2004

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Citation/Publisher Attribution

Tooley, P. W., Kyde, K. L., & Englander, L. (2004). Susceptibility of Selected Ericaceous Ornamental Host Species to *Phytophthora ramorum*. *Plant Disease, 88*(9), 993-999. doi: 10.1094/PDIS.2004.88.9.993
Available at: https://doi.org/10.1094/PDIS.2004.88.9.993

Susceptibility of Selected Ericaceous Ornamental Host Species to *Phytophthora ramorum*

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ABSTRACT

Tooley, P. W., Kyde, K. L., and Englander, L. 2004. Susceptibility of selected ericaceous ornamental host species to *Phytophthora ramorum.* Plant Dis. 88:993-999.

We assessed disease reactions of 51 species or varieties of ericaceous ornamental hosts to two isolates of *Phytophthora ramorum*, the causal agent of sudden oak death. Inoculation was performed with an A2 mating type U.S. isolate from rhododendron and the *P. ramorum* type culture of A1 mating type from Germany. For only one host were statistically significant differences in disease observed between the two isolates. Several different inoculation methods were compared. The 51 hosts tested varied widely in susceptibility, ranging from 0% to over 90% leaf area infected. Two cultivars of *Vaccinium macrocarpon* (cranberry) showed no disease, while three cultivars of *Kalmia latifolia* (mountain laurel) were all highly susceptible. The results indicate that many ornamental hosts grown in the United States are susceptible to *P. ramorum* under artificial inoculation conditions. Inoculum density studies with two susceptible host species showed that *P. ramorum* is capable of producing disease symptoms over sporangium concentrations ranging from 100 to 5,000 sporangia per ml. Mean numbers of chlamydospores forming in host tissue of 21 hosts ranged from 2 to over 900 chlamydospores per 6-mm-diameter leaf disk. Whether hosts showing susceptiblity under the experimental conditions used in this study would become infected with *P. ramorum* in the presence of inoculum under natural conditions is unknown.

Additional keywords: chlamydospore, host range, inoculum density, sudden oak death

Phytophthora ramorum (S. Werres, A.W.A.M. de Cock & W.A. Man in't Veld) sp. nov. causes sudden oak death (SOD), which is a canker disease of certain oak species that has killed thousands of trees in California since 1995 (13,28) and has moved northward into southwestern Oregon (17). In Europe, *P. ramorum* is described by Werres et al. (34) as causing a dieback of *Rhododendron* and *Viburnum* spp. but since its initial characterization in Europe, researchers have considered it to be the same pathogen species as that causing oak mortality in California (28). The ever-widening host range of *P. ramorum* contains 28 species in 13 plant families (13,21,26), including two highly valued forest species, Douglas-fir and coast redwood (11,24). On most reported ornamental hosts, *P. ramorum* causes foliar leaf spot or dieback symptoms in contrast to the bleeding cankers seen on trunks and limbs of its major hosts, tanoak (*Lithocarpus densiflorus*) and coast live oak (*Quercus agrifolia*) in California (13).

Although pathogens from the United States and Europe are considered to be *P. ramorum*, some important genetic differences between the subpopulations, such as mating type, have been noted (3,18,33). Since its initial description from the Netherlands and Germany (34), *P. ramorum* has been reported from several additional European countries (14,19,23,25), causing much concern and resulting in the establishment of European regulatory measures (1).

In the United States, vast amounts of plant material, including many *P. ramorum* hosts, are shipped from west to east within the nursery industry (15). Concern about movement of *P. ramorum* eastward on ornamentals or other plant-related materials, such as wood products and bay laurel wreaths, led to the establishment in 2001 of state and federal regulations restricting movement of *P. ramorum* hosts out of infested areas of California (5,6,27). In 2003, new reports of *P. ramorum* infecting nursery crops were issued from Oregon, Washington, and Canada, as well as from regions of California not then under quarantine (18,26; J. Jones, *personal communication*).

In March 2004, *P. ramorum* was identified on camellias at a nationwide nursery supplier in California, which had over the previous year, shipped potentially infected material to numerous garden centers in 39 states (32). In response, numerous states very quickly banned imports of plant material from California consisting of hosts and associated hosts of *P. ramorum*, or in some cases, all plant material. On April 9, 2004, the USDA Animal and Plant Health Inspection Agency (APHIS) implemented new restrictions on interstate movement of hosts and associated articles from all commercial nurseries in California, outside the 12 quarantined counties as well. Laboratory-confirmed positive *P. ramorum* samples were identified from nursery stock in Florida, Georgia, Louisiana, and Virginia, in addition to Oregon and Washington State (J. Jones, *personal communication*). These findings have alarmed growers, state regulators, foresters, and others concerned about the well-being of landscape plants and oak forests in the United States and resulted in new survey efforts directed at determining whether *P. ramorum* is now present in additional states. Knowledge of the reaction to *P. ramorum* of common ericaceous plant species grown or planted in the United States will provide information about the likelihood of various hosts to become infected and potentially contribute to new outbreaks of SOD. Information on symptomatology and susceptibility will assist in state or federal surveys that attempt to identify the pathogen in new regions.

The plant family Ericaceae contains over 100 genera and over 3,350 taxa (4), including many that are important in the nursery trade. The genus *Rhododendron* alone comprises over 1,000 species with several thousand varieties in existence that show adaptation to various environmental zones throughout the United States (9,10,30). However, zones are not exclusive, varieties grown in the Pacific Northwest, for example, may also be grown in New England states. Other ericaceous hosts, such as mountain laurel (*Kalmia latifolia*), are native to the eastern United States and are also grown in the west.

Since SOD is a relatively new disease, methods for screening such diverse hosts for susceptibility are not well established (28,29). Our primary objectives were to

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Accepted for publication 30 April 2004.

Publication no. D-2004-0617-01R

develop a screening method for determining susceptibility of ericaceous ornamental hosts to *P. ramorum*, and evaluate the reactions of ericaceous plant species or cultivars important in the nursery industry in the United States. As part of developing a screening method, our objective was to examine the range of inoculum density over which *P. ramorum* can infect susceptible ericaceous host species. Another objective was to evaluate the propensity of *P. ramorum* to sporulate on ericaceous hosts, thereby quantifying the ability of specific hosts to produce inoculum that might spread the disease. Our results provide information that can help predict which eastern host species may support growth of *P. ramorum* and provide inoculum for new outbreaks of the disease to occur.

MATERIALS AND METHODS

Host plants. Material was obtained from cooperators in Rhode Island, Oregon, and elsewhere (see acknowledgements) and purchased commercially. Species and cultivars were chosen to represent the diversity within the family Ericaceae while focusing on hosts of commercial importance (Table 1). Within *Rhododendron*, a very large genus, we included members of the major groups of deciduous and evergreen azaleas, lepidote and elepidote rhododendrons, and species representing varied geographic origins of members of the genus. Plants were maintained in a greenhouse and fertilized periodically with nutricote controlled release fertilizer, type 360/70 (Florikan, Sarasota, FL). Miracid Professional (21-7-7 formulation; Scotts-Sierra Horticultural Products Co., Marysville, OH) was applied to rhododendrons and azaleas in the spring. Plants were transported by van to a Biosafety Level 3 containment facility where experiments were performed. Inside the containment facility, plants were maintained in a containment greenhouse with an automatic drip watering system set for 5 min of watering two times per day on every third day.

Cultures and inoculation methods. Cultures of *P. ramorum* were obtained from cooperators in California and Germany (see acknowledgements) and maintained on Rye A (7) and 20% V8 juice agar at 18 to 20°C in darkness. The two isolates used were BBA 9/95 (mating type A1, isolated in 1995 from Germany, host *Rhododendron catawbiense*, *P. ramorum* type culture) and Pr-52 (mating type A2, isolated in 2000 from Felton, California, host *Rhododendron* cv. 'Gomer Waterer'), which have been described in previous studies (28,34).

P. ramorum sporangia were produced by cutting 10 to 15 6-mm-diameter plugs from the margin of a 10- to 14-day-old V8 agar culture with a cork borer and placing them into 20 ml of 1% soil extract (made

Table 1. Unadjusted and adjusted percentage leaf area infected as measures of susceptibility of 51 ericaceous plant hosts inoculated with two isolates of *Phytophthora ramorum*[a]

		Unadjusted % leaf area infected			Adjusted % leaf area infected[b]		
		Isolate			Isolate		
Host	Common name and origin or parentage[c]	BBA 9/95	Pr-52	Mean[d]	BBA 9/95	Pr-52	Mean[d]
Arctostaphylos uva-ursi	Bearberry; NE U.S. native	38.9	47.2	43.0	15.5	27.2	21.4
Gaultheria procumbens	Wintergreen; E U.S. native	6.4	5.9	6.1	1.9	2.3	2.1
Gaylussacia baccata	Black huckleberry; E U.S. native	22.9	12.1	17.5	7.3	2.6	4.9
Gaylussacia frondosa	Blue huckleberry; E. U.S. native	11.7	27.7	19.7	7.7	13.3	10.5
Kalmia angustifolia	Sheep laurel; NE U.S. native	5.4	1.9	3.6	0.8	0.5	0.6
K. latifolia 'Madeline'	Mountain laurel; cv. of U.S. native	59.0	67.7	63.3	49.6	66.1	57.9
K. latifolia 'Minuet'	Mountain laurel; cv. of U.S. native	96.2	93.3	94.8	96.2	88.1	92.1
K. latifolia 'Olympic Wedding'	Mountain laurel; cv. of U.S. native	71.3	27.1	49.2	70.4	22.5	46.5
Leucothoe axillaris 'Greensprite'	Greensprite doghobble; cv. of SE U.S. native	3.2	4.6	3.9	0.1	1.0	0.6
L. fontanesiana	Highland doghobble; Massachusetts, New York, and SE U.S. native	4.7	1.4	3.0	2.2	0.5	1.4
Pieris floribunda	Mountain fetterbush; SE U.S. native	9.5	10.9	10.2	3.8	4.7	4.2
P. japonica	Japanese pieris; Japan	5.8	33.7	19.7	1.7	26.9	14.3
Rhododendron 'Aglo'	Aglo rhododendron; *R. minus*, compact form × *R. dauricum* hybrid	30.5	20.4	25.5	17.5	9.8	13.6
R. arborescens	Smooth azalea; E U.S. native	9.4	8.8	9.1	2.6	1.5	2.1
R. calendulaceum	Flame azalea; E North American native	28.9	28.6	28.8	12.5	10.7	11.6
R. carolinianum	Carolina azalea; Tennessee, North Carolina, South Carolina, and Connecticut native	10.8	8.5	9.6	3.8	2.1	3.0
R. catawbiense	Catawba rhododendron; E U.S.native	18.2	8.2	13.2	8.4	3.0	5.7
Rhododendron 'Chinoides'	Chinoides rhododendron; *R. ponticum* hybrid	11.6	25.4	18.5	3.4	13.1	8.2
Rhododendron 'Cunningham's White'	Cunningham's White rhododendron; *R. caucasicum* × *ponticum* var. *album*	48.7	27.8	38.3	42.0	22.7	32.4
R. dauricum PJM type	Seedling of Siberian species	24.8	28.3	26.5	7.4	11.8	9.6
Rhododendron 'Delaware Valley White'	Delaware Valley White azalea; *R. mucronatum* hybrid	20.9	27.3	24.1	13.4	10.2	11.8
Rhododendron 'Exbury hybrid'	Exbury hybrid rhododendron; parentage unknown	9.1	10.7	9.9	7.5	6.4	6.9
Rhododendron 'Girard's Fuchsia'	Girard's Fuchsia azalea; ('Sandra Ann' × ['Herbert' × 'Girard's Hot Shot']) × 'Sandra Ann'	22.8	13.6	18.2	2.7	1.6	2.2
Rhododendron 'Girard's Rose'	Girard's Rose azalea; (['Fedora' × 'El Capitan'] × ['Boudoir'] × 'Boudoir')	5.0	14.5	9.7	1.1	1.0	1.0

(*continued on next page*)

[a] Intact leaves on whole host plants were dip inoculated with a suspension of 5,000 sporangia per ml of *P. ramorum* and incubated in a dew chamber at 20ºC in darkness for 7 days.
[b] Mean percent leaf area infected multiplied by the proportion of leaves becoming infected in each set of 25 inoculated leaves.
[c] cv. = cultivar, E = eastern, N = northern, NE = northeastern, SC = southcentral, and SE = southeastern.
[d] Overall mean for each host over the two isolates used for inoculation.
[e] Minimum significant difference (MSD) for comparing interaction means (i.e., host × isolate means), Tukey's studentized range test ($P = 0.05$).
[f] Minimum significant difference (MSD) for comparing main effects means (i.e., means over both isolates), Tukey's studentized range test ($P = 0.05$).

by autoclaving 10 g of garden soil in 1 L of distilled water, vacuum-filtering through Whatman no. 1 filter paper, and then re-autoclaving) in 9-cm-diameter petri dishes for 48 h at 20°C in darkness. Sporangia formed in abundance on and around each agar plug and were collected by applying Parafilm to the petri dish perimeters, vigorously shaking them horizontally for 10 sec to dislodge sporangia, and then pouring through four layers of cheesecloth to remove mycelial fragments. Sporangia suspensions were quantified with a hemacytometer and by counting the number of sporangia present in several 5-µl drops sampled randomly from the suspension, and adjusted to 5,000 sporangia per ml. When counts below 5,000 per ml were obtained, spores were concentrated by centrifugation at 254 × *g* in a tabletop centrifuge for 5 min at 15°C.

To develop a screening method for *P. ramorum*, we experimented with several different types of inoculation. Mycelial suspension was produced by removing a 2.5-cm^2 area of hyphae from the surface of a 20% V8 juice agar culture to a depth of approximately 2 mm with a scalpel and grinding in a glass tissue grinder with 10 ml of sterile distilled water until finely macerated. Plant wounding was performed by cutting off the terminal 3 mm of leaf tissue with a scissors.

Dipping plants in a mycelial suspension was compared with dipping in a sporangia suspension. Plants were inoculated by dipping limbs (or when plants contained less than 25 leaves, the entire plants) containing groups of at least 25 leaves into a sporangia suspension (5,000 per ml) or mycelial suspension in gallon-size plastic zip-lock bags. The foliage was immersed completely to ensure saturation with inoculum, agitated gently in the inoculum, and then gently shaken before removal from the bags to remove excess inoculum. To prevent drying of inoculum on the leaves, plants were placed immediately into a 20°C dew chamber and incubated for 7 days in darkness. Following symptom development, isolation attempts were made from lesion margins onto PARP selective medium (20).

For each host/isolate combination in all experiments, four sets of at least 25 leaves were inoculated and when possible, all four sets chosen were located on the same plant. After 7 days of incubation in the dew chamber at 20°C in the dark, the areas of leaves and *P. ramorum* lesions were measured. For each set of at least 25 leaves that were inoculated with a given isolate, 25 leaves were chosen randomly and assessed for disease symptoms. To assess disease in initial studies, leaf outlines were traced with a pencil onto white paper. With the leaf held in place, a pencil point was pressed through the leaf at points 2 to 3 mm apart along the lesion margins, making dots on the paper. The leaf was then removed, and the dots were connected, displaying the lesion shape within the already traced leaf shape. Leaf and lesion areas were then measured (cm^2) with a hand-held digital planimeter (model KP-82N;

Table 1. (*continued from preceding page*)

		Unadjusted % leaf area infected			Adjusted % leaf area infected[b]		
		Isolate			Isolate		
Host	**Common name and origin or parentage[c]**	**BBA 9/95**	**Pr-52**	**Mean[d]**	**BBA 9/95**	**Pr-52**	**Mean[d]**
Rhododendron 'Glacier'	Glacier azalea; Glenn Dale hybrid 'Malvatica' × 'Yozakura'	9.1	29.1	19.1	1.0	3.6	2.3
Rhododendron 'Gloria'	Gloria azalea; sport of 'Dorothy Gish'	18.0	13.2	15.6	1.5	2.4	1.9
Rhododendron 'Hino Crimson'	Hino Crimson azalea; 'Amoenum' × 'HinodeGiri'	0.8	0	0.4	0	0	0
R. indicum 'Macrantha'	Macrantha azalea; Japan	11.3	5.3	8.3	1.5	0.4	0.9
Rhododendron 'Inga'	Inga azalea; sport of 'Helmut Vogel'	5.9	19.2	12.6	1.2	2.0	1.6
R. macrosepalum	Big sepal azalea; S Japan	3.8	1.8	2.8	0.3	0.2	0.2
Rhododendron 'Marilee'	Marilee azalea; cv. from open-pollinated *R. nakaharai*	7.9	16.3	12.1	1.3	2.4	1.9
R. maximum	Rosebay rhododendron; E U.S. native	5.6	3.7	4.7	3.0	1.5	2.2
R. micranthum	N China native	36.1	21.7	28.9	19.5	7.3	13.4
R. minus	Piedmont rhododendron; SE U.S. native	9.4	8.8	9.1	3.6	3.0	3.3
Rhododendron 'Nova Zembla'	Nova Zembla rhododendron; 'Parson's grandiflorum' × 'Mars'	34.4	54.1	44.3	26.9	40.2	33.6
Rhododendron 'PJM'	PJM rhododendron; *R. carolinianum* × *R. dauricum* var. *sempervirens*	8.9	5.9	7.4	1.6	0.3	0.9
Rhododendron 'Purple Gem'	Purple Gem azalea; *R. fastigiatum* × *carolinianum*	43.6	41.1	42.3	10.7	6.1	8.4
Rhododendron 'Purple Splendor'	Purple Splendor azalea; Gable hybrid (*R. poukhanense* × 'Hexe')	3.5	0.1	1.8	0.4	0	0.2
Rhododendron 'Roseum Elegans'	Roseum elegans rhododendron *R. catawbiense* hybrid	20.4	10.5	15.4	10.9	5.7	8.3
R. vaseyi	Pinkshell azalea; U.S. native	30.5	14.1	22.3	28.8	13.6	21.1
R. viscosum	Swamp azalea; E and SC U.S. native	7.1	1.9	4.5	1.4	1.1	1.3
R. yakushimanum 'Ken Janeck'	Ken Janeck rhododendron; seedling of S Japan species	6.4	4.0	5.2	3.1	0.5	1.8
R. yedoense var. *poukhanense*	Korean azalea; Korea, Japan	6.9	4.7	5.8	4.3	1.6	3.0
Umbellularia californica	California bay laurel; U.S. native	21.3	28.3	24.8	20.8	25.0	22.9
Vaccinium angustifolium	Lowbush blueberry; NE and NC U.S. native	8.8	8.3	8.6	2.9	4.1	3.5
V. corymbosum 'Bluecrop'	Highbush blueberry; commercial cv. of E and SC U.S. native	0.9	1.6	1.2	0.5	1.0	0.7
V. corymbosum 'Duke'	Highbush blueberry; commercial cv. of E and SC U.S. native	1.8	1.4	1.6	0.3	0.4	0.4
V. corymbosum 'Weymouth'	Highbush blueberry; commercial cv. of E and SE U.S. native	2.2	2.8	2.5	1.3	2.7	2.0
V. macrocarpon 'Crowley'	Cranberry; commercial cv. of U.S. native	0	0	0	0	0	0
V. macrocarpon 'Stevens'	Cranberry; commercial cv. of U.S. native	0	0	0	0	0	0
Zenobia pulverulenta	Honeycup; SE U.S. native	2.7	1.8	2.2	0.4	0.3	0.3
Overall mean		17.3	16.8		10.3	9.3	
MSD value (Tukey's test, $P = 0.05$)			28.8[e]	18.9[f]		22.6[e]	14.8[f]

Topcon, Inc., Pleasanton, CA). To expedite the measuring process, midway through these studies we adopted the use of ASSESS (22) software to measure leaf and lesion areas by placing infected leaves on a flatbed scanner, scanning them, and saving the scans as JPEG files. Files were then opened in ASSESS and hue and intensity settings were adjusted to highlight and measure (cm^2) leaf and lesion areas. The two methods of measuring leaf and lesion areas were compared statistically (31) by measuring a set of 35 infected leaves of *Pieris floribunda* with both methods and no significant differences ($P = 0.05$) were observed.

Disease was expressed as percent leaf area infected. In initial studies, the number of leaves infected by *P. ramorum* out of randomly chosen groups of 25 leaves of several hosts was analyzed by analysis of variance (ANOVA) and was found to differ significantly. Thus, we determined that the number of leaves infected would serve as an additional measure of resistance or susceptibility to *P. ramorum*. To produce a measure of resistance/susceptibility that incorporated the number of leaves becoming infected along with the percent leaf area infected for leaves that did become infected, we calculated an adjusted percent leaf area infected by multiplying the mean percent leaf area infected for each set of 25 inoculated leaves by the proportion of the 25 leaves that had become infected for each set. Unadjusted and adjusted percent leaf area was analyzed using ANOVA within the GLM procedure in SAS (31).

Inoculum density studies. Seven sets of 25 randomly preselected leaves on whole plants of bearberry (*Arctostaphylos uva-ursi*) and 'Minuet' mountain laurel (*K. latifolia* 'Minuet') were inoculated with sporangia suspensions containing 0, 50, 100, 500, 1,000, 2,000, 3,000, 4,000, or 5,000 sporangia per ml of *P. ramorum* isolate Pr-52. Inoculated plants were incubated in a dew chamber at 20°C for 7 days in darkness after which lesion and leaf areas were measured with ASSESS software as described above. The total lesion area produced by *P. ramorum* for each set of 25 inoculated leaves at each inoculum level was calculated and plotted against inoculum concentration. The data were subjected to regression analysis and analysis of covariance using PROC GLM in SAS (31).

Chlamydospore production. Leaf disks (6 mm in diameter) were removed with a paper punch and immersed in 5 ml of a *P. ramorum* sporangia suspension (5,000 sporangia per ml in 1% of soil extract) in 6-cm-diameter petri dishes for 48 h at 20°C in darkness. The disks were then rinsed with several changes of sterile distilled water and incubated in a submerged fashion in 5 ml of sterile distilled water for an additional 12 days at 20°C in darkness. The number of chlamydospores in the leaf tissue (abaxial and adaxial leaf surfaces) was then counted under a dissecting microscope with grids etched onto 6-mm-diameter clear acetate circles to facilitate counting. Only chlamydospores observable in the first few layers of plant cells on upper and lower leaf disk surfaces were counted and it was determined with a scalpel that disks were thick enough to prevent the seeing "through" of the leaf section and counting some chlamydospores twice. An inoculating loop was passed over the surface of each leaf disk prior to counting to scrape away any chlamydospores formed superficially on the disks or intertwined in superficial hyphae and not in the plant tissue. Three sets of eight leaf disks were inoculated and assessed per host/isolate combination, with *Rhododendron* 'Cunningham's White' leaf disks included in each set as a positive control.

RESULTS

Inoculation methods. Inoculation of tissue (wounded or nonwounded) with *P. ramorum* sporangia was compared with inoculation (wounded) with a suspension of mycelium (Table 2). Significant differences were observed in unadjusted and adjusted percent leaf area infected among five azalea varieties tested, 'Gloria' and 'Girard's Fuchsia' were the most susceptible and 'Inga' and 'Purple Splendor' were the least susceptible (Table 2). Inoculation treatments differed significantly for percent leaf area infected and adjusted percent leaf area infected, with the mycelium/wounding method causing the most disease over all the azaleas tested, followed by the two sporangial inoculation methods (wounding versus nonwounding) which did not differ significantly from one another. A marginally significant host × treatment interaction was observed ($P = 0.0759$) for percent leaf area infected and adjusted percent leaf area infected. Since the sporangia nonwounding method resulted in the same degree of overall infection as did the sporangia wounding method, we chose to use the nonwounding method in further studies. Even though more disease was produced with mycelial suspension as inoculum, we chose to use sporangia in further studies because they represent a natural form of inoculum.

Plant inoculations. In addition to the five azaleas initially screened, we screened 46 additional hosts with the sporangia, nonwounding method. These hosts varied widely in their reaction to inoculation with two isolates of *P. ramorum* (Table 1). Symptomatology varied among hosts, with most disease reactions characterized by the presence of spreading brown or black lesions which occurred randomly over the leaf surface area (2). Dieback symptoms were occasionally observed in that lesions were present on petioles and stems; however, we did not attempt to quantify dieback symptoms. Unadjusted percentage leaf area infected varied from above 95% for *K. latifolia* 'Minuet' (mountain laurel) to 0% for *Vaccinium macrocarpon* (cranberry). The most susceptible hosts for

Table 2. Comparison of three different types of inoculation of five different azaleas with isolate BBA 9/95 of *Phytophthora ramorum*[a]

	Unadjusted % leaf area infected				Adjusted % leaf area infected[b]			
	Method of inoculation				Method of inoculation			
Host	Mycelium wound	Sporangia wound	Sporangia nonwound	Overall mean[c]	Mycelium wound	Sporangia wound	Sporangia nonwound	Overall mean[c]
'Girard's Fuchsia'	25.5	12.5	22.8	20.2	8.5	1.6	2.7	4.3
'Glacier'	15.4	15.4	9.1	13.3	3.2	5.2	1.0	3.1
'Gloria'	31.7	23.3	18.0	24.3	4.7	6.1	1.5	4.1
'Inga'	8.1	2.2	5.9	5.4	1.0	0.3	1.2	0.8
'Purple Splendor'	13.6	5.5	3.5	7.5	1.1	0.7	0.4	0.7
Overall	18.8	11.8	11.9	6.6[d]	3.7	2.8	1.4	2.3[d]
MSD value		21.9[e]		10.0[f]		7.5[e]		3.4[f]

[a] Leaves were dip inoculated with a mycelial suspension or with 5,000 sporangia per ml of *P. ramorum* isolate BBA 9/95 and incubated in a dew chamber at 20°C in darkness for 7 days. Wounding was achieved by cutting off the last 3 mm of the leaf with scissors.
[b] Mean percent leaf area infected multiplied by the proportion of leaves becoming infected in each set of 25 inoculated leaves.
[c] Overall mean for the three different inoculation methods.
[d] Minimum significant difference (MSD) value for comparing main effects of inoculation treatments over all cultivars, Tukey's studentized range (HSD) test ($P = 0.05$).
[e] Minimum significant difference for comparing interaction means, Tukey's studentized range (HSD) test ($P = 0.05$).
[f] Minimum significant difference for comparing main effects means, Tukey's studentized range (HSD) test ($P = 0.05$).

unadjusted percent leaf area infected included three *K. latifolia* cultivars, 'Cunningham's White' rhododendron, other *Rhododendron* species, and bearberry (*A. uva-ursi*) (Table 1). In contrast, the least susceptible hosts included cranberry and highbush blueberry (Table 1). While the native species, *K. latifolia*, was highly susceptible to *P. ramorum*, its relative, *K. angustifolia* (sheep laurel), was among the most resistant species tested with a mean adjusted percent leaf area infected value of just 0.6% (Table 1). U.S. native species of *Rhododendron* varied in susceptibility to *P. ramorum*, with the most susceptible being *R. vaseyi* (21.1% adjusted percent leaf area infected) and the least susceptible being *R. viscosum* (1.3% adjusted percent leaf area infected). Species of Asiatic origin also varied in susceptibility, but some were less susceptible, such as *R. yakushimanum* 'Ken Janeck' (1.8% adjusted percent leaf area infected) and *R. macrosepalum* (0.2% adjusted percent leaf area infected).

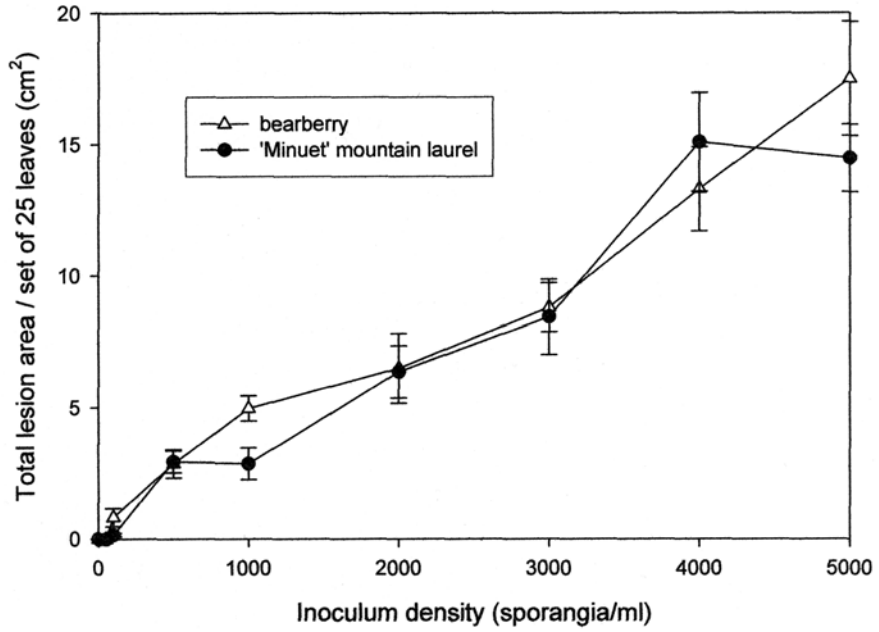

Fig. 1. Responses of Minuet mountain laurel (*Kalmia latifolia* 'Minuet') and bearberry (*Arctostaphylos uva-ursi*) to increasing density of *Phytophthora ramorum* sporangia (0 to 5,000 sporangia per ml) in nonwounding, dip inoculation of intact plants followed by 7 days of incubation in a dew chamber at 20°C in darkness. The total amount of infected leaf area obtained at each inoculum density was plotted against inoculum density. Bars represent standard errors based on seven data points (each data point representing a group of 25 inoculated leaves) at each concentration for each host. Linear regression equations for each curve are given in the text, with adjusted R^2 values of 0.81 for both hosts.

For unadjusted and adjusted percent leaf area infected, significant differences ($P < 0.0001$) were observed among host main effects but not isolate main effects, and a statistically significant host × isolate interaction was observed ($P < 0.0001$). When interaction means were compared using Tukey's studentized range test, a statistically significant ($P = 0.05$) difference was observed between isolates in unadjusted and adjusted percent leaf area infected for only one host (*K. latifolia* 'Olympic Wedding') (Table 2). *P. ramorum* was reisolated from host lesion margins onto PARP selective medium from all hosts except those showing no characteristic disease symptoms.

Inoculum density relationships. Inoculum density studies showed that *P. ramorum* was capable of infecting bearberry and 'Minuet' mountain laurel over a wide range of inoculum levels and as low as 100 sporangia per ml (Fig. 1). No dis-

Table 3. Number of *Phytophthora ramorum* chlamydospores produced on leaf disks of selected ericaceous hosts

	Number of chlamydospores per 6-mm-diameter leaf disk[a]						
	Isolate BBA 9/95			Isolate Pr-52			Overall
Host	Top[b]	Bottom	Total	Top[b]	Bottom	Total	mean[c]
Arctostaphylos uva-ursi	156	55	211	259	102	361	286
Gaultheria procumbens	709	240	949	592	287	879	914
Kalmia angustifolia	2	1	3	7	2	9	6
Pieris japonica	204	86	290	218	150	367	328
Rhododendron arborescens	41	37	78	16	13	29	53
R. carolinianum	110	127	237	95	110	205	221
R. catawbiense	64	72	136	59	145	204	170
Rhododendron 'Cunningham's White'	280	205	485	312	162	474	479
Rhododendron 'Delaware Valley White'	190	131	321	151	121	272	297
Rhododendron 'Girard's Rose'	97	98	195	188	247	434	315
Rhododendron 'Gloria'	102	57	159	92	76	168	164
R. maximum	66	51	117	71	47	118	118
R. minus	46	38	84	55	37	92	88
Rhododendron 'Roseum elegans'	152	126	278	149	117	266	272
Umbellularia californica	359	96	455	782	139	921	688
Vaccinium angustifolium	3	1	4	8	6	14	9
V. corymbosum 'Duke'	1	1	2	2	0	2	2
V. corymbosum 'Blue Crop'	1	1	2	2	0	2	2
V. macrocarpon 'Crowley'	7	5	12	9	7	16	14
V. macrocarpon 'Stevens	1	1	2	2	1	3	3
Zenobia pulverulenta	1	3	4	2	4	6	5
Tukey MSD value ($P = 0.05$)[d]	113	51	132	113	51	132	96

[a] Leaf disks were inoculated by placing in a suspension of 5,000 *P. ramorum* sporangia per ml for 48 h followed by 12 days incubation at 20°C in darkness. Data are means of 24 leaf disk counts (3 replications of 8 leaf disks) for each host/isolate combination.

[b] Numbers of chlamydospores formed on upper (abaxial) and lower (adaxial) surfaces were counted under a dissecting microscope and combined to form the total number of chlamydospores per disk.

[c] Total chlamydospore mean for each host averaged over both isolates.

[d] Minimum significant differences for comparing means via Tukey's studentized range test ($P = 0.05$).

ease was observed at 0 or 50 sporangia per ml. Bearberry showed a significant linear slope component ($Pr > F = 0.0004$) and a nonsignificant ($Pr > F = 0.5241$) quadratic component. The linear regression equation describing the relationship between total lesion area (y) and inoculum density (x) was $y = 0.422 + 0.00326x$ with an adjusted R^2 value of 0.81. Mountain laurel 'Minuet' was also found to have a significant linear slope component ($Pr > F < 0.0001$) and nonsignificant quadratic component ($Pr > F = 0.5921$). The linear regression equation describing the relationship between y and x was $y = 0.1637 + 0.00312x$ with an adjusted R^2 value of 0.81.

Chlamydospore production. Mean chlamydospore numbers produced in 6-mm-diameter leaf disks ranged from 914 in *Gaultheria procumbens* to only 2 chlamydospores produced in tissue of 'Duke' and 'Bluecrop' highbush blueberry following 12 days of incubation at 20°C in darkness (Table 3). The numbers of chlamydospores forming on upper and lower surfaces of the leaf disks were assessed and combined for the total. Significant host effects, isolate effects, and a host × isolate interaction were observed for upper surface chlamydospores produced, lower surface chlamydospores produced, and the total number of chlamydospores produced per leaf disk. Averaged over all hosts, isolate Pr-52 produced significantly ($P = 0.01$) greater numbers of chlamydospores than did isolate BBA 9/95 for upper surface, lower surface, and total chlamydospores. For both isolates, significantly ($P = 0.01$) greater numbers of chlamydospores were produced on upper leaf surfaces compared with lower leaf surfaces.

DISCUSSION

We evaluated the reactions of 51 ericaceous ornamental hosts to two isolates of *P. ramorum* and found that host species and cultivars differ widely in their reactions. After comparison of several inoculation methods, the method we adopted involved dipping host plants into a suspension of 5,000 sporangia per ml followed by 7 days of incubation in a dew chamber at 20°C in darkness. This method is advantageous because it does not require wounding of the host, which might give the pathogen an advantage and result in greater susceptibility than would occur in nature. Sporangia play a major role in dispersal of many *Phytophthora* species, including *P. ramorum*, in which dispersal is hypothesized to occur via wind-splashing rain (12). Thus, using sporangia for inoculation seemed more natural compared with another described method of pinning agar plugs containing hyphae to leaves of host plants (28).

The conditions we used for incubation during our screening tests, 7 days at 20°C in darkness, are unnatural in the degree of darkness to which plants are subjected. Plants subjected to sustained darkness may be more vulnerable to pathogen infection because of their inability to photosynthesize during the incubation period and perhaps also because of physiological effects on other phenomena such as stomate opening and closing. However, in spite of these conditions, we obtained a wide range of susceptibility among the hosts tested, indicating that incubation conditions were not sufficient to overcome resistance of many hosts.

On the basis of mean adjusted percent leaf area infected, averaged over both isolates used for inoculation, cranberry (*V. macrocarpon*), 'Hino Crimson' azalea, and highbush blueberry (*V. corymbosum*) were the most resistant hosts tested, while mountain laurel (*K. latifolia*) and some common *Rhododendron* cultivars were among the most susceptible hosts tested. California bay laurel that sustained approximately 25% leaf area infected in our tests, is thought to play a significant role in providing inoculum for SOD epidemics in California, where it grows commonly as an understory tree in oak forests. Although not as susceptible in terms of percent leaf area infected compared with other susceptible hosts, we and other researchers (12) have observed leaf disks of this host to sustain very high levels of sporangia production when inoculated and placed in water or 1% soil extract. The fact that this host does not rapidly become necrotic when infected with *P. ramorum* may contribute to its ability to provide sustained spore production in the forest ecosystem.

The use of adjusted percent leaf area infected altered disease ratings for some hosts compared with unadjusted percent leaf area infected. For example, *Rhododendron* 'Glacier' showed a mean unadjusted percent leaf area infected of 19.1% but a mean adjusted percent leaf area infected of only 2.3%, showing that the adjustment for the number of leaves of each group of 25 with lesions had a strong effect in some cases. Adjusted percent leaf area infected is likely a more accurate measure of susceptibility or resistance of a given host because it reflects the number of leaves infected as well as the severity of disease on the infected leaves.

The two isolates used for inoculation did not produce significantly different amounts of disease when averaged over all hosts, even though a statistically significant host × isolate interaction was observed. However, for only one host (*K. latifolia* 'Olympic Wedding') was a statistically significant difference observed between isolates for unadjusted and adjusted percent leaf area infected. Thus, in most cases, hosts responded similarly to the two isolates used. This may be surprising given that the two isolates represented genetic extremes within *P. ramorum*. Isolate BBA9/95 originated from *R. catawbiense* in Germany, is part of the European subpopulation of *P. ramorum*, and is of the A1 mating type, while isolate Pr-52 originated from rhododendron in California and is of A2 mating type. Certain differences have been reported to exist between the United States and European *P. ramorum* populations, (3,16) including higher levels of virulence and growth over a wider range of temperatures for European isolates. However, we did not observe major differences between the two isolates used in this study in virulence on the range of hosts tested.

Inoculum density studies showed that *P. ramorum* could infect susceptible hosts bearberry and 'Minuet' mountain laurel with inoculum concentrations ranging from 100 to 5,000 sporangia per ml. The response curve for infection as a function of sporangia concentration showed the same linear slope, and thus, a similar response to increasing inoculum density for both hosts. The minimum spore concentration allowing *P. ramorum* infection to occur was 100 sporangia per ml with both hosts. Determination of this lower limit of inoculum concentration for *P. ramorum* infection of ornamental hosts may prove useful in efforts of APHIS to develop pest risk assessment (PRA) models for spread of *P. ramorum* to new areas. Furthermore, since infection was shown to be possible with only 100 sporangia per ml, the concentration we chose (5,000 sporangia per ml) for routine screening could thus be considered moderate or high and the screening test considered fairly stringent.

Results of chlamydospore quantification studies showed that, in general, susceptible hosts were able to support formation of more chlamydospores in their tissue compared with hosts deemed to be more resistant. An exception was *G. procumbens* (wintergreen). Although appearing to have low susceptibility in the screening tests (mean adjusted percent leaf area infected of 2.1) it supported the largest number of chlamydospores in tissue of any of the hosts tested. This result is significant because it indicates that certain hosts that may sustain very low infection levels can still serve as potent reservoirs of inoculum for *P. ramorum*. Were this phenomenon widespread, it could result in mildly infected hosts producing large amounts of chlamydospore inoculum, which might contribute to epidemics in new areas where these hosts were transported. However, several other hosts in the resistant range, such as highbush blueberry and cranberry, produced virtually no chlamydospores, indicating that host physiology affects the propensity for chlamydospores to form in the tissue. The physiological factors conditioning high or low chlamydospore production in different plant host species should be investigated further. California bay laurel sustained the second highest number of chlamydospores of any host tested, indicating its propensity to support

inoculum production that may contribute to SOD epidemics in areas where it is present along with susceptible oak species.

Our results, documenting the susceptibility of numerous ornamental hosts to *P. ramorum*, will be useful to the states already involved in *P. ramorum* surveys (8) and to those that will be involved in future surveys initiated in response to the recent spread of the pathogen to the eastern United States (32). Knowledge of host reactions under greenhouse conditions may help in choosing target hosts for surveying in nursery settings. Surveyors could focus on finding *P. ramorum* in association with such highly susceptible hosts such as *K. latifolia*, which grows widely in the eastern United States naturally and as a valued landscape plant. Whether the results from our containment greenhouse studies will be paralleled by natural occurrence is unknown. However, some of the hosts we have reported as susceptible, *K. latifolia* and *Pieris japonica*, have since our work began, been reported as *P. ramorum* hosts under natural conditions (26,33; J. Jones, *personal communication*). Thus, our results may be useful in predicting which additional plant species may prove to serve as natural hosts for *P. ramorum*. In the future, additional ericaceous species as well as members of additional plant families should be tested for their susceptibility to *P. ramorum* to determine the likelihood of their serving as natural hosts for the pathogen.

ACKNOWLEDGMENTS

We are very grateful to D. Rizzo and S. Werres for providing the isolates of *P. ramorum* used in this study. A special thanks is owed to D. Rizzo and J. Davidson for providing essential help in learning to work with *P. ramorum*. We thank S. Oak, U.S. Forest Service, Asheville, NC and S. Gordon of Kinney Azalea Gardens, Kingston, RI for assistance in choosing and providing host plants for this study. We thank L. Lamari for considerable help in using the ASSESS software program. We also thank Bell Nursery, Historyland Nursery, Yoder Brothers, Oregon Azaleas, and K. Kosta of the California Department of Food and Agriculture for donating plant material for these studies. We owe much thanks to D. Berner for in-depth guidance on statistical analysis of our data and J. Phillips for input on experimental design. We thank C. Cavin for much assistance in the containment greenhouse and D. Koogle for assistance in plant maintenance.

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