parts of the United States which were surveyed.

3) *P. citricola.*—It was isolated from rhododendron roots, crowns, and branches in California, North Carolina, Ohio, Oregon, and Rhode Island.

4) *P. lateralis* Tucker & Milb.—Isolates forming sessile, laterally attached, thin-walled chlamydospores (hyphal swellings) in VA and HS were considered to be this species. They differ from the original description of this species in growing at a high temp (14). Thick-walled chlamydospores, sporangia, and oospores were not observed. These isolates differed from *P. cinnamomi* in the attachment of chlamydospores, in forming a rosette, appressed-colony type on VA, and not producing sporangia when flooded with nonsterile soil extract. Cultures were isolated from rhododendron crowns in Ohio and Pennsylvania.

5) *P. cryptogea.*—Isolates which produced nonpapillate sporangia, occasionally in VA but abundantly in HS cultures, and characteristic clusters of hyphal swellings (5) were referred to this species. It was only isolated from rhododendron roots in Ohio.

6) *P. citrophthora.*—This species was isolated from lesions on rhododendron branches in Ohio and Rhode Island. It is the most common *Phytophthora* spp. isolated from *Pieris japonica* (Thunb.) D. Don and *Euonymus* spp. in Ohio (2).

7) *P. gonapodyides* Petersen.—Isolates were characterized by occasional nonpapillate sporangia in HS, and groups of thickened hyphae (16) in both VA and HS. Isolates grew slowly on VA and produced a fluffy colony. It was isolated occasionally from rhododendron roots and from the crown of *Sciadopitys verticilata* Sieba Zucc., a *Freemontia* sp. and a *Rhamnus* sp. in nurseries in the Pacific northwest.

8) Other species.—*P. megasperma* Drechs. was isolated once from rhododendron rooted cuttings. *P. parasitica* Dast. was isolated frequently from greenhouse azaleas in Florida, Illinois, and Ohio but never from hardy rhododendrons.

Virulence of Phytophthora spp. to a susceptible variety.—The mean root rot rating of rhododendron hybrid Purple Splendour inoculated with various *Phytophthora* isolates is presented in Table 1. Data of *P. citrophthora, P. gonapodyides, P. lateralis, P. megasperma,* and *P. parasitica* were similar to those of *P. cryptogea,* and therefore were not included. These six species caused only slight root damage.

Isolates of *P. cinnamomi* from rhododendron or other ericaceous plants killed significantly more plants ($P = 0.01$) than isolates of *P. cinnamomi* from nonericaceous plants or the other eight species. There was no significant difference ($P = 0.05$) in virulence to hybrid Purple Splendour among isolates from rhododendron and those of other ericaceous plants. Root rot ratings of isolates of *P. cinnamomi* from nonericaceous plants was similar to those from the other eight species. Root rot ratings of isolates of *P. cinnamomi* from rhododendron, grouped according to origin within the United States, did not differ significantly. Two rhododendron isolates of *P. cinnamomi* from The Netherlands were as virulent as those from the United States.

Virulence of Phytophthora spp. to a resistant variety.—None of the English Roseum plants inoculated with any of the *Phytophthora* species were killed. Four months after inoculation, feeder roots and some large roots were rotted by all isolates. Root rot ratings of isolates of all species were similar ($P = 0.05$). Crowns of these English Roseum plants were not invaded by any of the isolates and new roots were regenerated from the crown in the top 2-4 cm of the container mix.

DISCUSSION.—*P. cinnamomi* is the most important *Phytophthora* sp. involved in the rhododendron root rot syndrome, and it occurs throughout the rhododendron production centers of North America. It has a broad host range (20) and was found on many other nursery crops. The rhododendron isolates of *P. cinnamomi* from the entire sampled area were identical in morphology and virulence on the two hybrids tested. Therefore, it is likely that *P. cinnamomi* has been moved frequently across the United States on "healthy appearing" plants with root rot. Isolates of *P. cinnamomi* from other ericaceous plants were as virulent to cultivar Purple Splendour as were the rhododendron isolates. These plants were produced in nurseries that specialize in ericaceous plants, including rhododendron. Five isolates from nonericaceous plants (*Juniperus* sp., a *Cornus* sp., an *Ilex* sp. and a *Cryptomeria* sp.) were significantly less virulent ($P = 0.05$) on Purple Splendour than isolates from ericaceous plants. Three of these isolates were isolated from plants in nurseries that did not specialize in

TABLE 1. Relative virulence to rhododendron cultivar Purple Splendour of *Phytophthora* species isolated from rhododendron, other ericaceous and nonericaceous plants

Species	Source[a]	Number of isolates	Percentage kill	Mean root[b] rot rating	Standard error
Phytophthora cinnamomi		41	53	4.1	0.1
P. cactorum		10	4	2.3	0.2
P. citricola		9	7	2.7	0.3
P. cryptogea		2	0	2.5	0.6
P. cinnamomi	Ohio	7		3.9	0.3
	West Coast	17		4.0	0.2
	Southeast	9		4.2	0.3
	Northeast	8		4.5	0.3
	Other-ericaceous	9	56	4.1	0.2
	Non-ericaceous	5	7	2.6	0.3

[a]Refers to isolates from rhododendron from all parts of the United States, unless indicated otherwise.
[b]Determined with a least squares analysis, based on three replicates per isolate.

ericaceous plants. This work confirms an earlier report (4) that some strains of *P. cinnamomi* are not highly virulent on rhododendron but could be on other crops. Virulence of these isolates to plants from which they were cultured was not tested. There appears to be no evidence for variation in pathogenicity among isolates from ericaceous plants.

In a previous report, *P. citricola* was considered as serious as *P. cinnamomi* (4) on rhododendron in Ohio. However, *P. citricola* was not found in any other area as a root pathogen, although it is a dieback pathogen in several areas, confirming a report from Germany (9). It is not known why it is not a root pathogen outside of Ohio. More intensive surveys might reveal its presence on roots elsewhere. *P. citricola* has been reported as the major root pathogen in walnut (3) and plays a significant role in the Pieris dieback and root rot complex (2).

P. cactorum, basically, is a stem dieback pathogen on rhododendron (18), but it can cause damage to roots and crowns in field plantings where flooding occurs. *P. cambivora* found in Denmark (8) was not detected in this study. *P. citrophthora* is considered a minor dieback pathogen on rhododendron. It is the major species involved in Pieris dieback and root rot (2). *P. cryptogea, P. lateralis, P. megasperma,* and *P. gonapodyides* probably are minor root pathogens similar to *Pythium splendens* Brawn, *P. irregulare* Buisman, and *P. carolinianum* Mathews, which were also found in this study. These four *Phytophthora* spp. were only isolated from crowns of very susceptible rhododendron hybrids growing in container media with poor drainage and under excessive moisture conditions. Based on our observations these four *Phytophthora* spp. could probably be controlled by using media with adequate drainage. However, the other *Phytophthora* spp. may not be controlled by drainage alone. More information is needed on the effects of drainage properties of container media on root rot incidence.

White first observed differences in susceptibility of rhododendron hybrids and species to Phytophthora root rot (19). The hybrid English Roseum is resistant to all eight *Phytophthora* spp. Roots of this hybrid may be rotted by all species but roots regenerate from the crown in the top 2- to 4-cm layer of soil. Under high temp (30 C) and extremely high moisture conditions, some English Roseum plants were killed by *P. cinnamomi* in several nurseries visited. However the amount of kill in this variety was minor, compared to that in the more susceptible types. A detailed evaluation of resistance of rhododendron hybrids and species is being published elsewhere (6).

LITERATURE CITED

1. DAVISON, E. M. 1972. Phytophthora cinnamomi on ornamentals in South Australia. Plant Dis. Rep. 56:290.
2. GERLACH, W. W. P., H. A. J. HOITINK, and C. W. ELLETT. 1974. Dieback of Pieris japonica caused by Phytophthora citricola, P. citrophthora and Botryosphaeria dothidea. Phytopathology 64:1368-1370.
3. GREEN, R. J., and R. G. PRATT. 1970. Root rot of black walnut seedlings caused by Phytophthora citricola. Plant Dis. Rep. 54:583-585.
4. HOITINK, H. A. J., and A. F. SCHMITTHENNER. 1969. Rhododendron wilt caused by Phytophthora citricola. Phytopathology 59:708-709.
5. HOITINK, H. A. J., and A. F. SCHMITTHENNER. 1970. Phytophthora species involved in rhododendron wilt and sources of resistance. Phytopathology 60:1296.
6. HOITINK, H. A. J., and A. F. SCHMITTHENNER. 1974. Resistance of rhododendron hybrids, species and other Ericaceae to Phytophthora cinnamomi. Plant Dis. Rep. 58:650-653.
7. JEFFERS, W. F., C. E. COX, R. A. JEHLE, L. O. WEAVER, O. D. MORGAN, and J. F. MOORE. 1951. Notes on occurrence of some plant diseases in Maryland during 1950. Plant Dis. Rep. 35:385-386.
8. KOCH, J. 1971. Rhododendron blight caused by Phytophthora cambivora. Friesia 9:418-425.
9. KRÖBER, H. 1959. Phytophthora cactorum (Leb. et Cohn) Schroet. var. applanata Chest. als Erreger einer Zweigkrankheit an Rhododendron. Phytopathol. Z. 36:381-393.
10. RATTINK, H. 1971. Jaarverslag. Instituut voor Plantenziektenkundig Onderzoek. Wageningen, The Netherlands. p. 37-39
11. ROTH, L. F., and E. G. KUHLMAN. 1963. Field tests of the capacity of Phytophthora root rot to damage Douglas fir. J. For. 61:199-205.
12. SAUTHOFF, W. 1963. Phytophthora cinnamomi as disease agent of Rhododendron. Gartenwelt 63:275-276.
13. SAVAGE, E. J., C. W. CLAYTON, J. H. HUNTER, J. A. BRENNEMAN, C. LAVIOLA, and M. E. GALLEGLEY. 1968. Homothallism, heterothallism, and inter-specific hybridization in the genus Phytophthora.

Phytopathology 58:1004-1021.
14. TUCKER, C. M., and J. A. MILBRATH. 1942. Root rot of Chamaecyparis caused by a species of Phytophthora. Mycologia. 34:94-103.
15. WATERHOUSE, G. M. 1963. Key to the species of Phytophthora de Bary. Commonw. Mycol. Inst. Mycol. Pap. No. 92.; Commonwealth Mycol. Inst., Kew, Surrey, England. 22 p.
16. WATERHOUSE, G. M. 1970. The genus Phytophthora de Bary. Commonw. Mycol. Inst., Mycol. Pap. No. 122. Commonwealth Mycol. Inst., Kew, Surrey, England. 70 p.
17. WHITE, R. P. 1929. Progress with rhododendron wilt. N. J. Agric. Exp. Stn., Dept. Plant Pathol., Nursery Disease Notes. 1:8.
18. WHITE, R. P. 1930. Two Phytophthora diseases of rhododendron. Phytopathology 20:131.
19. WHITE, R. P. 1936. Summary of nine years' experience with rhododendron wilt. Plant Dis. Rep. 20:204-207.
20. ZENTMYER, G. A., and W. A. THORN. 1967. Hosts of Phytophthora cinnamomi. Calif. Avocado Soc. Yearb. 51:177-186.