# Predisposing Effects of Soil Moisture Extremes on the Susceptibility of Rhododendron to Phytophthora Root and Crown Rot

N. S. Blaker and J. D. MacDonald

Graduate student and assistant professor, respectively, Department of Plant Pathology, University of California, Davis 95616.
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## ABSTRACT

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One-year-old rhododendrons were subjected to various soil-water regimes and inoculated with motile zoospores of *Phytophthora cinnamomi*. In the absence of stresses due to drought or flooding, plants of cultivar Purple Splendour developed severe root and crown rot following inoculation with $10^4$ or $10^5$ zoospores per plant, whereas the cultivar Caroline remained free from symptoms and was relatively resistant. However, if Caroline plants were drought stressed to leaf water potentials of $\leq -16$ bars, or their roots were flooded for 48 hr before inoculation with *P. cinnamomi*, they developed severe symptoms of root and crown rot. Soil moisture extremes that commonly occur both in nursery and landscape plantings can predispose normally resistant rhododendrons to root and crown rot caused by *P. cinnamomi*.

Root and crown rot of *Rhododendron* spp., which is caused primarily by *Phytophthora cinnamomi* Rands (7), is considered the most serious disease of rhododendrons in the United States (8,13). Serious disease losses can occur in both nursery and landscape plantings (5,13) and, although it usually affects 1- to 3-yr-old rhododendrons (13,20), older plants also may be infected and killed, especially following periods of excessive rainfall if planted in poorly drained soil (13).

Since the first report of Phytophthora root and crown rot of rhododendron in 1930 (20), research has focused on the development of various controls (6,9,20). Recently, a large number of *Rhododendron* species and hybrids were screened for susceptibility to *P. cinnamomi* (8) and some were found to be relatively resistant. However, this resistance has not always held up; rhododendrons considered to be resistant were found severely diseased in a nursery planting (15).

In nurseries, plant roots can be exposed to many forms of environmental stress, flooding and drought being among the most common. Such stresses result from the difficulty of achieving precise irrigation management in containerized or field-grown crops and both forms of stress have been reported to predispose agronomic plants to Phytophthora root rots (3,12).

The purpose of this study was to determine whether the flooding and drought stresses that frequently occur in nurseries and landscape situations could affect the development or severity of Phytophthora root and crown rot of a woody ornamental such as rhododendron.

## MATERIALS AND METHODS

**Inoculation procedure.** One-year-old rhododendron plants of two cultivars, Caroline and Purple Splendour, were obtained from a commercial nursery. Cultivar Caroline is reported to be highly resistant to Phytophthora root rot and cultivar Purple Splendour is reported to be extremely susceptible (8). The plants were grown in 15-cm-diameter plastic pots containing steamed UC soil mix (16) for a minimum of 8 wk before their use in experiments. All plants were maintained in a greenhouse and were watered daily with deionized water except when water was an experimental variable. Every 6–8 wk the plants were fertilized with a commercial-grade fertilizer formulated for ericacous plants (Best® Rhododendron, Azalea, and Camellia Food, 8-12-6; Occidental Chemical Co., Houston, TX 77027).

An isolate of *P. cinnamomi* pathogenic to rhododendrons (isolate 544, from H. A. J. Hoitink, Ohio Agric. Res. and Development Center, Wooster 44691) was used in inoculation trials. Plants were inoculated by adding a measured number of motile zoospores to the soil in each pot. Zoospores were obtained by cutting 7-mm-diameter agar disks from the margins of 3- to 4-day-old colonies grown in cleared V-8 agar, and placing them in a cleared V-8 broth (100 ml of V-8 juice, 1 g $CaCO_3$, and 900 ml of distilled water) for 24 hr. Abundant sporangium formation was induced by removing the disks from the V-8 juice broth, rinsing them three times with distilled water and incubating them (~10 disks per dish) in the dark for 3–4 days at 25 C in petri dishes containing 20 ml of 2% nonsterile soil extract. The sporangia were induced to release zoospores by chilling the petri dishes containing the disks to 9 C for 1 hr, rewarming them to 25 C, and allowing ~1 hr for release. Zoospores were separated from the mycelial disks by filtration through four layers of cheesecloth and immediately transferred to the plants. In the standard inoculation, the plants were inoculated by first wetting the soil to saturation with distilled water and then applying a measured volume of suspension, containing a total of $10^4$ or $10^5$ motile zoospores, evenly to the soil surface around each plant. The soil was allowed to drain briefly to assure zoospore entry into the root zone after which pots were placed in watertight containers and additional water was added to fully saturate the soil. Saturation was maintained for 24 hr after inoculation to maximize the opportunity for motile zoospores to contact the plant roots. Following 24 hr of saturation, the soil was allowed to drain freely and the daily watering regime was resumed. Uninoculated control plants of each cultivar were treated identically, except that distilled water was added in place of the zoospore inoculum.

A subjective rating system, based on severity of wilt and canker development, was used to assess disease development. Plants with symptoms were rated on a scale of 0–5: 0 = no visible symptoms, 1 = youngest flush of leaves slightly wilted, 2 = both youngest leaves and lower leaves wilted, 3 = leaves wilted and stem canker $\leq$ 2 cm above soil line, 4 = plants severely wilted and stem canker $>$ 2 cm above soil line, and 5 = dead plant. Data were analyzed by one-way analysis of variance.

**Effect of drought stress.** In experiments to test the effects of drought, water was withheld from groups of plants for 8–10 days until they wilted, while a similar group of plants received daily

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irrigation. At 0900 hr on the day of inoculation, a fully expanded leaf from the penultimate flush of leaves was removed from each plant and placed in a pressure chamber to estimate leaf water potential ($\Psi$) (1). The leaf $\Psi$ of nonstressed plants ranged from $-2$ to $-6$ bars $\Psi$. Wilted plants with $\Psi$ values of $-16$ to $-20$ bars were selected for use as drought stressed plants. A total of six plants was selected for each group. All plants were irrigated 1 hr after the leaf $\Psi$ measurements, and inoculated approximately 1 hr after irrigation. As before, the soil remained saturated for 24 hr following inoculation with zoospores. Groups of uninoculated stressed and nonstressed plants served as controls. Following the inoculation procedure, all plants were returned to a normal irrigation schedule and observed for symptoms of disease development.

**Effect of flooding stress.** To expose plants to flooding stress before inoculation, the pots in which they were growing were placed in watertight containers with sufficient water added to maintain 0.5 cm of standing water on the soil surface. Five plants in each treatment were held under continuously flooded conditions for 24 or 48 hr, after which time they were removed from the watertight containers, allowed to drain freely, and inoculated immediately by adding motile zoospores as described above. Five plants not previously flooded also were similarly inoculated at the same time. Following the addition of zoospores, all plants were held in saturated conditions for 24 hr as described previously and then returned to a normal irrigation regime to observe disease development. An equal number of uninoculated plants were exposed to identical flooding periods to serve as controls.

## RESULTS

In the absence of a drought or flooding stress prior to inoculation, the two rhododendron cultivars differed in susceptibility to root and crown rot. For example, when plants were inoculated with $10^4$ zoospores of *P. cinnamomi* per plant, those of cultivar Purple Splendour had typical root and crown rot symptoms (Fig. 1) within 2–3 wk, whereas those of cultivar Caroline were not visibly affected by the pathogen. An increase in the inoculum concentration from $10^4$ to $10^5$ zoospores per plant increased both the severity of disease and the rate of symptom development in Purple Splendour plants (Fig. 2), but had no effect on those of Caroline. Although Caroline (Fig. 2) plants had no symptoms, the pathogen was readily reisolated from within root tissue, indicating that it was capable of infecting or at least persisting in the roots of this cultivar.

**Effect of drought stress.** When plants of the highly susceptible cultivar Purple Splendour were inoculated at the time of stress relief, both the drought stressed and nonstressed plants developed obvious disease symptoms in as little as 6 days and all were killed within 35 days (Fig. 3). Thus, there was no distinguishable effect of the stress on the incidence or severity of disease. Disease development in the more resistant Caroline, however, was greatly affected by drought stress. In the previously stressed plants, disease symptoms became evident between 21 and 35 days after inoculation and steadily increased in severity over the remaining period of the experiment (Fig. 3). In contrast, the nonstressed Caroline plants remained symptomless the entire 90 days. Although the Caroline plants were rendered susceptible to disease by drought stress, the severity and rate of symptom development in stressed Caroline plants were considerably less than in those of Purple Splendour.

**Effect of flooding stress.** Submersion of the root systems of Purple Splendour plants in water for either 24 or 48 hr prior to the inoculation treatment again had no distinguishable effect on the rate of subsequent disease development; ie, the inherent susceptibility of the cultivar invariably allowed rapid and severe disease development in all the inoculated treatments (Fig. 4A). Statistical analysis of the effect of prior flooding on disease development in Purple Splendour plants showed no significant differences between the level of disease in those that had received any of the treatments. In contrast, Caroline plants showed a significant response to the preinoculation flooding treatment (Fig. 4B). While a preinoculation flooding of only 24 hr resulted in a slight increase in disease in comparison to plants which were not flooded prior to inoculation, the difference was not statistically significant. However, Caroline plants that had been flooded for 48 hr prior to inoculation rapidly developed severe disease symptoms severe disease symptoms (Fig. 4B).

## DISCUSSION

In the absence of stress, plants of rhododendron cultivars Purple Splendour and Caroline differed greatly in susceptibility to root and crown rot caused by *P. cinnamomi* (8). However, our results

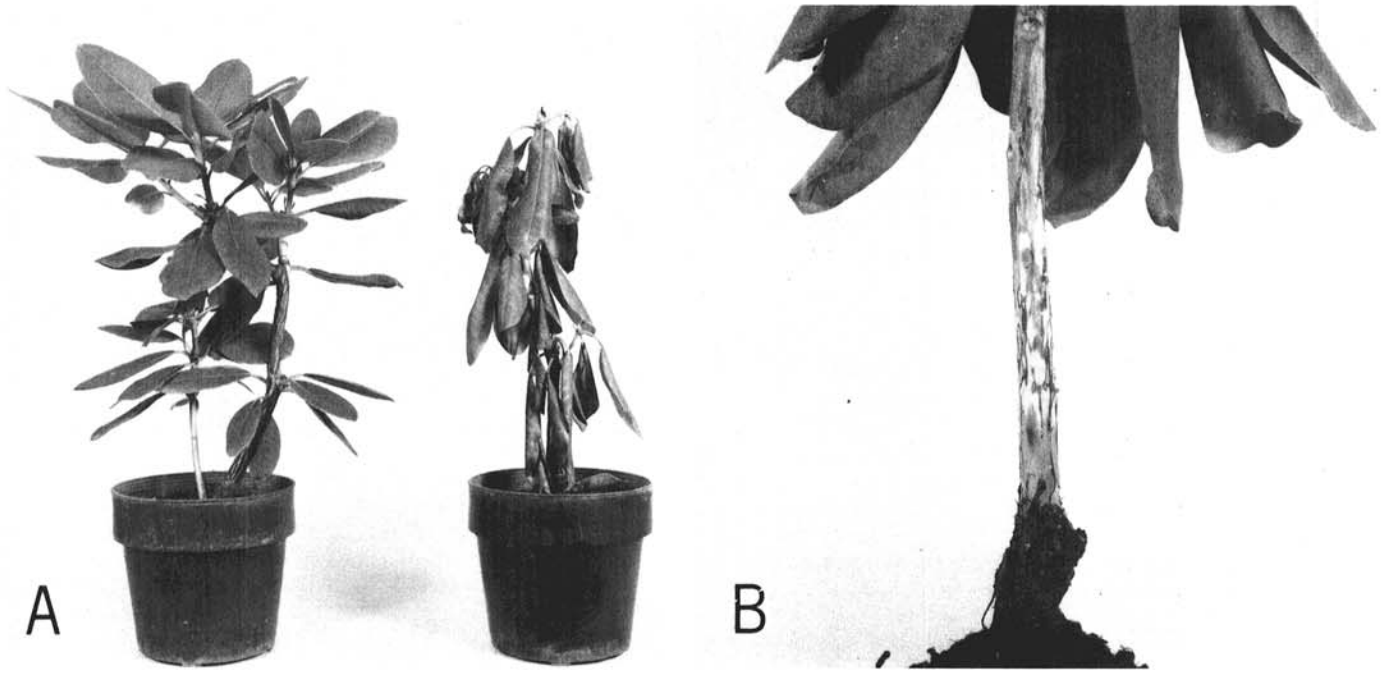


**Fig. 1.** Symptoms of Phytophthora root and crown rot on rhododendron. **A,** Healthy plant on the left with a symptom rating of 0 and severely diseased plant on the right with a symptom rating of 5 (see text for explanation of rating system). **B,** Close-up of severely diseased plant with bark removed showing a stem canker extending above the soil line.

demonstrated that the resistance usually present in Caroline was reduced by episodes of either drought or flooding stress. The levels of stress that reduce resistance were not considered excessive due to the rapid and complete recovery of uninoculated plants of both cultivars following resumption of normal irrigation. In fact, the levels of stress employed in these experiments are considered typical of the seasonal stresses to which nursery crops are sometimes exposed. Because drought and flooding stresses acting on the host prior to inoculation increased the severity of disease, they are considered to be examples of predisposition.

Although drought and flooding stresses have been implicated in the development of Phytophthora root rot on other plant species, this is the first time both stresses have been clearly shown to predispose a woody ornamental to Phytophthora root and crown rot. Duniway (3) showed that safflower plants could be predisposed to root rot caused by *P. cryptogea* by a single episode of water stress, and that water stress was capable of reducing the effectiveness of genetic resistance in the cultivar Biggs. At the opposite extreme of soil moisture, Kuan and Erwin (12) found that the longer alfalfa plants were held under flooded conditions, the more severe were the symptoms induced by *P. megasperma*. The predisposing effect on alfalfa could be observed following flooding intervals as short as 24 hr. Phytophthora root rot frequently has been associated with excessive soil moisture and poorly drained soils. Although this association generally was attributed to the effect of high soil moisture on the biology of the organism (4), our study and the work of Kuan and Erwin (12) demonstrate that a host component may be involved when increased disease results from saturated soil conditions.

The mechanisms by which these stresses operate to predispose plants are not clearly understood. In experiments with Phytophthora root rot of citrus, Stolzy et al (17) found that the length of time that the soil was saturated was more important than the number of saturation episodes. Plants whose roots were subjected to 8-hr saturation periods three times per month or grown in $O_2$-deficient atmospheres, were considered less able to withstand the effects of root rot due to impaired generation and growth of new roots. Although impaired regeneration of roots may be important when plants are held in chronically $O_2$-deficient conditions, this hypothesis cannot explain the results of our experiments in which plants were subjected to a single flooding episode and, after inoculation, were returned to a normal irrigation schedule. Thus, shortly after the introduction of the pathogen, the soils drained freely and were adequately aerated (16).

Although soil $O_2$ was not limiting in the postinoculation period in these experiments, $O_2$ deficiency in the rhizosphere prior to inoculation was felt to be a contributing factor in the observed predisposition. Under conditions of high transpirational demand that occurred in the greenhouse, some of the plants held in flooded soils for 48 hr prior to inoculation actually wilted, suggesting severe $O_2$ deficiency in the roots (2). Following inoculation, it was observed that such plants suffered much more rapid and serious disease development than those flooded for similar periods of time that did not wilt.

A second hypothesis concerning the effect of $O_2$ stress was developed by Kuan and Erwin (12). They found that alfalfa roots retrieved from flooded soils exuded great quantitites of sugars and amino acids, and attracted greater numbers of zoospores than nonflooded roots. They attributed the greater exudation of chemotactically active substances from roots under such conditions to an impaired functioning of root cell membranes under $O_2$-deficient conditions, and hypothesized that the predisposition of alfalfa resulted from an enhanced attraction of zoospore inoculum to flooded roots. Injuries to root cell membranes also can be caused by drought stresses and may result in increased root exudation (11,19), but there is no direct evidence to indicate whether this results in increased zoospore attraction.

The extremely fine, fibrous nature of rhododendron roots made it difficult to obtain root samples from the plants and to observe zoospore attachment in the various stress treatments. However, our attempts to cause disease in plants of Caroline by increasing inoculum density were not successful, which suggests that factors other than attraction of inoculum also may be involved in predisposition. Oxygen deficiency and drought stress can disrupt many metabolic pathways in plants (2,10,14,18), and could function in predisposition by impairing a plant's ability to resist pathogenic invasion. Unfortunately rhododendron was not considered suitable for a detailed examination of such questions.

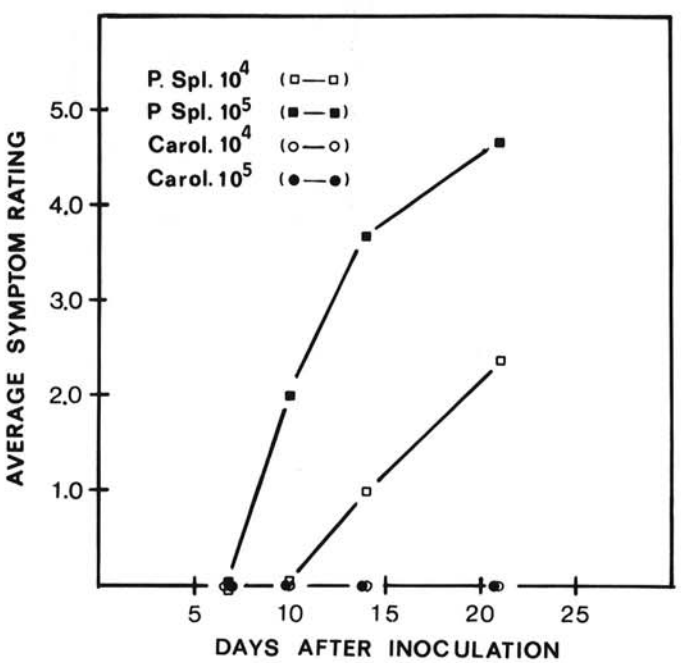


**Fig. 2.** Effect of inoculum concentration on development of root and crown rot in the rhododendron cultivars Purple Splendour (P. Spl.) and Caroline (Carol.). Plants grown in the absence of stress were inoculated with $10^4$ or $10^5$ motile zoospores of *Phytophthora cinnamomi* as described in the text, and disease symptoms were rated on a scale of 0 (healthy plant) to 5 (dead plant). Averages were based on three replications and the least significant difference at $P = 0.05$ was 1.75.

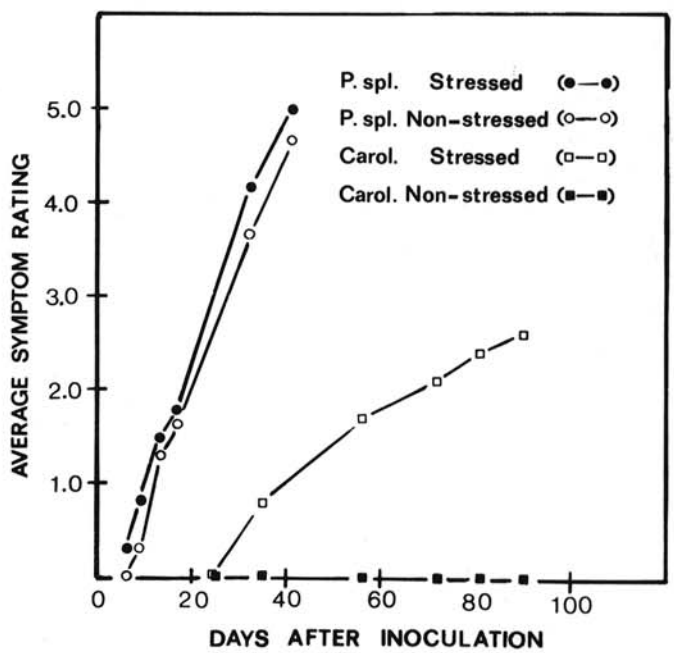


**Fig. 3.** Effect of a preinoculation drought stress on the development of Phytophthora root and crown rot in rhododendron cultivars Purple Spendour (P. Spl.) and Caroline (Carol.). Stressed plants had leaf water potentials ($\Psi$) ranging from −16 to −20 bars, while nonstressed plants had $\Psi$ values ranging from −2 to −6 bars. Disease symptoms were rated on a scale of 0 (healthy plant) to 5 (dead plant). Averages were based on six replications and the least significant difference, $P = 0.05$, was 1.57.

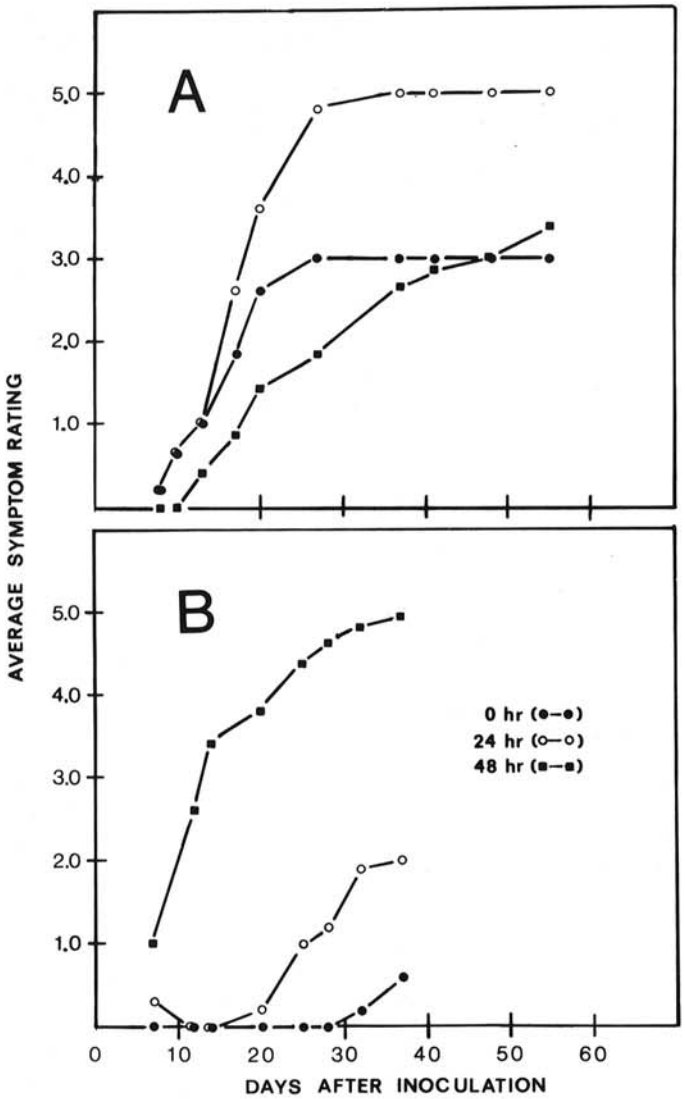


**Fig. 4.** Effect of preinoculation flooding on the development and severity of Phytophthora root and crown rot of the rhododendron cultivars **A,** Purple Splendour and **B,** Caroline. Plants were held under flooded soil conditions for 0, 24, or 48 hr prior to inoculation with zoospores of *Phytophthora cinnamomi*. Disease symptoms were rated on a scale of 0 (healthy plant) to 5 (dead plant). Averages were based on five replications and at $P = 0.05$, there was no significant difference between plants that received flooding treatments in Purple Splendour, while in Caroline, the least significant difference was 2.36.

It is evident from our experiments that opposite extremes of soil moisture, which occur periodically in the nursery and landscape environment, can predispose an otherwise resistant rhododendron cultivar to Phytophthora root rot. Knowledge of such factors not only could be important in the production and maintenance of high-quality plants in the nursery, but also in methods of artificial inoculation used to detect resistance. Although the nature of the root rot resistance observed in rhododendrons (8) is not well characterized, it must be stable enough to withstand stresses typical of the nursery environment before it will have a significant impact on the development of new cultivars.

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