Overwintering and Survival of *Phytophthora parasitica*, Causing Dieback of Rhododendron

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ABSTRACT

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Two isolates of *Phytophthora parasitica* from rhododendron survived <3 days at temperatures below −4.5 C, and <11 days at temperatures between 0 and −4 C on a thermal gradient plate. At temperatures between 1 and 2 C, the fungus survived 21 to over 40 days in infected leaf disks depending on tissue collection date, and was recovered from 80–100% of leaf disks after 40 days at temperatures above 4 C. Cold acclimation of colonized leaf disks for 3 days at 4 C before placement on the gradient plate had no effect on fungal tolerance to cold temperatures. In the winter of 1980–1981, *P. parasitica* overwintered in infected leaf disks buried 5 cm deep in a layer of pine bark (called a container base) upon which potted nursery plants are placed, but not in infected leaf disks on the surface of the container base or on the surface of a pine bark medium in 2.4-L pots resting on the container base. The fungus survived in buried infected leaf disks during the 1981–1982 winter as well. Low percentages of *P. parasitica* were recovered from infected main stems and roots of hybrid rhododendron plants and infected leaf disks on the surface of the container base in 1981–1982. *P. parasitica* was recovered from the naturally infested pine bark from March through December, but not following periods of cold weather in January and February. Mycelium, chlamydospores, and sporangia of *P. parasitica* were observed in overwintered leaf disks that were buried 5 cm deep in the container base of pine bark during the 1981–1982 winter season. Colonies of *P. parasitica* originated from chlamydospores in the leaf disks.

Phytophthora parasitica Dast. (*P. nicotianae* Breda de Hann var. *parasitica* [Dast.] Waterh.) is one of five *Phytophthora* species that cause a foliar dieback disease of hybrid rhododendron (2). Phytophthora dieback is severe under commercial production schemes utilizing shade houses and overhead sprinkler irrigation (8). Infected leaf debris is an important inoculum source in rhododendron-growing areas during the summer months when *Phytophthora* is active (8) and may be an important overwintering site for *P. parasitica*.

Several *Phytophthora* species have been shown to overwinter in infected plant debris. For instance, *P. citrophthora* survives Ohio winters in infected leaf debris of *Pieris japonica* (Thunb.) D. Don. (3) and *P. cinnamomi* can overwinter in North Carolina in roots of *Abies fraseri* (Pursh) Poir if soil temperatures remain above 0 C (1).

Low temperatures are inhibitory to survival of *P. parasitica* and other *Phytophthora* species (1,3,5,7,9,10). In nonsterile soil, *P. parasitica* was killed after one winter in Berlin, West Germany, where there were 58 days with maximum temperatures below 0 C (7). Survival of chlamydospores of *P. parasitica* in soil (4) and water (5) was best at temperatures of 9 or 12 C.

Our study was initiated to characterize the effects of cold temperatures on the ability of *P. parasitica* to survive in rhododendron leaf tissue, and to examine overwintering sites of *P. parasitica* under nursery conditions in North Carolina.

MATERIALS AND METHODS

Two isolates of *P. parasitica* from rhododendron in North Carolina (317 and 324) were used for inoculation of rhododendron leaf disks in cold inactivation studies. Isolates were maintained on cornmeal agar (CMA) plates at 25 C until needed. Isolate 317 was used for overwintering studies in the nursery.

Leaf disk inoculations. Leaves of hybrid rhododendron cultivar Nova Zembla were collected from new growth flushes 2–3 wk after leaves had fully expanded and were stored at 5 ± 2 C until needed.

Leaves were collected several times during the year. Leaf disks (10-mm diameter) were cut from leaves with a cork borer, surface sterilized in 70% ethanol for 1 min, and rinsed three times with sterile deionized water (SDW). Leaf disks were arranged, abaxial side upward, on moist sterile towels in polystyrene boxes that had been surface sterilized by immersion in a 0.5% sodium hypochlorite solution.

Zoospore suspensions were used for leaf disk inoculations. Agar disks from the margins of 3- to 5-day-old colonies of *P. parasitica* growing on CMA were flooded with clarified lima bean extract broth (50 g of frozen lima beans per liter) for 3 days. Mycelial mats were then rinsed twice with SDW and maintained in a flooded condition for 24 hr at 25 C. During this time, abundant sporangia were formed. The sporangium-bearing mycelial mats were rinsed two or three times with SDW, refrigerated for 15 min at 4 C, and returned to room temperature. Concentrations of zoospores released after 30–45 min were adjusted according to hemacytometer counts and pipetted onto leaf disks (10–15 ml per 100 leaf disks). After incubation in polystyrene boxes at room temperature for 7–10 days, the leaf disks became necrotic and were fully colonized.

Cold inactivation. Inactivation of two isolates of *P. parasitica* by cold temperature was studied using a thermal gradient plate (TGP) described previously by Benson (1). A temperature gradient of approximately 12 C was maintained on the aluminum plate that was located in a 5 ± 2 C cold room. The TGP was divided into a 10 × 10 arrangement of 100 grids (6.4 × 6.4 cm each). Leaf disks were inoculated with either *P. parasitica* isolate 317 or 324 and placed on 2% water agar (WA) in 5-cm-diameter polystyrene petri dishes (10 disks per dish). Dishes were constructed with tightly fitting lids to prevent desiccation. Dishes containing leaf disks were placed on five alternate rows along the grid. Thermometers imbedded horizontally into water agar in plastic dishes were placed on adjacent rows, and temperatures along the TGP were recorded daily. Exposure temperatures on rows containing leaf disks were estimated by interpolation from linear regression analysis of temperature data recorded on the adjacent rows. Five dishes for each of the two isolates of *P. parasitica* tested were randomized along each gradient row. Leaf disks were removed (one leaf disk per dish) at intervals of one to several days and plated on $P_{10}PP$

medium (2). The experiment was repeated several times at two temperature ranges and results were consistent between experiments.

Overwintering studies in the nursery. Overwintering of *P. parasitica* was examined during the winters of 1980–1981 and 1981–1982 in a research nursery in Raleigh. The test area was a lath house (50% shade) with a layer of pine bark (called a container base) upon which potted nursery plants are placed. During the winter of 1980–1981, leaf disks colonized by *P. parasitica* were placed in nylon mesh sacks (25 disks per sack) and either secured to the surface of the container base, buried 5 cm deep in the container base, or placed on the surface of pine bark potting media in 2.4-L plastic pots placed on the container base. Pine bark was mounded around the pots about 10 cm up the side of the 15-cm-tall pot. Overwintering sites were randomized in three replicate test areas in the lath house. Leaf disks were collected every 3 wk from December 1980 to May 1981 and plated on $P_{10}PP$ medium (2) amended with 75 μg of hymexazol ($P_{10}PPH$) per milliliter to inhibit *Pythium* spp. Percent recovery of *P. parasitica* from infected leaf disks was determined after 1 wk.

Overwintering of *P. parasitica* in leaf disks during the winter of 1981–1982 was examined in the same three nursery sites. In addition, shoots and roots of 1-yr-old hybrid cultivar Nova Zembla rhododendron plants (average 20–25 cm tall) in 2.4-L pots were inoculated with *P. parasitica*. CMA disks containing the fungus were placed on wounded terminal shoots of plants and incubated under mist (15 sec of mist every 5 min) for 2 days. Inoculated plants were then incubated in humidity chambers in a greenhouse for 3 days. Necrosis spread 5–7 cm down the stem during this time, but disease progress stopped when plants were moved to the nursery area in November. At the time of shoot inoculation, the roots of the same plants were inoculated with a zoospore suspension of *P. parasitica* (50,000 zoospores per plant). Pots were immersed in water and zoospores were applied to the pot surface while the potting medium was flooded. Pots were then allowed to drain into saucers. Twenty-four inoculated plants and nylon mesh sacks (28–30 leaf disks per sack) were randomized in three replicate areas in the lath house.

Survival of *P. parasitica* in leaf disks and intact shoot and root tissues was determined by sampling at 3-wk intervals from November 1981 to May 1982. Twenty-five leaf disks from each site and pieces of leaf tissue from the leading edges of lesions on shoots of potted plants were plated on $P_{10}PPH$ medium and assayed for fungal growth after 3–7 days. Three leaf disks from each sack were incubated in moist chambers at room temperature for 1 wk before plating. Two samples (20–30 cm^3 each) containing roots and medium from each plant were washed under tap water, blotted dry, cut into 1- to 2-cm pieces, and plated on $P_{10}PPH$ medium.

A thermograph in the lath house was used to record ambient air temperature 20 cm above container base level and bark temperature 5 cm deep in the container base.

Thin sections of buried leaf disks from each sampling were stained with cotton blue and examined microscopically for propagules of *Phytophthora*. Buried leaf disks also were blended 30 sec in SDW and 50 μg of pimaricin per milliliter and plated on PARP medium (6) amended with 50 μg of hymexazol per milliliter (PARPH). Plates were incubated 2 days in the dark at room temperature, rinsed with tap water to remove debris, and examined under the microscope for *P. parasitica* colonies.

Presence of *Phytophthora* in the pine bark container base in the lath house was detected by a rhododendron bioassay. Samples (100 cm^3) of pine bark in 250-ml beakers were flooded with 50 ml of deionized water, so that the water level was approximately 1 cm above the bark. Ten 10-mm-diameter leaf disks of rhododendron cultivar Nova Zembla were surface sterilized and floated adaxial side up above each bark sample. A cheesecloth net (four layers) on one end of a 5-cm-long section of 6-cm-diameter PVC pipe was used to keep bark pieces submerged below leaf disk baits, although any released zoospores could easily move up through the net. Leaf-disk baits were floated above bark samples for 3 days at room temperature, rinsed in deionized water, and plated on $P_{10}PPH$ medium. Plates were examined 3–5 days afterward for growth of *P.*

parasitica from leaf disks. Twenty pine bark samples were taken in the test area every 3–4 wk from February 1981 to June 1982. In January and February of 1982, additional pine bark samples were incubated in the greenhouse for 12 or 24 days before assaying. Bark samples also were blended 30 sec in SDW and plated on PARPH or $P_{10}PPH$ medium. Plates were incubated in the dark for 2–3 days at 20–25 C, rinsed under tap water to remove debris from the medium surface, and examined for presence of colonies of *P. parasitica*.

RESULTS

Cold inactivation. Cold temperature had a direct effect on survival of *P. parasitica* in rhododendron leaf disks (Figs. 1 and 2). Leaf disks on WA in sealed dishes did not desiccate during experiments, although at temperatures below −4 C, WA and leaf disks froze in some dishes. *P. parasitica* survived <3 days at temperatures below −4.5 C. At temperatures between −4 and 0 C, the fungus was not recovered after 6–11 days.

In two experiments, *P. parasitica* was killed within 21 to 33 days at temperatures of 1–2 C. In a third experiment, survival at 1–2 C was 40% after 40 days, when the experiment was terminated. In an experiment comparing survival of *P. parasitica* in leaf disks
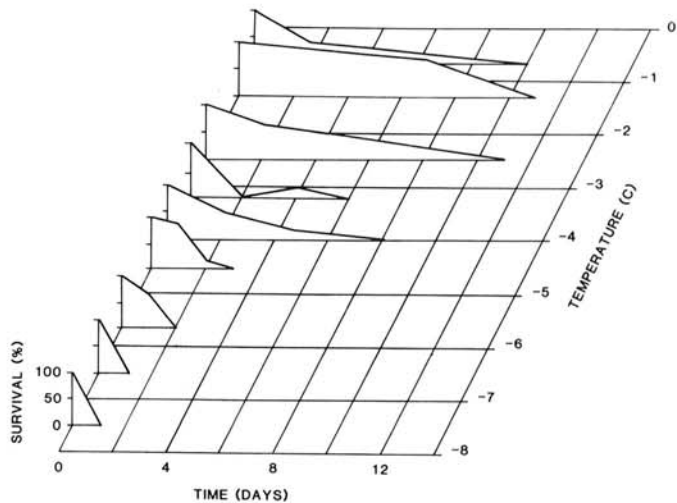

Fig. 1. Survival of *Phytophthora parasitica* in leaf disks in relation to temperatures below 0 C. Fully colonized leaf disks were incubated on water agar in sealed dishes on a thermal gradient plate and then assayed for the presence of *P. parasitica* after various time intervals.
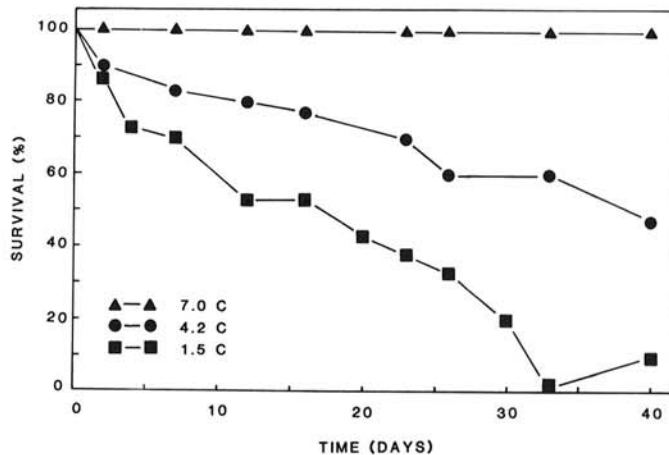

Fig. 2. Survival of *Phytophthora parasitica* in leaf disks of rhododendron at temperatures above 0 C on the thermal gradient plate. Fully colonized leaf disks were incubated on water agar in sealed dishes and assayed over 40 days for the presence of *P. parasitica*.

harvested at different times of the year, the fungus survived 28 to 33 days at 1–2 C in leaf disks harvested in October, but only half as long in leaf disks harvested in July. However, there were no differences in fungal survival between batches of leaf disks at temperatures below 0 C.

P. parasitica survived 27 to 40+ days at 4–5 C. After 40 days, percent survival in two experiments was 60 and 80%. At ≥7 C, the fungus was recovered consistently from 100% of the leaf disks over a 40-day period. Rates of inactivation were similar between the two isolates at all temperatures tested.

P. parasitica in leaf disks was acclimated to cold by incubating colonized leaf disks at 4 C for 3 days before placement of the TGP. No differences in rate of inactivation were observed between cold-acclimated and nonacclimated leaf disks.

Survival in leaf disks. *P. parasitica* survived the winter of 1980–1981 in rhododendron leaf disks buried 5 cm deep in the container base of pine bark at the nursery, but did not survive in leaf disks on the surface of the container base or on the surface of the pine bark potting medium in 2.4-L pots placed on the container base (Fig. 3A). Percent recovery from buried leaf disks was extremely variable within replicates, and at several sampling dates was not statistically significant from leaf disks on the surface of the container base or pots even though the fungus was not recovered from leaf disks on the container base and on the surfaces of pine bark in the pots after the first sampling date (19 December 1980). By 13 January, leaf disks on the container base and surfaces of pine bark in the pots were dried out, but leaf disks buried in the container base remained wet throughout the winter. Percent

recovery of *P. parasitica* declined as buried leaf disks decayed. *Pythium* spp. and saprophytic microorganisms were isolated frequently from buried leaf disks.

Generally, air temperatures 20 cm above the container base fluctuated more than bark temperatures measured 5 cm deep in the container base. During the 3 wk before the first samples were taken, air temperatures reached 0 C or below on 10 days. Air temperature reached 0 C or below on 20 days between 19 December 1980 and 13 January 1981, and was below −7 C 12 times during this period. The lowest air temperature recorded during this time was −12.8 C. Air temperatures reached freezing or below on 76 days during the winter of 1980–1981.

Pattern of inactivation of *P. parasitica* in leaf disks in the nursery was similar during the winter of 1981–1982 (Fig. 3B). Percent recovery from buried leaf disks throughout the winter was greater than 70% after 5–6 mo. Leaf disks on the container base and surfaces of pine bark in the pots dried out within 3 wk after placement in the nursery, and percent recovery declined rapidly. Some leaf disks that had dried out on the surfaces of pine bark in the pots and container base were incubated for 1 wk in moist chambers at room temperatures before they were assayed for *P. parasitica*. This incubation did not enhance recovery of the fungus. After 25 November 1981, *P. parasitica* was recovered from 1.3% of the leaf disks that had been on the surface of pine bark in the pots. Percent recovery from leaf disks on the container base surface was 5% or less after 15 December 1981.

Air temperatures reached 0 C or below on 16 days between 13 November and 15 December 1981 (the first two sampling dates).
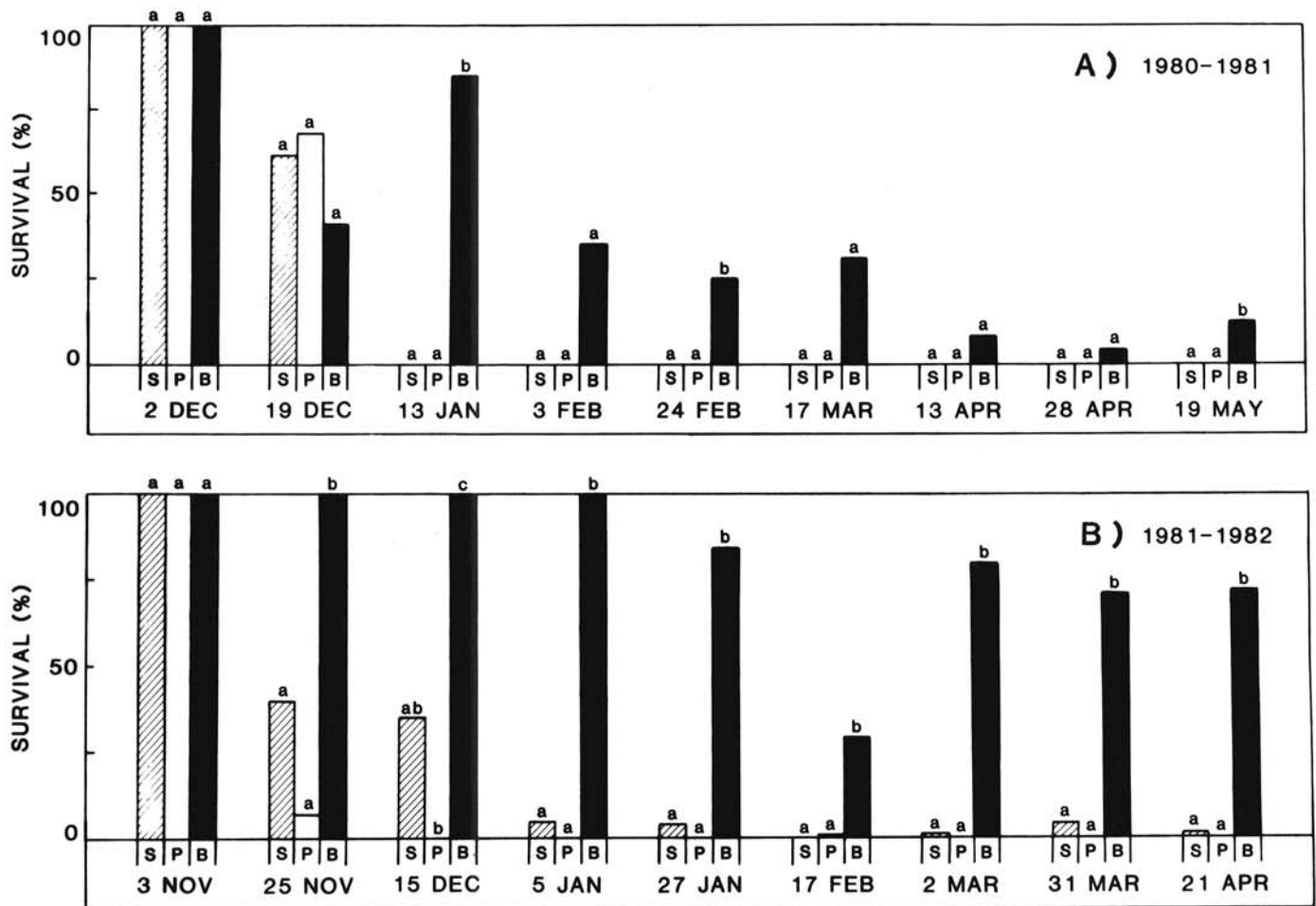

Fig. 3. Percent recovery of *Phytophthora parasitica* from infected leaf disks of rhododendron during **A,** 1980–1981 and **B,** 1981–1982 winter seasons. Infected leaf disks in nylon mesh sacks were placed in a lath house, either on the surface of a container base of pine bark (S), on the surface of pine bark potting medium in 2.4-L plastic pots (P) placed on the container base, or buried 5 cm deep in the container base (B). Leaf disks were placed in the lath house on 2 December 1980 and 3 November 1981, and assayed for recovery of *P. parasitica* every 3 wk. Survival percentages within a sampling date with different letters (above the bar) are significantly different ($P = 0.05$) according to Duncan's new multiple range test.

The low temperature during this time was −5 C. Minimum air temperatures were 0 C or below on 16 days between 15 December 1981 and 5 January 1982. Temperatures were below −7 C on 2 days. The time period between 5 January and 27 January 1982 included 21 days with minimum temperature at or below 0 C; 7 of 21 days were below −7 C. The lowest temperature during the winter of 1981–1982 was −15.6 C, which occurred on two consecutive days. Air temperatures reached freezing or below on 63 days during the winter of 1981–1982. Bark temperatures 5 cm deep in the container base reached 0 C or below only twice (actual values: −1.1 and 0 C) during the 1981–1982 winter.

Survival propagules in leaf disks. Several types of fungal propagules were observed in thin sections of leaf disks buried 5 cm deep in the container base during the winter of 1981–1982. Nonseptate mycelium was observed frequently growing intercellularly in leaf tissue. Papillate sporangia of *P. parasitica* were present on the surface of leaf disks and chlamydospores were observed both on the surface and imbedded in leaf tissue. Several thick-walled, oosporelike propagules were also present, although none could be identified as *Phytophthora*. When leaf disks were ground up and plated on PARP medium after burial in the container base of pine bark from November 1981 to April 1982, several colonies of *P. parasitica* were observed growing from chlamydospores.

Survival in intact plants. *P. parasitica* was recovered from the colonized main stems of at least one of three plants at seven of eight sampling times during the winter of 1981–1982. The fungus was not recovered from lesions on leaves or petioles of the same plants. These plant parts generally became dry and brittle after infection.

P. parasitica was isolated at low frequencies from plant roots sampled during the winter of 1981–1982. After 5 mo of winter conditions in the lath house, *P. parasitica* was recovered from roots of 8 of 24 plants assayed.

Every day for 3 wk in January 1982 (5 January–27 January) the minimum daily temperature reached 0 C or below. *P. parasitica* could not be recovered from infected main stems or roots immediately following this period, but was recovered from both stems and roots at 3-wk intervals thereafter.

Survival in pine bark. *P. parasitica* was first recovered from the container base of pine bark using the leaf disk bioassay on 28 April 1981, 3 mo after the assay was initiated. The fungus was recovered regularly from 20–80% of the container base samples through 6 January 1982. The fungus was not recovered again from the container base until mid-March, when it was isolated from 1 of 200 leaf disks used to assay 20 container base samples. Recovery reached 75% by 8 May 1982. *P. parasitica* was not recovered from the container base samples, taken in January and February 1981, that were incubated in the greenhouse for 12 or 24 days before assaying.

The fungus was never recovered from the container base by plating the bark pieces directly on several selective media. Plates were frequently overrun by *Pythium* spp. as well as several different bacteria inhabiting the pine bark.

DISCUSSION

Phytophthora parasitica did not survive in infected leaf tissue of rhododendron at temperatures below 0 C in the laboratory. Trujillo and Marcley (10) reported that chlamydospores of *P. parasitica* in soil were killed within a few minutes at temperatures below 0 C. Moisture levels at which chlamydospores were maintained were not reported. Holdaway and Tsao (4), examining recovery of a citrus isolate of *P. parasitica* from soils by dilution assays, rarely recovered the fungus after 14 days at 1 C. Less than 50% of the chlamydospores of *P. parasitica* stored in water at 1 C for 4 days were viable (5). Sneh and McIntosh (9) reported that mycelium of *P. cactorum* rapidly lysed when exposed to −7 C. Mycelium and chlamydospores of *P. cinnamomi* are equally sensitive to subzero temperatures (1). In this study, 4 C pretreatment did not acclimate *P. parasitica* to cold temperatures on the TGP, although *P. citrophthora* was acclimated by pretreatment at 4 C and survived to −21 C (3).

On the TGP, *P. parasitica* survived temperatures between 1–2 C (but not at 0 C or below) twice as long in leaf tissue harvested in October than in leaves harvested in July. Leaf tissue removed in the fall had begun to harden-off and apparently provided a better overwintering site at temperatures above 0 C than the more succulent mid-summer leaf tissue.

In the nursery, air and bark temperatures were not consistently below freezing, as they were on the TGP, but the repeated occurrence of subzero temperatures and drying conditions were sufficient to kill *P. parasitica* in leaf disks placed above the container base surface in the nursery during both winters. *P. cinnamomi* did not overwinter in North Carolina in infected root segments of *A. fraseri*, naturally infested soil, or colonized oat grains when soil temperatures (measured at 10 cm deep) dropped to 0 C or below (1).

Desiccation of leaf disks markedly reduced survival of *P. parasitica* in leaf disks placed on the surface of pine bark in the container base or in pots placed in the container base in the nursery. This was confirmed in laboratory studies under controlled environments (8). Similarly, Gerlach et al (3) did not recover *P. citrophthora* from air-dried leaves of *P. japonica*. However, we recovered *P. parasitica* from main stem tissue of intact plants that did not dry out as did other infected plant parts.

The fungus survived both winters buried 5 cm deep in leaf disks in the container base of pine bark at the nursery. Leaf disks under bark remained moist. Occasionally, the top 2–10 cm of the container base froze, but temperatures at 5 cm infrequently dropped to below freezing. *P. cinnamomi* survived under similar conditions in North Carolina (1). The decline in percent recovery of *P. parasitica* over the winter was due in part to decomposition of the leaf disks. A similar decline in recovery has been reported for *P. citrophthora* (3). After 4–5 mo, leaf disks began to break apart, and leaf tissue was increasingly colonized by *Pythium* and saprophytic bacteria.

The fungus could not be recovered from the naturally infested container base during the winter months using rhododendron leaf baits, but was readily recovered during the warmer months. Recovery during the winter was not enhanced by incubating bark samples in the greenhouse for 12 or 24 days before assaying for presence of *P. parasitica*, indicating that the fungus was not surviving in the container base, that survival propagules were in a dormant state, or that sporangia and zoospores were not being formed.

P. parasitica was not recovered from stem or root tissues on intact plants after 3 wk in January 1982 where ambient temperature reached below 0 C every day, but was again recovered after temperatures warmed up in February 1982. Cold temperature may have induced an imposed dormancy on the fungus such that recovery by plating or by baiting was not possible until dormancy was broken by warmer temperatures.

Attempts to identify a survival propagule in the container base by plating bark pieces were unsuccessful. However, mycelium, chlamydospores, and sporangia were observed throughout the winter of 1981–1982 in infected leaf disks buried in the container base. *P. parasitica* can survive for long periods as chlamydospores (4,5). Colonies of *P. parasitica* were observed growing on PARP medium from chlamydospores isolated from buried leaf disks that had wintered in the nursery from November 1981 to April 1982. Mycelium characteristic of *Phytophthora* was observed frequently in buried leaf disks and also may be important in survival of the fungus in leaf tissue. Indirect evidence for mycelial survival of *Phytophthora* spp. has been reported (3). A few sporangia were observed on leaf disks during the winter of 1981–1982, but viability of these spores was not determined. Sporangia formed at low frequency on leaf disks during incubation after inoculation, but prior to placement in the nursery. It is probable, therefore, that sporangia observed on leaf disks were formed during incubation, and contributed little to survival of *P. parasitica* in rhododendron leaf tissue. Sporangia of *P. cactorum* in soil survived temperatures of −8 to −10 C for several hours, but germination of sporangia decreased from 85% to <10% within 8–14 days at temperatures of −4 to −29 C (9). Similarly, no growth was observed from sporangia

of *P. citrophthora* after exposure to temperatures of −21 C (3).

In this study, survival of *P. parasitica* in infected leaf tissue of rhododendron was favored most by temperatures above 0 C and burial in a container base of pine bark where infected leaf tissues remained moist during the winter months.

LITERATURE CITED

1. Benson, D. M. 1982. Cold inactivation of *Phytophthora cinnamomi.* Phytopathology 72:560-563.
2. Benson, D. M., and Jones, R. K. 1980. Etiology of rhododendron dieback caused by four species of *Phytophthora.* Plant Dis. 64:687-691.
3. Gerlach, W. W. P., Hoitink, H. A. J., and Schmitthenner, A. F. 1976. Survival and host range of *Phytophthora citrophthora* in Ohio nurseries. Phytopathology 66:309-311.
4. Holdaway, B. F., and Tsao, P. H. 1971. Survival of *Phytophthora parasitica* in soils. (Abstr.) Phytopathology 61:1321.
5. Holdaway, B. F., and Tsao, P. H. 1972. Longevity of chlamydospores of *Phytophthora parasitica* in water. Plant Dis. Rep. 56:971-973.
6. Kanniwischer, M. E., and Mitchell, D. J. 1978. The influence of a fungicide on the epidemiology of black shank of tobacco. Phytopathology 68:1760-1765.
7. Krober, H. 1980. Üeberdauerung einiger *Phytophthora*-Arten im Boden. (Survival of some *Phytophthora* species in soil.) Z. Pflanzenkrankh. Pflanzenschutz 87:227-235. (In German).
8. Kuske, C. R., and Benson, D. M. 1983. Survival and splash dispersal of *Phytophthora parasitica*, causing dieback of rhododendron. Phytopathology 73:1188-1191.
9. Sneh, B., and McIntoch, D. L. 1974. Studies on the behavior and survival of *Phytophthora cactorum* in soils. Can. J. Bot. 52:795-802.
10. Trujillo, E. E., and Marcley, M. 1967. Effect of soil temperature and moisture on survival of *Phytophthora parasitica* and *Pythium aphanidermatum.* (Abstr.) Phytopathology 57:9.