Episodic Abiotic Stress as a Potential Contributing Factor to Onset and Severity of Disease Caused by *Phytophthora ramorum* in *Rhododendron* and *Viburnum*

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ABSTRACT

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Phytophthora ramorum attacks many forest and nursery species, primarily causing trunk or stem cankers, foliar blight, and dieback, and in some species root infection has been demonstrated. However, the abiotic and edaphic factors that influence infection and disease development are unresolved. Root infection by *P. ramorum* and the potential for mild abiotic stress in disease predisposition was examined with *Rhododendron* hybrid Cunningham's White and *Viburnum tinus* cv. Spring Bouquet. To impose water stress in a uniform and synchronous manner, osmotic stress induced with 0.2 M NaCl was selected. Roots were exposed to NaCl for 16 to 24 h in modified hydroponic culture or standard potting soil, removed from the NaCl, and then inoculated with zoospores. In the hydroponic regime, disease symptoms developed in *Rhododendron* and *V. tinus* plants within 1 week after inoculation of salt-stressed roots, whereas symptom development was delayed in nonstressed, inoculated plants. Microscopic examination of roots from both species revealed that their apices were covered with sporangia of *P. ramorum*. On potted *Rhododendron* plants inoculated by applying zoospores directly to the soil, stem lesions developed rapidly in salt-stressed plants, with death of the plant occurring within 4 weeks after inoculation. Nonstressed plants survived for 6 to 8 weeks before succumbing to disease, and symptom development in these plants was delayed by 1 to 2 weeks relative to the inoculated, salt-stressed plants. A postinfection episode of salt stress to inoculated roots in the hydroponic regime resulted in significantly faster development of stem lesions in *Rhododendron* relative to nonstressed, inoculated plants.

Sudden oak death (SOD), or ramorum blight, is an emergent disease of mostly woody plants along the Pacific coast of North America (20). The primary species of economic and ecological importance are red oaks (*Quercus*, section *Erythrobalanus*), tanoaks (*Lithocarpus densiflorus*), California bay laurel (*Umbellularia californica*), and various ornamental and landscape plants such as *Rhododendron*, *Camellia*, and *Viburnum* species. The causal agent initially was isolated from *Rhododendron* and *Viburnum* in Europe in 1993 and was characterized as *Phytophthora ramorum*, a new species, in 2001 (45). In North America, *P. ramorum* infection of nursery plants has produced immense economic and regulatory consequences (38). Within California ecosystems, this pathogen appears to be most damaging to coast live oak, *Quercus agri-*

folia, and tanoak, both of which are keystone species in coastal forests (11,36,37). Lethal girdling cankers can be found near the base of large coast live oak trees, and anywhere on the main bole and branches of tanoaks of any age.

Unlike in many other host species, the *P. ramorum* cankers in live oak and tanoak do not appear to extend below the soil line. Infections of California bay laurel mainly result in nonlethal leaf lesions, which can provide a primary source of inoculum for other species. Here, too, natural infections of *P. ramorum* appear to be largely restricted to the foliage or aboveground portions of the plant. However, in the past several years it has become apparent that root infections occur, as the pathogen can be isolated from roots of naturally infected trees (34) and has been isolated from roots and associated soil from other host species, in some cases with little or no symptom expression (2,35,40,46).

Artificial inoculation of roots of *Rhododendron* sp. and other species with *P. ramorum* indicates that roots are indeed susceptible and the pathogen moves readily into shoots (35,39,40). Thus, there is strong evidence for the capacity of *P. ramorum* to attack both roots and shoots in some species, but why the propensity for one over the other in a given context is unclear. *P. ramorum* also is reported to

cause asymptomatic infections, but how these infections become active is largely speculative (13).

For all plant diseases caused by *Phytophthora* spp., water status of the host and the environment critically influence disease occurrence and severity (18,19). Water potential influences pathogen behavior and the formation and activity of infective propagules (18). However, water stress and other abiotic factors, including transient episodes of stress such as soil salinity and root anoxia, affect root physiology to directly compromise host resistance, making roots and, in some cases, shoots more vulnerable to disease (7). Cycles of soil moisture extremes dramatically impact the severity of root and crown rot of *Rhododendron* caused by *Phytophthora cryptogea* (3), and water stress imposed by deficit irrigation or salinity has a similar effect in a number of other *Phytophthora*-plant interactions (4,17,26,27).

In light of evidence of a role for root infections in the disease cycle of ramorum blight (35,40), together with the possibility that such infections may remain asymptomatic, a better understanding of specific edaphic and abiotic factors that contribute to the soilborne phase will inform management and risk assessment for this important disease. Such factors may strongly influence the physiology of both host and pathogen, and thus could have a strong effect on inoculum thresholds necessary for infection, the extent and significance of root infection in various hosts (40), the consistency/reliability of pathogenicity tests for the assessment of host range and cultivar resistance, and the efficacy of chemical treatments to manage disease (25,44).

In this study, roots of *Rhododendron* sp. and *Viburnum tinus*, two important ornamental hosts that are affected by ramorum blight in nurseries and landscapes, were infected by zoospores of *P. ramorum*, giving rise to running lesions extending into stems and leaves. To determine if an experimentally imposed abiotic stress can enhance disease development caused by *P. ramorum*, we selected brief osmotic stress by exposing roots to NaCl. Salt stress was selected because of the ease of imposing and removing the stress in a uniform and synchronous manner. We examine how osmotic stress to roots prior to inoculation can predispose plants to strongly enhance symptom development caused by *P.*

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ramorum, and discuss this in relation to the potential for abiotic root stress to activate asymptomatic infections. A preliminary report of this work has been published (39).

MATERIALS AND METHODS

Experimental formats, plants, and stress regimes. Liners of *Rhododendron* hybrid Cunningham's White and potted plants of *V. tinus* cv. Spring Bouquet were donated by Monrovia Nursery Co. (Azusa, CA) and Hines Nurseries, Inc. (Winters, CA), respectively. Plants were maintained in their original container or transferred to 4-in pots in a temperature-controlled greenhouse until used in experiments. *Rhododendrons* were maintained in Sunshine Pro Premium potting soil (Sun Gro Horticulture Canada CM Ltd.), and *V. tinus* plants were maintained in a steamed "UC Mix" type potting soil consisting of equal parts of composted redwood, #2 washed sand, peat moss, and white pumice, and approximately 1 kg each of oyster shell calcium and dolomite lime per cubic meter.

Experiments were conducted in two formats: a modified hydroponic culture, and in potted plants in soil. For hydroponic experiments, *Rhododendron* and *V. tinus* plants maintained in the greenhouse were removed from their pots and the roots gently rinsed with DI water to remove soil particles. In initial experiments, plants were placed into 200-ml beakers containing 50 ml of 0.5× Hoaglands solution for 2 days before exposing them to treatments. The plants were carefully positioned such that only the roots were immersed to minimize contact of the lower stems with the treatment solutions. In later experiments, the stems at the base were wrapped with foam strips to provide a tight seal to support the plants in 50-ml Falcon tubes, with only the roots in contact with the solution. Plants were exposed to the following four treatment regimes: (i) non-inoculated; (ii) inoculated; (iii) salt-stressed, noninoculated; and (iv) salt-stressed, inoculated. Plants in hydroponic culture and selected for salt treatment were transferred to beakers or Falcon tubes to expose the roots to 0.2 M NaCl/0.02 M CaCl₂/0.5× Hoaglands solution (50 ml) for 16 h, rinsed in 0.5× Hoaglands solution, and then returned to 0.5× Hoaglands solution. Potted plants were treated with 50 ml of 0.2 M NaCl/0.02 M CaCl₂ for 16 h, and then flushed with DI water to reduce the salt in soil solution. Nonstressed controls in both experimental formats were similarly treated, but without NaCl/CaCl₂ in the treatment solutions.

Plants in the hydroponic regime were maintained at room temperature (25 ± 2°C) under fluorescent lamps in the laboratory. Potted plants were maintained in a controlled access growth chamber at 22°C with 12-h day length and 70% relative

humidity under fluorescent and incandescent lights at 150 µmol m⁻² s⁻¹.

Inoculation and evaluation methods. Two isolates of *P. ramorum* from the UC Davis collection maintained by D. M. Rizzo were used, #264 isolated from California bay laurel leaves, Marin County, CA, in 2002, and #397, isolated from *Ranunculus* spp., Sonoma County, CA, in 2003. These isolates were chosen because both were found to be pathogenic on *Rhododendron* and *V. tinus*, and both grow and sporulate well in culture.

For the modified hydroponic culture experiments, roots were inoculated by placing them in 0.5× Hoaglands solution containing zoospore suspensions of *P. ramorum* at a final concentration of 2×10^2 , 2×10^3 , or 2×10^4 zoospores/ml. Potted plants were inoculated by pouring 50 ml of 0.5× Hoaglands solution containing 2×10^3 or 2×10^4 zoospores/ml at the base of each plant.

Disease was assessed by measuring the length of the lesion along the main stem from the base or soil line and expressed directly as lesion length or as a percentage of the total stem length from the base/soil line of the plant to the tip of the apical meristem. The latter assessment (% stem lesioned) was determined by digital image analysis of stems with the aid of APS Assess software. Percent stem lesioned was used for some experiments in the final analysis because of slight variation in the size of plants and because some individual plants within a treatment had lesions that involved the entire length of the stem prior to the termination of an experiment (generally 3 weeks after inoculation). Reisolation of the pathogen from the leading margin of the lesion on pimarcin-ampicillin-rifampicin-PCNB (PARP) selective medium (22) indicated that this measure provided a good estimate of pathogen colonization and disease. In all initial experiments, the presence of *P. ramorum* was confirmed by its isolation from symptomatic roots, leaves, or stems of inoculated plants on PARP agar and by culture morphology.

Physiological assessment of salt stress.

A physiological effect on plants experiencing an episode of salt stress to the roots is a change in the rate of transpirational water loss, which can be measured by the change in weight of the plant over time (9,42). Potted *Rhododendron* plants were equilibrated to the ambient temperature (25 ± 2°C) and relative humidity conditions of the laboratory overnight. At approximately 0900 h, the plants were watered with 50 ml of DI water (control) or 50 ml of 0.2 M NaCl/0.02 M CaCl₂. The pots were immediately sealed with plastic bags to minimize water evaporation from the soil and roots, leaving the shoots uncovered to allow for transpirational water loss. Plants were weighed every 30 min for 5 h and then at 9 and 24 h from the initiation of the

experiment. The difference in weight between first and second measurements (0 and 30 min) was used to calculate an initial rate of water loss in mg h⁻¹. Subsequent measurements determined the loss of weight between each incremental period, and the rate of weight loss (mg h⁻¹) for each time period was expressed as a percentage of the initial rate.

Comparison of abscisic acid (ABA) and salt in predisposition. To see if ABA affected the susceptibility of *Rhododendron* to *P. ramorum* in hydroponic culture, the following two treatment regimes were added: ABA-treated, inoculated; and ABA-treated, noninoculated. ABA ((±)-abscisic acid, Sigma #A1049) was dissolved in a small volume of ethanol, diluted with 0.5× Hoaglands, and added to the root solutions of *Rhododendron* plants to achieve a final ABA concentration of 10 µg/ml (32 µM). This is a concentration within the range reported in previous studies to simulate the predisposing effect of NaCl on plant roots to infection (6). Plants were inoculated 24 h later by immersing the roots in a zoospore suspension (2×10^3 per ml) of *P. ramorum* isolate #264 in 0.5× Hoaglands solution. Plants were evaluated for stem lesion development at 7, 14, and 21 days after inoculation as previously described.

Impact of delayed postinfection root stress on symptom development. To determine if a postinfection stress episode can stimulate symptom development, roots were immersed in zoospore suspensions for 24 h at low inoculum levels of 10^2 or 10^3 zoospores/ml. Plants were transferred to fresh 0.5× Hoaglands solution and incubated an additional 48 h. Root stress was then imposed by transfer to 0.5× Hoaglands solution containing 0.2 M NaCl/0.02 M CaCl₂. After 24 h, the salt solution was removed, the roots flushed with DI water to remove salts, and the plants returned to 0.5× Hoaglands solution. As a positive control, a set of plants was inoculated by immersion of the roots in a concentrated zoospore suspension (10^5 /ml), the same used to create the diluted inocula for the other treatments. All plants were returned to the growth chamber and evaluated for stem lesion development continuously over a 3-week period. Two experiments were performed, and both *P. ramorum* isolates (#264 and #397) were used in each experiment. There was not a statistically significant difference between these isolates in lesion development, and thus the data are reported as composite results.

Experimental design and analysis.

The general experimental design was complete randomized or randomized complete block, with four to seven individual plant replicates for each treatment. Experiments were performed at least twice or as indicated. The stem lesion data did not satisfy the ANOVA criterion for normality, and thus were analyzed by the Wilcoxon or

Kruskal-Wallis rank sums tests. Lesion data expressed as a percentage of the stem length were transformed using the arcsine square root function. Level of significance was set at $\alpha = 0.05$. Statistical analyses were performed using JMP software version 7.0 (SAS Institute, Cary, NC).

RESULTS

Hydroponic culture experiments. In initial experiments to establish the hydroponic system for root infection studies, both *P. ramorum* isolates readily infected *Rhododendron* and *V. tinus* roots. Root rot, stem lesions, and leaf wilting developed in all inoculated plants of both species. However, the rate at which symptoms developed was markedly different between stressed and nonstressed plants. Within 1 week after inoculation with 2×10^2 zoospores/ml, *Rhododendron* plants that had received a preinoculation salt stress had developed stem lesions sixfold greater (χ^2 , 5.40; $P = 0.02$) than those developing in the nonstressed plants (Fig. 1). Inoculated plants that experienced an episode of salt stress collapsed and became completely necrotic (Fig. 2A). As expected, disease development was faster at the higher inoculum levels (2×10^3 zoospores/ml). Although during the course of the first week of observation the predisposing effect of salt stress was visually apparent, there was not a statistical difference between stressed and nonstressed plants exposed to the higher inoculum level at the two time points when lesions were measured.

As the infection advanced, leaves collapsed and became necrotic. Within 2 weeks, the stem surfaces of all inoculated plants were completely necrotic. Although healthy *Rhododendron* root systems are dark colored, the inoculated roots became noticeably darker in color over time and were necrotic at the termination of the experiment. Salt-stressed, but noninoculated controls were similar in appearance to the nonstressed, noninoculated controls, except for occasional marginal leaf necrosis in a few plants.

In contrast, stem lesions on inoculated *Viburnum* plants were not as obvious; however, roots were necrotic and the leaves permanently wilted (Fig. 2B). Plants that were not salt stressed but inoculated developed ramorum blight symptoms gradually, and the symptoms were less severe than in the salted/inoculated plants. By 2 weeks after inoculation, symptoms on the non-salt-stressed, inoculated plants were as severe as those on the salt-stressed, inoculated plants at 1 week after inoculation. Microscopic examination of roots from both species revealed that their apices were covered with newly developed sporangia of *P. ramorum*. *P. ramorum* was isolated from all symptomatic tissues—roots, stems, and leaves—on PARP, affirming that *P. ramorum* zoospores can infect *Rhododendron* and *V. tinus* plants in this experimental regime and that the pathogen moves systemically into shoots and leaves.

Potted plant experiments. Results with potted plants were different from those

with hydroponic plants in that the two *P. ramorum* isolates differentially affected the two host species. Isolate #264 was highly virulent on potted *Rhododendron* plants, similar to hydroponic plants. Disease developed in salt-stressed roots with death of the plant occurring within 4 weeks. Nonstressed plants survived for 6 to 8 weeks before succumbing to disease, and symptom development in these plants was delayed by 1 to 2 weeks relative to the inoculated, salt-stressed plants. Isolate #397 caused only limited stem lesions after 8 weeks on a few salt-treated *Rhododendron* plants, and only with the highest inoculum level tested (2×10^4 zoospores/ml).

Potted *Viburnum* plants were relatively resistant to ramorum blight following root inoculation with either isolate under our experimental conditions. Disease had developed 8 weeks after inoculation only with isolate #264 and only on one salt-stressed plant. On this plant, roots had decayed, leaves were wilted, and the stem had a lesion that covered approximately 40% of the stem length.

Physiological assessment of the salt stress effect. To determine if a physiological impact of the salt treatment could be discerned in the potted plant format, we monitored changes in weight loss due to transpiration as a nondestructive whole plant measure of stress. In both *Rhododendron* and *V. tinus*, there was not a clear difference between salt-treated and control plants over the time course of observation in the incremental rate of transpirational water loss expressed as a percentage of the initial rate (data not shown). During the first 5 h of measurement, rates of transpirational weight loss in both salted and control plants fluctuated somewhat, but any differences were not significant. Between 9 and 24 h, the transpiration rates leveled off to a similar level in both treatments. At the termination of the experiment (24 h), salt-stressed *V. tinus* and *Rhododendron* plants had similar rates of water loss, to about one-third of the initial rate. The general pattern is consistent with the plants displaying a circadian rhythm, where after about 6 h (late afternoon) the stomata are beginning to close. We did not take additional measurements beyond 24 h because of our primary interest in the response pattern prior to inoculation during the period of salt exposure and predisposition onset.

Exogenous ABA increases susceptibility of *Rhododendron* to *P. ramorum*. Both ABA and salt treatment of *Rhododendron* roots prior to inoculation significantly increased disease development relative to the nontreated control. At 7 days after inoculation, salt and ABA treatments were significantly different from the inoculated control (Kruskal-Wallis test, $\chi^2 = 15.5$, $P = 0.0004$; Fig. 3). After this period, lesion size in the inoculated controls was similar to that in the other treatments. At 14 days

Fig. 1. Stem lesion development following root infection of nonstressed and salt-stressed *Rhododendron* hybrid Cunningham's White by *Phytophthora ramorum*. Experiments conducted in the hydroponic regime and the data are composite results with *P. ramorum* isolates #264 and #397 (isolates were not significantly different in virulence in this disease assay format in *Rhododendron*). Values are the percentage of the primary stem length that is necrotic, and each value is the mean and SE of four plants. Data were transformed by the arcsine square root function and analyzed by the Wilcoxon rank sums test. The average stem length of plants used in these experiments was 43 ± 2 mm. 2×10^2 zoospores/ml: 1 week, * = column pair significantly different (χ^2 , 5.40; $P = 0.02$); 2 weeks, ns = column pair difference not significant ($\chi^2 = 2.19$, $P = 0.14$). 2×10^3 zoospores/ml: 1 week, ns = column pair difference not significant ($\chi^2 = 2.61$, $P = 0.11$); 2 weeks, * = column pair significantly different ($\chi^2 = 3.94$, $P = 0.05$).

after inoculation, lesion size was not significantly different between the ABA-treated and control plants ($\chi^2 = 0.77$, $P = 0.38$), and by 21 days after inoculation, disease severity was similar in all treatments. Lesion development following salt stress also was significantly different from the control at 14 days after inoculation ($\chi^2 = 9.77$, $P = 0.002$).

Impact of delayed postinfection root stress on symptom development. To see if abiotic stress can stimulate root infections to give rise to systemic lesions, we exposed *Rhododendron* roots in the hydroponic regime to low inoculum levels of *P. ramorum* prior to salt treatment. Figure 4 illustrates that plants inoculated with 10^2 zoospores/ml and then 72 h later exposed to an episode of salt stress developed significantly larger stem lesions than the non-stressed controls when measured after 3 weeks (Wilcoxon rank sums test, $\chi^2 = 8.63$, $P = 0.003$). In one experiment, inoculated but nonstressed plants at the lower inoculum level did not show any symptoms even at 3 weeks; however, in a second experiment, the nonstressed, inoculated plants displayed symptoms but less than those in the salt-stress, inoculated plants (Figure 4 represents the average of the two experiments). With 10^3 zoospores/ml, the difference between control and salted plants was not significant at the 3-week time point ($\chi^2 = 2.05$, $P = 0.15$). At the end of each experiment, the presence of *P. ramorum* was confirmed by isolation on PARP in all inoculated plants that were symptomatic and nonsymptomatic.

DISCUSSION

The importance of root infection by *P. ramorum* in the overall etiology and epidemiology of SOD and ramorum blight of various host species is unclear. Although roots were suspected as a likely infection court following isolation of the pathogen from naturally infected roots in some hosts, it was relatively recent that root infection was firmly established in controlled studies in *Rhododendron* and other species, suggesting a soil phase in the disease cycle (24,35,40). *P. ramorum* can be found in the xylem underlying phloem lesions of tree hosts (8) and readily colonizes xylem elements following infection of roots of *Rhododendron* sp. (35). The pathogen appears to move rapidly via this pathway into shoots and leaves. The results of our experiments indicate that, similar to other *Phytophthora* species, *P. ramorum* has the capability to rapidly exploit roots following an episode of root stress. In addition, our results provide evidence that a postinfection stress episode can accelerate symptom expression, pointing to the potential role that abiotic stress could play in the activation of asymptomatic infections.

Whether abiotic root stress factors into the epidemiology and severity of SOD and

ramorum blight under field conditions remains to be seen. Tree water potentials were not related to the incidence of SOD in woodlands (41). High temperatures and

solar radiation (14) and, possibly, low leaf water potentials (16) are the principal eco-physiological factors that reduce over-summering survival of *P. ramorum* in Cali-

Fig. 2. A, The effect of a preinoculation episode of salt stress on disease development following inoculation of roots with *Phytophthora ramorum* in *Rhododendron* hybrid Cunningham's White. Plants were maintained in a hydroponic regime during the experiment and roots were inoculated with zoospores (2×10^2 /ml) following a 16 h exposure to NaCl. Photo taken 1 week after inoculation. **B,** *Viburnum tinus* cv. Spring Bouquet plants showing the effect of 16 h salt stress prior to inoculation with zoospores (2×10^3 /ml) of *P. ramorum* isolate #397. Plants were maintained as in (A), 1 week after inoculation. Non-inoculated control and salted plants (not shown) were healthy, similar in appearance to *Rhododendron* plants (A) with the corresponding treatments.

fornia bay laurel leaves, a primary source of inoculum for SOD epidemics in mixed evergreen forests in California. The occurrence of *P. ramorum* in forests tends to be favored by closed canopies and cool, moist microclimates (30,33). Thus, if water stress does occur, it likely inhibits disease development in these situations.

The difference in susceptibility of *V. tinus* plants between hydroponic and potted plant experiments and the difference in response of potted *Rhododendron* plants to

the two isolates were unexpected. In the latter case, the isolate from California bay laurel (#264) was highly virulent on potted plants, whereas only limited disease development was observed in these plants with the isolate from *Ranunculus* (#397). There was no indication of a loss of virulence with the latter, as the potted plant experiments were conducted prior to or concurrently with the hydroponic culture experiments. The soil environment under our experimental conditions may be less con-

ducive to infection than the hydroponic regime. We were unable to follow up on this aspect, so we are cautious about interpreting too much from these results. Nonetheless, it would be of interest to know the extent to which there are isolate differences in *P. ramorum* with respect to their occurrence and behavior in the soil environment and ability to initiate root infections.

The absence of a measurable effect of salt treatment on the transpiration rates in *Rhododendron* and *Viburnum* under our experimental conditions does not preclude a physiological impact on the roots and/or shoots. Leaves of *Viburnum* plants wilted within a few hours of exposure to salt, and rapidly recovered upon return to 0.5× Hoaglands. *Rhododendron* leaves did not wilt during the stress treatment, but a few plants displayed marginal leaf necrosis from salt. It is possible that under greenhouse conditions and higher light intensity, an effect of salt on transpiration would be apparent. However, the treatments were imposed under the fluorescent lights and laboratory humidity and temperature that we used for the stress induction phase throughout this study, conditions sufficient to predispose *Rhododendron* and *V. tinus* plants to infection by *P. ramorum*. Nonetheless, the physiological impact of the stress regime is transient and does not appear to perturb these species severely. In tomato, a more tractable model for the experimental study of predisposition than the ornamental species used in this study, plants begin to recover from a predisposed state about 4 to 6 h after removal of the roots from the salt-stress treatment (15).

Abiotic stresses such as those that predispose to *Phytophthora* root rots induce a degree of water stress in plants with a concomitant increase in levels of ABA in roots and shoots (42). A role for ABA in disease and predisposition is supported by studies with ABA-modified plants (1,28). A pre-inoculation ABA treatment of roots in mesophytic species such as tomato mimics the predisposing effect of salt stress, and ABA-deficient mutants do not display a corresponding predisposition phenotype as ABA-normal plants (5,6,15). Studies in potato (21) and soybean (10,29,31) demonstrated that ABA pretreatment of plants abrogated race-cultivar resistance to *Phytophthora* spp. and suppressed phytoalexin accumulation, and that ABA-synthesis inhibitors enhanced resistance. The results with ABA in *Rhododendron* reported in this study are concordant with findings in other systems. There has been resurgence in interest in ABA in disease processes in recent years, where it has been examined with different pathosystems incorporating ABA-mutants and abiotic stress in a few cases (23). In *Arabidopsis*, ABA treatment or drought stress induces susceptibility to *Pseudomonas syringae* pv. *tomato* (Pst DC3000) and to the downy mildew patho-

Fig. 3. Lesion development in nonstressed, salt-stressed, and abscisic acid (ABA)-treated (32 μ M) *Rhododendron* hybrid Cunningham's White plants maintained in the hydroponic regime. Roots were inoculated 24 h after initiation of the first treatment with zoospores (2×10^3 /ml) of *Phytophthora ramorum* isolate #397. Each value is the mean and SE of 18 (*P. ramorum* + ABA) or 20 (*P. ramorum* and *P. ramorum* + salt) plants from four experiments. See text for description of statistical analysis.

Fig. 4. The impact of delayed postinfection salt stress treatment on *Phytophthora ramorum* symptom development in *Rhododendron* in a hydroponic regime. Stem lesion data are the composite results for isolates #264 and #397 and are presented for two inoculum levels with symptoms evaluated 3 weeks after inoculation. Each value is the mean and SE of 22 plants from two experiments. * = column pair significantly different by Wilcoxon rank sums test, $\chi^2 = 8.63$, $P = 0.003$. ns = not significantly different ($\chi^2 = 2.05$, $P = 0.15$).

gen *Hyaloperonospora parasitica* (32). Recently, an important study illustrated how effectors from *Pst* DC3000 co-opt ABA biosynthesis and signaling in *Arabidopsis* to cause disease (12). Salicylic acid-mediated responses associated with systemic acquired resistance to *Pst* appear to be a target for ABA, whether it is applied exogenously or induced by NaCl stress (43,47).

In our study, salinity (osmotic) stress was selected to examine predisposition to *P. ramorum* because this stress can be rapidly imposed and alleviated for experimental studies. Imposition of osmotic stress and inoculation were temporally separated to minimize any direct impact on zoospore behavior, thus focusing the predisposing effect of the stress as largely a result of altered host physiology. Other predisposing stressors such as water deficit, anoxia (waterlogging), and possibly cold stress, which also impact ABA-regulated processes, are candidates for future study as they are more likely to occur in nursery, shipment, and transplanting operations. It seems reasonable that activation of asymptomatic infections by episodic stress could contribute to the sudden onset of ramorum dieback and blight in some situations. In an open-air simulated container nursery system with contaminated overhead irrigation water, *P. ramorum* was frequently detected in root balls from asymptomatic *Rhododendron* and *Viburnum* plants (46). The complicating influence of predisposing stress on disease onset and severity is an additional consideration for risk assessment in nurseries impacted by *P. ramorum*.

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