Survival and Splash Dispersal of *Phytophthora parasitica*, Causing Dieback of Rhododendron

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ABSTRACT

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Phytophthora parasitica survived in air-dried, rhododendron leaves for 1–2 hr. Survival rate rapidly declined after 1–3 days and the fungus could not be recovered after 18–22 days. Sporangium production on leaf disks that had been air-dried prior to placing in water was reduced to 0.2–21% of that of nondried leaf disks. Zoospores were not released from infected leaf disks that were air-dried for 3 hr or more and then flooded. Sporangia and zoospores were formed on infected leaf disks held at a high relative humidity (RH). Maximum zoospore production occurred between 24 and 48 hr in flooded conditions. In nursery studies, the initial incidence of lesions induced on rhododendrons by *Phytophthora* was affected by weather conditions. Even though plants were irrigated daily, rainfall was significantly correlated with lesion incidence from June to mid-September and lesion numbers were greatest after periods of rain preceded by days with several hours of RH ≥90%. Incidence of initial lesion induction by *Phytophthora* on 1- and 2-yr-old plants grown in pots resting on a naturally infested layer of pine bark (called a container base) followed a negative dispersal gradient with height from the base, indicating that propagules of *Phytophthora* were splashed up from the container base onto the leaves of the plants.

Phytophthora dieback is part of a complex of disease symptoms on rhododendron caused by several *Phytophthora* species. In the United States, *P. cactorum* (Leb. and Coyn) Schroet., *P. heveae* Thomp., *P. citricola* Sawada, and *P. parasitica* Dast. (*P. nicotianae* Breda de Haan var. *parasitica* [Dast.] Waterh.) cause foliar lesions on rhododendron (4,9). These species, as well as *P. cinnamomi* Rands, *P. cryptogea* Pethyb. and Laff, *P. lateralis* Tucker and Milb., *P. megasperma* Drechs., and *P. gonapodyides* Petersen can also cause root rot symptoms on rhododendron (4,9). *P. cinnamomi* is the most prominent root rot pathogen in this complex (9). A similar complex involving *Phytophthora* has been reported on *Pieris japonica* (Thunb.) D. Don. in Ohio (7).

Rhododendrons are produced in containerized culture in North Carolina. For weed control in the nursery, plants are placed on a pine bark, gravel, or black plastic base (called a container base). Overhead sprinkler irrigation is common and plants are fertilized regularly to promote lush growth. Under these growing conditions, Phytophthora dieback has become a severe problem in some North Carolina nurseries (4).

Symptoms appear as necrotic lesions on young foliage and stems. Primary dieback lesions form 2–3 days after infection by *Phytophthora* (4). Although mature leaves are not penetrated directly, the pathogen can colonize mature leaves from infected young tissue (4).

Epidemics of Phytophthora dieback depend on the survival of the fungus in infected leaf debris, and the production of sporangia and subsequent zoospore release from this debris. Relative humidity (RH) and moisture affect both sporulation and dissemination of *Phytophthora* species. Conditions of high RH or free water are essential for sporangia development of several *Phytophthora* species (4,8,10,16). Free water, in the form of rain or sprinkler irrigation, enhances the spread of foliar diseases caused by *Phytophthora* spp. (1,8,10,11). Fungal propagules can be disseminated in splash droplets and wind-blown rain (8,10,12,13).

The purpose of this study was to examine the effects of moisture on survival and sporulation of *P. parasitica* in infected rhododendron leaf tissue, and to characterize the role of splashing water in the dissemination of propagules of *Phytophthora* on rhododendrons grown under nursery conditions. A portion of this study has been reported (14).

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MATERIALS AND METHODS

Inoculum production and leaf disk inoculation. Stock cultures of *P. parasitica*, isolate 317, from hybrid rhododendron with dieback symptoms was maintained on cornmeal agar at 25 C. Zoospore inoculum was produced as previously described (15). Leaf disks cut from young leaves of rhododendron cultivar Nova Zembla (1–3 wk after fully expanded) were surface sterilized with 70% ethanol for 1 min, followed by three rinses in sterile distilled water (SDW) and inoculated for use in survival studies as previously described (15).

Effect of drying on fungal recovery. Survival of *P. parasitica* in infected, air-dried rhododendron leaves was examined by incubating the fully colonized, turgid leaf disks (13-mm diameter) in partially open polystyrene boxes and allowing them to dry at 4, 12, 20, and 30 C. Leaf disks were considered air-dried at 20–23% moisture (w/w) compared to turgid leaf disks. At this moisture content, the tissue was curled and brittle. In one experiment, disks were air-dried at room temperature before incubation at the above temperatures. Leaf disks were sampled every 1–3 days for 18–24 days, weighed, and submerged in P_{10}PP agar medium (pimaricin content changed from 100 to 10 mg/L), a modified medium of Eckert and Tsao (6) that is selective for isolation of *Phytophthora*. Growth of *P. parasitica* from leaf disks was recorded 5–7 days later. Three replicates of four to five disks each were sampled at each temperature. Moisture content of air-dried disks was determined as the percent of the weight of the fully colonized and turgid disks before drying.

Effect of drying on sporulation. Sporangium formation of *P. parasitica* in air-dried rhododendron leaf tissue was studied using fully colonized leaf disks (13-mm diameter) placed in partially open polystyrene boxes and incubated as described above. Samples were taken every 1–4 days over 9–21 days. Sporangium formation was induced by rehydrating leaf disks in SDW for 3 days at 25 C. After being stained 1 min with 0.1% crystal violet, sporangia along the periphery of the abaxial surface of the disk were counted in three to six microscope fields (2.95 mm^2 per field) on each of 10–15 disks per

treatment.

Effect of leaf moisture on zoospore production. Sporangium production and subsequent zoospore release were examined on infected rhododendron leaf disks (10-mm diameter) under four moisture regimes, in sterile 90-mm-diameter polystyrene petri dishes (15 leaf disks per dish). Leaf disks were: air-dried (based on percent of weight of turgid disks); air-dried and rehydrated after incubation by flooding with 15 ml SDW for 12 or 24 hr; flooded during incubation with 15 ml SDW; or kept under high RH conditions by placing leaf disks on a wire-mesh rack above moist towels.

Leaf disks were sampled at intervals over 72 hr by counting the number of zoospores released from sporangia produced under the four moisture regimes. Zoospore release was induced by flooding leaf disks with 15 ml of SDW, followed by chilling at 4 C for 15 min, and incubation at 25 C for 1 hr. Leaf disks were then removed from the incubation dishes and water containing zoospores was rinsed into beakers with 10 ml of SDW. Spores adhering to the sides and bottoms of dishes were gently dislodged by using a rubber scraper. Zoospores were stained with cotton blue and six hemacytometer counts were made per treatment. Zoospores released during the initial incubation of leaf disks under the four moisture regimes were counted also and combined with counts from chilled leaf disks. All laboratory experiments were repeated at least twice.

Nursery studies. Incidence of primary dieback lesions on hybrid rhododendron (cultivars Purple Splendour and Chionoides White) was observed in the nursery from 3 June to 11 October 1981. Pots were spaced on 90-cm centers on a layer of pine bark (called a container base) that was naturally infested with several different species of *Phytophthora*. Plants were irrigated twice daily by overhead sprinklers (0.6 cm/day), and fertilized with 100 kg of 16-9-12 slow-release N-P_2O_5-K_2O per hectare to encourage growth of new foliage.

Plants were examined every 2–3 days for Phytophthora-induced lesions. Heights of leaf lesions above the container base were measured. Leaves with lesions were removed after measurement to prevent secondary spread of inoculum. Plants defoliated or killed by *Phytophthora* during the experiment were replaced by plants of the same height class.

Zoospores of *P. parasitica* (3 L of 7,000–10,000 zoospores per milliliter of suspension) were applied to the container base of pine bark in the test site on 14 July and 8 August to increase disease incidence. Plants were irrigated for 1 hr prior to and after applying zoospores to facilitate propagule dispersal and infection.

Phytophthora was isolated from lesions on rhododendron leaves by plating infected leaf pieces on P_{10}PP agar medium. *Phytophthora* species were identified according to the morphological characteristics described by Waterhouse (18).

Rainfall, temperature, and RH data were used to compare effects of these environmental factors on disease incidence.

RESULTS

Effect of drying on fungal recovery. Percent recovery of *P. parasitica* from infected, air-dried leaf tissue decreased over time. Comparisons between temperatures were not made since drying time varied considerably with temperature. Recovery from leaf disks air-dried at room temperature was 92–100% when leaf disks were plated within 1–2 hr of reaching an air-dried condition. *Phytophthora parasitica* was recovered from less than 30% of the leaf disks at all temperatures 1–3 days after tissue reached an air-dried condition. Recovery from air-dried leaf disks incubated at 30 C dropped to 17% or less after 1 day and the fungus was not recovered after 9–15 days. Death of *P. parasitica* in air-dried leaf disks occurred within 18–22 days at all temperatures, although in one test, recovery from leaf disks incubated at 12 C was 10% after 18 days.

Effect of drying on sporulation. Sporangium formation on leaf tissue was significantly reduced after tissue was air-dried. When leaf disks were allowed to slowly air-dry at 4, 12, 20, and 30 C, the number of sporangia produced in air-dried tissue after immersion in SDW for 3 days was reduced to 0.2–21% of the number produced on nondried leaf disks. Once tissue reached air-dried levels, percent sporulation was not significantly different in subsequent samples over several days. Comparisons of spore production were not made between temperatures since drying rates varied. Leaf disks usually reached air-dry levels between 1 and 3 days in incubators at 12, 20, and 30 C, and between 4 and 10 days at 4 C. When fully colonized leaf disks were air-dried at room temperature, sporulation on leaf disks immersed in water 1–2 hr after becoming air-dry was reduced to 16% of sporulation on turgid leaf disks.

Effect of moisture on zoospore production. Zoospore production by *P. parasitica* in leaf disks was affected by moisture (Fig. 1). The fungus profusely formed sporangia when turgid leaf disks were incubated in flooded conditions. After 24 hr, zoospore production in flooded leaf disks was eight times that observed on leaf disks incubated under high RH. After 48 hr, zoospore production under flooded conditions increased to 11.5 times that under high RH conditions. The maximum number of zoospores was produced under flooded conditions after 48 hr at 30 C (61,250 zoospores per milliliter). At 20 C, maximum zoospore release occurred 72 hr after flooding (21,650 zoospores per milliliter). Sporangia formed under high RH conditions released zoospores when flooded and chilled, and zoospore production increased slightly over time. No zoospores were released from leaf disks that were at air-dry levels for 3 hr or more, even when these leaf disks were flooded for 12–24 hr before assaying for zoospore production.

Nursery studies. *Phytophthora parasitica* was isolated from 97% of the over 100 dieback samples plated, and *P. citricola* was isolated from 3% of the samples.

Incidence of lesions from June through September was affected by varying weather conditions (Fig. 2). Daily maximum and minimum temperatures from 1 June to 20 September were within a range conducive to *Phytophthora* development, averaging 31 and 19 C, respectively. During most of this time period, RH was ≥90% for 3–12 hr/day.

Periods of rainfall greatly affected lesion incidence. Correlation of rainfall with lesions observed after 3 days was highly significant ($P = 0.0001$). Relative humidity and cloud cover measured at a National Weather Service site 17 km distant, and maximum and minimum temperatures measured at the nursery were not correlated directly with lesion incidence. However, several hours of RH ≥90%, high percent cloud cover, and lower maximum daily temperatures were significantly correlated with rainfall ($P = 0.01$).

Lesion incidence increased 1–3 days after periods of rain, even though plants were irrigated daily, especially when RH levels prior to rainfall were high. Lesions were first observed 1–3 days after a heavy rain on 7 June 1981. Relative humidity was ≥90% for 6–18

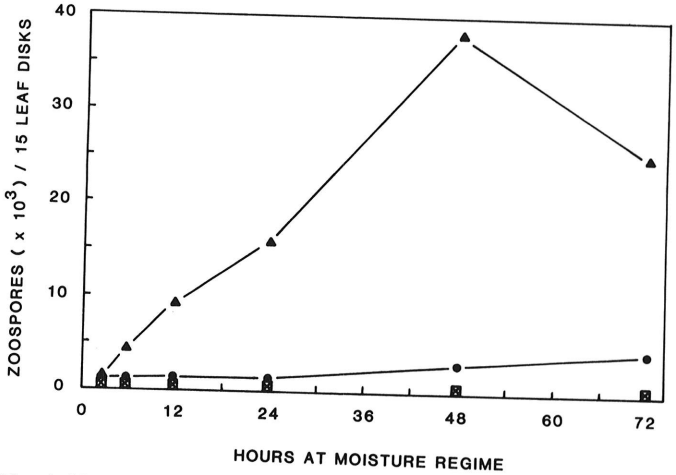

Fig. 1. Number of zoospores of *Phytophthora parasitica* per milliliter released from 15 fully colonized rhododendron leaf disks (10-mm diameter) under four moisture regimes: ▲ = flooded, ● = humid, □ = air dried, × = flooded after air drying. Leaf disks were flooded with SDW at 4 C for 15 min followed by 1 hr at 25 C prior to making zoospore counts. Counts were averaged over temperature.

hr/day the week prior to and following this rainfall. Lesions were also observed following a period of rain from 2–4 July. RH remained below 90% for 9 days prior to rainfall, and lesion incidence was low. Rain occurred every 1–5 days during the period 29 July–25 August, and RH was ≥90% for an average of 8 hr/day. New lesions were observed every 2–3 days during this time.

Zoospores applied to the container base enhanced disease incidence when infestation was followed by rain. Rain occurred 2 and 4 days after infestation on 13 July, and new lesions were observed on 22 July, 4–6 days after rain. Similarly, when the container base was infested on 8 August followed by rain on 8, 10, and 13 August, numerous lesions developed during the following week.

Lesions did not form after 15 September, although plants were examined through 11 October. Minimum daily temperatures were lower and rhododendron foliage began to harden off after mid-September.

Lesions were observed at intervals from 17 to 60 cm above the container base (Fig. 3). Flushes of leaves occurred at approximately 5-cm intervals on plants and the pattern of lesion occurrence mimicked this growth pattern. Accordingly, the data was grouped into 5-cm height classes before plotting. The average height of lesions was 30 cm from the container base. A negative dispersal gradient of lesions with height from the base (Fig. 3) was observed.

Infection by *P. parasitica* was not uniform among plants (Fig. 4). Throughout the season, 125 lesions were observed on twenty 2-yr-old plants while 436 lesions were observed on twenty 1-yr-old plants. Within each plant age class, the number of lesions per plant varied considerably. Seasonal lesion totals per plant ranged from 0 to 15 among 2-yr-old plants and from 3 to 48 among 1-yr-old plants.

DISCUSSION

Survival and sporulation of propagules of *P. parasitica* in infected rhododendron leaf tissue were reduced significantly by desiccation. Similarly, *P. citrophthora*, which causes a foliar blight of *Pieris*, could not be recovered from infected leaves in an air-dried condition (8). Benson and Jones (4) also reported that sporangia of *P. heveae* were not formed on air-dried rhododendron leaves.

Flooded conditions were optimal for both sporangium production and zoospore release by *P. parasitica*. It encouraged spread of dieback disease by allowing inoculum to build up quickly. Some sporangia were produced under conditions of high humidity, but were 8–11.5 times less abundant than on rhododendron leaves under flooded conditions after 24–48 hr. One hundred percent humidity is necessary for sporulation of *P. palmivora*, causing blight of papaya (10). Similarly, Trujillo (17), studying *P. colocasiae* causing Phytophthora blight of Taro, observed that the fungus sporulated best at 100% RH, and sporulation was decreased

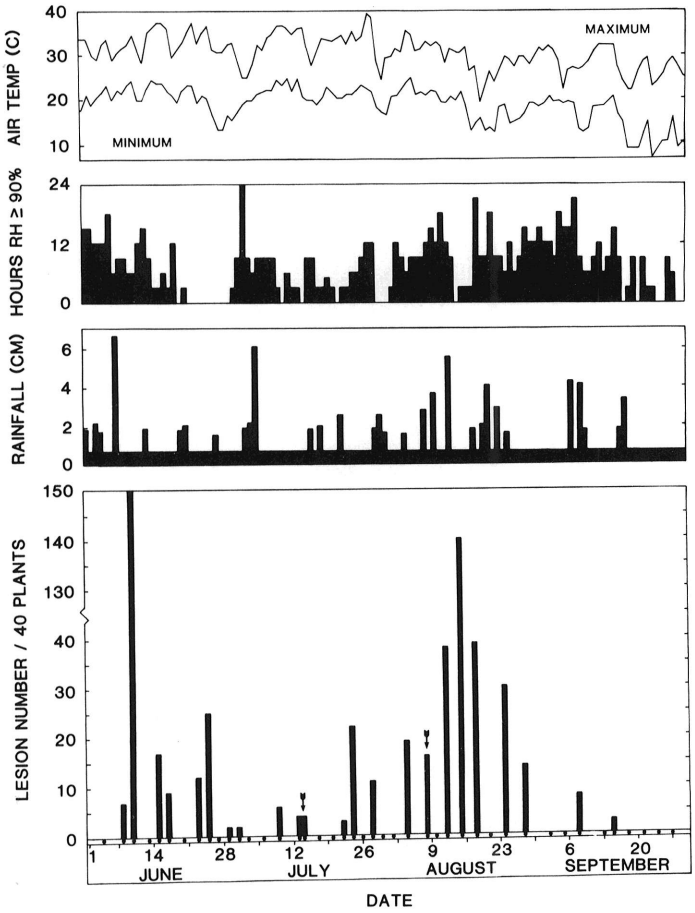

Fig. 2. Relationship weather data and the incidence of lesions caused by *Phytophthora parasitica* on hybrid rhododendron plants. Plants grown in pots were on a naturally infested container base of pine bark. Plants were examined for lesions every 2–3 days from June–October 1981 (indicated by dots). Leaves with lesions were removed to prevent secondary spread of disease. Zoospores of *P. parasitica* (3 L of 7,000–10,000 zoospores per milliliter) were applied to the container base twice, as indicated by arrows.

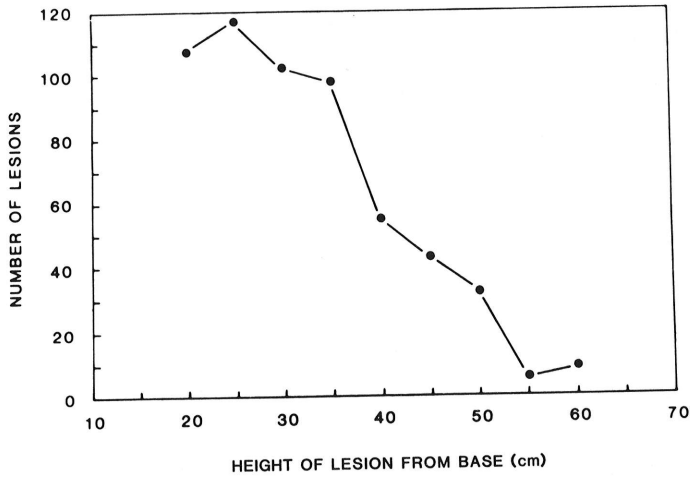

Fig. 3. Number of lesions of *Phytophthora parasitica* occurring on 40 hybrid rhododendron plants grown in pots (17–20 cm tall) on a naturally infested container base of pine bark from June–September 1981. Heights (centimeters) at which lesions occurred on plants were measured from the container base and grouped into 5-cm height classes.

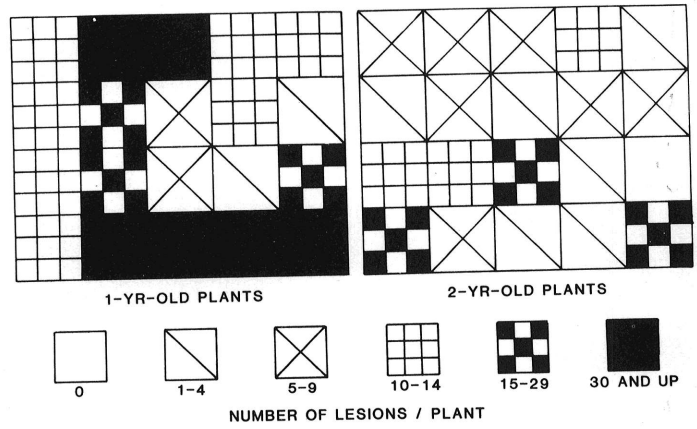

Fig. 4. Distribution of lesions of *Phytophthora parasitica* on 40 hybrid rhododendron plants grown in pots on a naturally infested container base of pine bark. One- and 2-yr-old plants averaged 40 and 63 cm tall from the container base, respectively. Each block represents one plant. Symbols within represent the number of lesions observed on that plant from June–September 1981.

fivefold when RH dropped to 90%.

Incidence of primary lesions caused by *Phytophthora* on rhododendrons grown under nursery conditions on a naturally infested container base of pine bark followed a negative dispersal gradient with height from the base. This relationship indicates that the inoculum source was on or in the container base. The fungus survives readily in abscised leaf tissue in pine bark under moist conditions (15). Abscised leaf tissue was also an important source of inoculum for *P. citrophthora* and *P. ilicis* causing dieback of *Pieris* and *Ilex opaca* Ait., respectively (5,8). Gerlach et al (8) demonstrated that during rainstorms, propagules of *P. citrophthora* were splashed up from colonized leaves of *P. japonica* on the soil surface onto plants, causing dieback infections of foliage. In this study, propagules of *Phytophthora* were splashed up to 60 cm from the infested container base, easily reaching susceptible rhododendron foliage on both 1- and 2-yr-old plants in pots.

Distribution of lesions of *Phytophthora* on plants of the same age and height class was uneven in the test plot. Generally, more lesions occurred on plants near areas that puddled during irrigation rainfall.

Periods of rainfall and sprinkler irrigation enhance conditions that encourage disease to become epidemic. Phytophthora blight of papaya (10), *Euonymus* (11), *Pieris* (8), and *Bougainvillea* (1) all became epidemic during periods of rainfall. In this research, the absence of lesions after the first zoospore infestation and irrigation (13 July), but presence of lesions after a rainfall 8 days later suggests that while daily irrigation may contribute to disease development, rain is much more important to dispersal of propagules of *Phytophthora*.

Lesion initiation was greatest after periods of rain when RH was $\geqslant$90% for several hours each day for several days prior to rainfall. High RH encourages growth and sporulation of *Phytophthora* spp. on infected tissue, and daily irrigation allows periods of wetness in which sporangia production can build up. Lesions of *Phytophthora* were not observed after mid-September. At this time, growth of plants stopped and minimum temperature frequently was below 15 C, a value known to suppress sporulation of *P. parasitica* (2).

Nursery practices, which remove or destroy sources of inoculum of *Phytophthora*, should effectively reduce initial incidence of Phytophthora dieback in commercial rhododendron nurseries. A well-drained container base and timing of irrigation to allow foliage to dry prior to nightfall should reduce the survival and sporulation of *Phytophthora* spp. and protective fungicidal sprays can be applied regularly to the foliage for control (3).

LITERATURE CITED

1. Alfieri, S. A., Jr. 1970. Bougainvillea blight: A new disease caused by *Phytophthora parasitica*. Phytopathology 60:1806-1808.
2. Aragaki, M., and Hine, R. B. 1963. Effect of radiation on sporangial production of *Phytophthora parasitica* on artificial media and detached papaya fruit. Phytopathology 53:854-856.
3. Benson, D. M. 1980. Chemical control of rhododendron dieback caused by *Phytophthora heveae*. Plant Dis. 64:684-686.
4. Benson, D. M., and Jones, R. K. 1980. Etiology of rhododendron dieback caused by four species of *Phytophthora*. Plant Dis. 64:687-691.
5. Buddenhagen, I. W., and Young, R. A. 1957. A leaf and twig disease of English holly caused by *Phytophthora ilicis* n. sp. Phytopathology 47:95-101.
6. Eckert, J. W., and Tsao, P. H. 1962. A selective antibiotic medium for isolation of *Phytophthora* and *Pythium* from plant roots. Phytopathology 52:771-779.
7. Gerlach, W. W. P., and Hoitink, H. A. J., and Ellett, C. W. 1974. Shoot blight and stem dieback of *Pieris japonica* caused by *Phytophthora citricola, P. citrophthora*, and *Botryosphaeria dothidea*. Phytopathology 64:1368-1370.
8. Gerlach, W. W. P., Hoitink, H. A. J., and Schmitthenner, A. F. 1976. *Phytophthora citrophthora* on *Pieris japonica*: Infection, sporulation, and dissemination. Phytopathology 66:302-308.
9. Hoitink, H. A. J., and Schmitthenner, A. F. 1974. Relative prevalence and virulence of *Phytophthora* species involved in rhododendron root rot. Phytopathology 64:1371-1374.
10. Hunter, J. E., and Kunimoto, R. K. 1974. Dispersal of *Phytophthora palmivora* sporangia by wind-blown rain. Phytopathology 64:202-206.
11. Keim, R., Klure, L. J., and Zentmeyer, G. A. 1981. A foliage blight of euonymus caused by *Phytophthora*. Calif. Agric. 35:16-17.
12. Kienholz, J. R. 1946. *Phytophthora* rot of pears under sprinkler irrigation at Hood River, Oregon. Plant Dis. Rep. 30:30-31.
13. Kliejunas, J. T., and Ko, W. H. 1976. Dispersal of *Phytophthora cinnamomi* on the island of Hawaii. Phytopathology 66:457-460.
14. Kuske, C. R., and Benson, D. M. 1982. Splash dispersal and cultural control of *Phytophthora parasitica* causing dieback of rhododendron. (Abstr.) Phytopathology 72:359.
15. Kuske, C. R., and Benson, D. M. 1983. Overwintering and survival of *Phytophthora parasitica* causing dieback in rhododendron. Phytopathology 73:1192-1196.
16. Kuske, C. R., Benson, D. M., and Jones, R. K. 1983. A gravel container base for control of Phytophthora dieback in rhododendron nurseries. Plant Dis. (In press).
17. Trujillo, E. E. 1965. The effects of humidity and temperature on Phytophthora blight of taro. Phytopathology 55:183-188.
18. Waterhouse, G. M. 1963. Key to the species of *Phytophthora* de Bary. Commonw. Mycol. Inst., Mycol. Pap. 92. 22 pp.