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Comparative Susceptibility of Plants Native to the Appalachian Range of the United States to Inoculation with *Phytophthora ramorum*

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Abstract

Phytophthora ramorum, cause of sudden oak death of trees or ramorum blight of other plant species, has an ever-increasing host range. Some geographic regions are considered to be at high risk of becoming infested with the pathogen, possibly causing plant mortality such as seen in native habitats of California and Oregon. One such region is the Appalachian range of the eastern United States, where known susceptible plants occur and climatic characteristics appear favorable for infections by this pathogen. We collected foliage of a range of plant species native to Appalachia in Kentucky during two summer seasons, and the foliage was shipped to Oregon for inoculation with *P. ramorum* to determine relative susceptibility. Leaves were needle-wounded and inoculated with either mycelium agar plugs or sporangia of a North American (A2 mating type) or European (A1 mating type) isolate. After 14 days incubation at 20°C in moist boxes, lesions caused by either inoculum type or isolate generally were comparable using digital photos and ASSESS software. Some genera, species, and cultivars within species were highly susceptible, while others were moderately susceptible or not susceptible. These results provide a basis for regional surveyors to select target hosts and to generate survey and management practices for nursery and forest areas.

Introduction

The discovery of sudden oak death or ramorum blight, caused by the pathogen *Phytophthora ramorum* (17), on a wide range of trees, shrubs, and ornamental plants in US nurseries, and nurseries and landscapes in several European countries, has resulted in regulation of the pathogen by quarantine to prevent geographic dissemination. Numerous plant species have been shown to be susceptible in nurseries and landscapes (1,2,5,6,7,9). Currently, nurseries are inspected to detect infected plants and thereby prevent dissemination of the disease geographically (7); however, geographic distribution of the pathogen has occurred both nationally and internationally, putting new regions and plant ecosystems at risk.

In 2004, 176 *P. ramorum*-positive sites were found in nurseries in 22 states, mainly resulting from dissemination of camellia plants from nurseries in California. National nursery surveys were performed in 48 states in 2005, and seven states were found to have nurseries with plants that tested positive for *P. ramorum* (8). While the number of US nurseries with infected plants decreased in 2005 compared to 2004, infected plants may have been shipped to many states and it has not been possible to recover all the plants shipped from *P. ramorum*-positive nurseries. Infected plants could serve as a source of inoculum for infection of plants in the urban and natural landscapes.

Native plants of the Appalachian range of the eastern US are especially at risk due to the favorable environmental conditions for the disease. Sudden oak death and ramorum blight are nursery and forest problems that could have a major impact on eastern forest systems should the pathogen be introduced. Based on the known host list (16), native and horticultural varieties of rhododendrons, viburnums, mountain laurel, maples, and other plants growing in central Appalachia could become infected with *P. ramorum*. However, little information is available about the pathogenicity and sporulation potential of *P. ramorum* on other eastern native plant species. Some information about the susceptibility of plants native to Oregon and California and of some ornamental plants has been published (1,5,8,11,12). Fifty-one plants important for the nursery industry, such as members of the Ericaceae (such as e.g. *Rhododendron*) and Caprifoliaceaee (e.g., *Viburnum*) have been evaluated for susceptibility to *P. ramorum* (16). Several groups of researchers in North America and in Europe are studying the susceptibility of horticultural species and varieties of *Viburnum spp*., *Rhododendron* spp. and mostly ornamental plants in other genera (3,4,5,14). Although some of this information is not yet published, it will add considerably to the understanding of *P. ramorum* and the diseases it causes on ornamental plants. In most of these studies, it was observed that susceptibility of the plants varied greatly between species and cultivars within species.

Table 1. List of plants tested for susceptibility to *Phytophthora ramorum*.

Plant scientific name	**Plant common name**	**Family**
Acer rubrum	Red maple	Aceraceae
Alnus serrulata	Common alder	Betulaceae
Amorpha fruticosa	False indigo bush	Fabaceae
Asimina triloloba	Pawpaw	Annonaceae
Betula nigra	River birch	Betulaceae
Calycanthus fertilis	Carolina allspice	Calycanthaceae
Carpinus caroliniana	American hornbeam	Betulaceae
Carya cordiformis	Bitternut hickory	Juglandaceae
Carya laciniosa 'Fayette'	Shellbark hickory 'Fayette'	Juglandaceae
Carya ovata	Shagbark hickory	Juglandaceae
Castanea pumila	Chinkapin	Fagaceae
Cephalanthus occidentalis	Buttonbush	Rubiaceae
Chionanthus virginicus	American fringetree	Olearaceae
Cornus amomum	Silky dogwood	Cornaceae
C. foemina	Swamp dogwood	Cornaceae
C. florida	Flowering dogwood	Cornaceae
Corylus americana	Hazelnut	Betulaceae
Euonymus americanus	Strawberry bush	Celastraceae
E. atropurpureus	Burningbush	Celastraceae
Fraxinus americana	White ash	Oleaceae
F. pennsylvanica	Green ash	Oleaceae
Halesia carolina	Carolina silverbell	Styracaceae
Juglans nigra	Black walnut	Juglandaceae
Ligustrum vulgare	Privet	Oleaceae

(continued)

Table 1. (continued)

Plant scientific name	Plant common name	Family
Lindera benzoin	Spicebush	Lauraceae
Liquidambar styraciflua	Sweetgum	Hamamelidaceae
Liriodendron tulipifera	Tulip poplar	Magnoliaceae
Nyssa sylvatica	Blackgum	Cornaceae
Ostrya virginiana	Ironwood	Betulaceae
Oxydendrum arboreum	Sourwood	Ericaceae
Prunus serotina	Black Cherry	Rosaceae
Rhamnus caroliniana	Carolina buckthorn	Rhamnaceae
Rhododendron cumberlandense	Cumberland red azalea	Ericaceae
R. maximum	Rosebay azalea	Ericaceae
Rhus copallinum	Winged sumac	Anacardiaceae
R. glabra	Smooth sumac	Anacardiaceae
R. typhina	Staghorn sumac	Anacardiaceae
Robinia hispida	Bristly locust	Fabaceae
Rosa setigera	Prairie rose	Rosaceae
R. palustris	Swamp rose	Rosaceae
Rubus occidentalis	Wild raspberry	Rosaceae
Sambucus canadensis	Elderberry	Caprifoliaceae
Staphylea trifolia	Bladdernut	Staphyeaceae
Symphoricarpos orbiculatus	Coralberry	Caprifoliaceae
Tilia americana	Basswood	Tiliaceae

In previous studies, the following species of trees native to eastern forests were found to be susceptible to infection by stem and leaf inoculations with agar plugs and/or suspensions of sporangia: northern red oak (*Quercus rubra*), white oak (*Q. alba*), cherrybark oak (*Q. pagoda*), chestnut oak (*Q. prinus*), laurel oak (*Q. laurifolia*), live oak (*Q. virginiana*), water oak (*Q. nigra*), willow oak (*Q. phellos*), sugar maple (*Acer saccharum*), and black walnut (*Juglans nigra*); however, susceptibility of the plants varied greatly between species. Although not native to eastern forests, coast live oak (*Q. agrifolia*) was also tested in this study (13).

Eastern US regions are currently being surveyed in an attempt to detect introductions from other regions, especially in areas surrounding nurseries where disease has been found. However, the relative susceptibility of many of the over-story, mid-story, and under-story plants in those areas is unknown, making field inspections extremely difficult due to the high diversity of plant species therein. Some knowledge of the susceptibility of eastern native plant species would be extremely useful to inspectors, and would generate management strategies for the nurseries and for eastern forests to reduce the risk of establishment of *P. ramorum*.

Thus, our objective was to determine the susceptibility to inoculation with *P. ramorum* of a sampling of plant species that represent several important species to the Appalachian range.

Cultures, Inoculation, and Disease Severity Ratings

The two isolates of *P. ramorum* used in this study were cultured originally from plants in an Oregon macro-propagation production nursery. Isolate 03-74-N10-A (N10A) was recovered from rhododendron (*Rhododendron* sp.) and is a

North American genotype of the A2 mating type. Isolate 03-74-D12-A (D12A) (European genotype, A1 mating type) was recovered from Doublefile viburnum (*Viburnum plicatum* var. *tomentosum* 'Mariesii') in the same nursery. Cultures were maintained and stored on agar slants at 20°C until used, and then were transferred frequently on dilute V8 juice agar plates (30 ml/liter of clarified V8 juice instead of the normal 150 to 200 ml/liter) (6). Sporangia used as inoculum were produced on dilute V8 juice agar plates, starting from a sporangial suspension spread on the plates, and incubated at 20°C for 10 days. Sporangia were removed from the plates by flooding with 5 ml of sterile distilled water and scraping the surface of the agar with the edge of a spatula. The aqueous suspension of cauducous sporangia was then poured into a beaker and gently swirled using a magnetic stirrer.

Prior to inoculation, leaves were wounded with a single needle probe on the upper side of a leaf, two wounds alternating on opposite sides of a midvein. In the first year of testing, two forms of inoculation were used: (i) Ten µl of sporangia from isolate N10A, containing 930 sporangia/ml, were pipetted onto each wound; or (ii) mycelium plugs (6-mm diameter) of N10A and D12A were inverted and placed onto the wound site on the upper side of the leaves. In the second year, only sporangia of N10A were used, and 10 µl of sporangial suspension (containing 1050 sporangia/ml) was pipetted into each wound. Four replicate leaves were inoculated in each treatment, plus a control, which was wounded and received an equivalent agar plug with no pathogen, or 10 µl of sterile distilled water.

Inoculated leaves were placed in plastic box chambers with moist vermiculite in the bottom to maintain high humidity. Leaves were allowed to sit for 2 h after inoculation at 21°C before they were sprayed with enough distilled water to provide some free-standing droplets. The sealed containers were then placed in a dark 20°C incubator for 14 days, then examined for severity of lesions. Lesion area, as a percentage of each total leaf area, was assessed quantitatively from digital photographs using the lesion assay software ASSESS (American Phytopathological Society, St. Paul, MN).

Lesion percentage data were transformed to arcsine-square root values and sporangia counts were log-transformed prior to analyses to normalize variances. Data from two years were combined since variance among trials was homogenous by Bartlett's test using Systat 11 (Systat Software Inc., Richmond, CA).

Transformed data were analyzed by one-way analyses of variance (ANOVA), but real data are presented in our results. Mean comparison tests were not used due to the extreme range of the data. Instead, treatment means of lesion percentages were compared on each cultivar using strict 95% confidence intervals. Means that did not overlap at the 95% confidence interval were considered statistically different.

Comparative Susceptibility of Inoculated Plants

Foliage of native plants from the Appalachian range in Kentucky (Table 1), collected from native stands and arboreta, were shipped overnight to Oregon where they were inoculated with mycelial plugs or sporangia of *P. ramorum*. Inoculations were performed in Oregon rather than in Kentucky due to quarantine restrictions. Quantitative estimates of lesion sizes (% of total leaf area with lesions resulting from inoculation) indicated considerable variation in susceptibility from either form of inoculum or isolate of *P. ramorum*. Results from one year of plug inoculations with two isolates on under-story and mid- to over-story plants are shown in Figures 1 and 2; data from two years of sporangial inoculations with only isolate N10A are shown in Figures 3 and 4. They indicate the high susceptibility of plants such as black cherry, black walnut, green ash, and the low susceptibility of plants such as red maple, hickory, sweetgum, etc. In some cases, inoculation with sporangia induced more infection than with mycelial plugs; in other cases, mycelial plugs induced larger lesions. Sporangia are a more natural form of inoculum, and results using sporangia are probably more indicative of true susceptibility than with mycelial plugs.

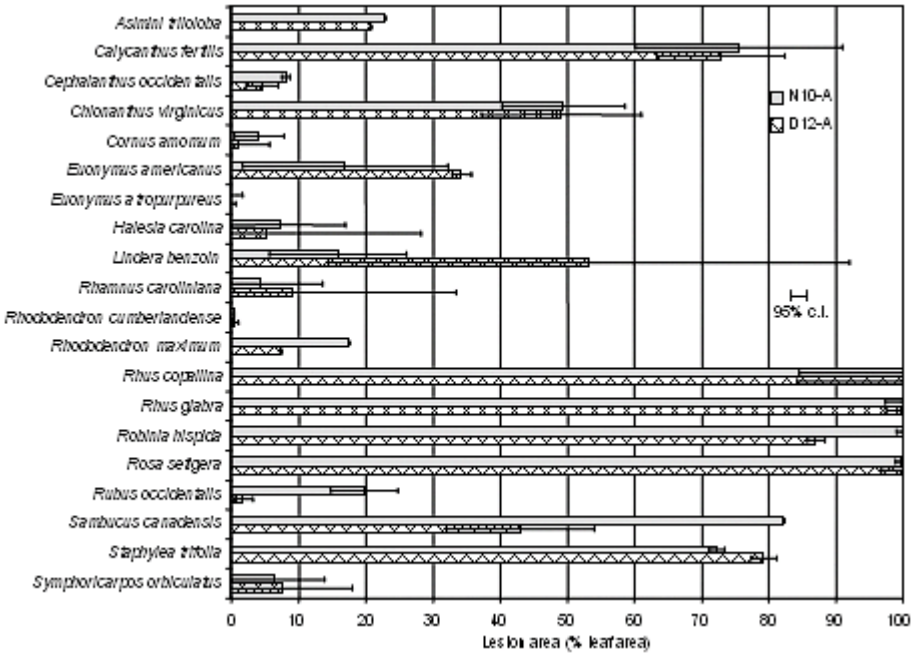

Fig. 1. Lesion areas of detached leaves from under-story plants plug-inoculated with *P. ramorum* isolates N10A and D12A. Percentages are based on the summed areas of four wound sites per leaf and are the averages of four replicate leaves per plant per isolate. Trial conducted once during 2005. Ninety-five percent confidence intervals are indicated in each bar.

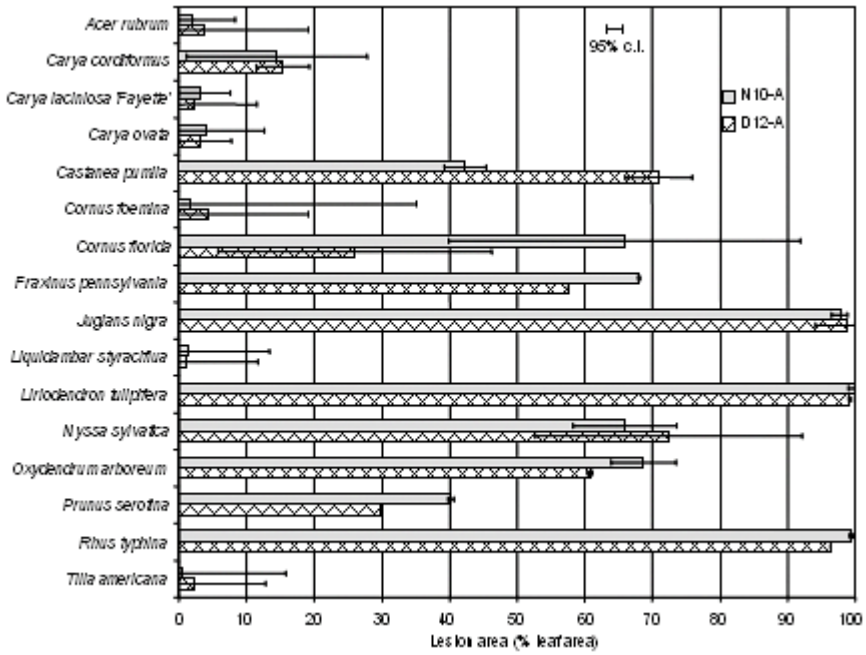

Fig. 2. Lesion areas of detached leaves from mid- and over-story plants plug-inoculated with *P. ramorum* isolates N10A and D12A. Percentages are based on the summed areas of four wound sites per leaf and are the averages of four replicate leaves per plant per isolate. These trials were conducted once during 2005. Ninety-five percent confidence intervals are indicated in each bar.

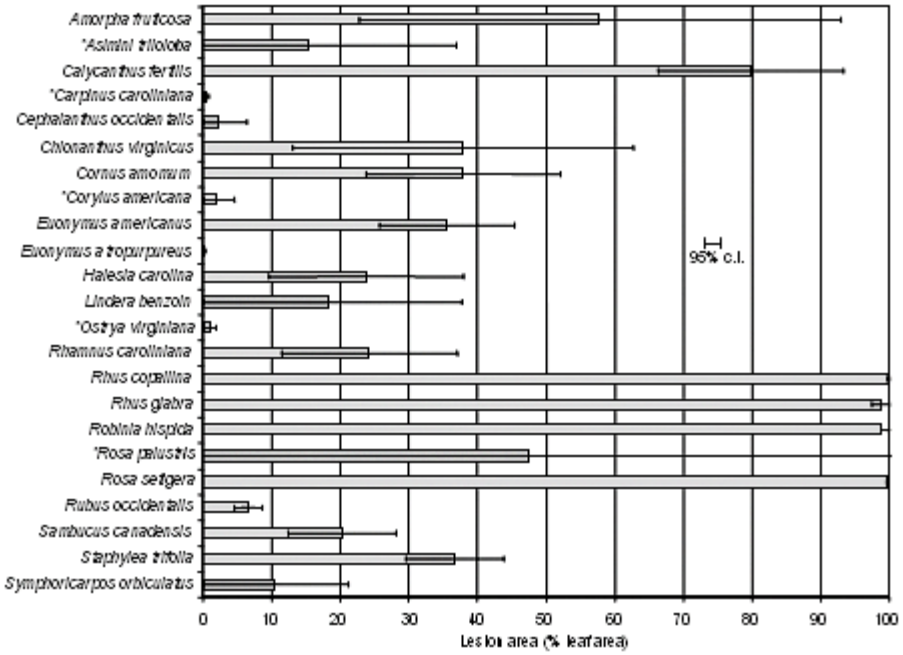

Fig. 3. Lesion areas of detached leaves from under-story plants inoculated with sporangia of *P. ramorum* isolate N10A. Percentages are based on the summed areas of four wound sites per leaf and are the averages of four replicate leaves per plant. Results are the means of two trials during 2005 and 2006, except where noted with asterisk (*), which were inoculated only once. Ninety-five percent confidence intervals are indicated in each bar.

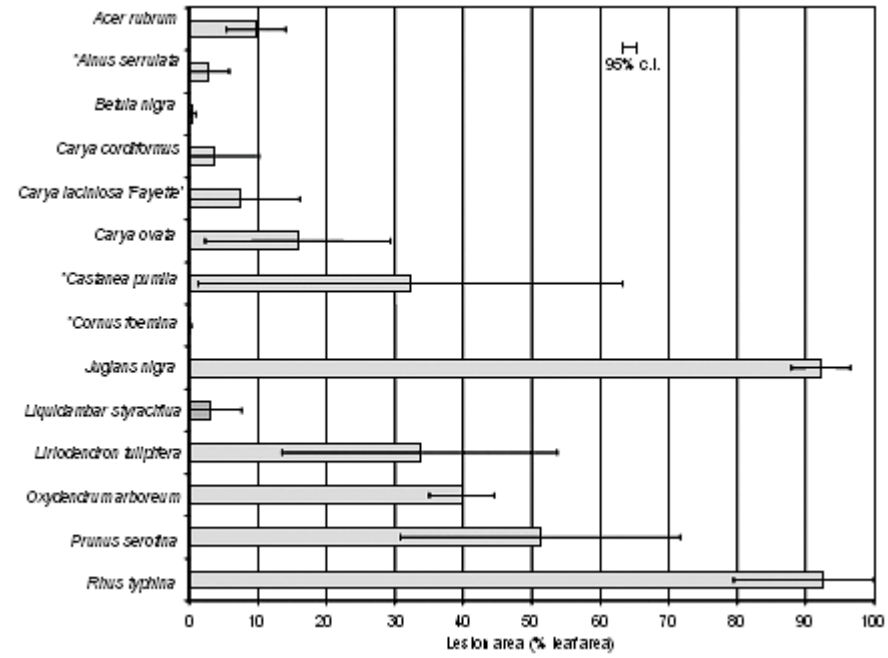

Fig. 4. Lesion areas of detached leaves from mid- and over-story plants inoculated with sporangia of *P. ramorum* isolate N10A. Percentages are based on the summed areas of four wound sites per leaf and are the averages of four replicate leaves per plant. Results are the means of two trials during 2005 and 2006, except where noted with asterisk (*), which were inoculated only once. Ninety-five percent confidence intervals are indicated in each bar.

Fig. 5. Relative susceptibility of native plants from the Appalachian range of the eastern United States to inoculation with sporangia of *Phytophthora ramorum*. One non-inoculated control leaf is on the left in all cases. Top to bottom: Spice bush, Prairie rose, Bladdernut, and Alder.

In 2005, using mycelial plug inoculum, lesion sizes between plant species were highly significant ($P < 0.001$). Lesion sizes induced by either isolate were not significantly different; however, the interaction of plant species × isolate was highly significant ($P < 0.001$). Highly significant differences were found in lesion sizes among the different host species induced by inoculation with N10A sporangia (combined two-year data).

Discussion

There was a high degree of variation in susceptibility between species of the native plants tested, and that information should be useful when surveys are conducted to detect introductions into the Appalachian range, especially near nurseries that were found to have infected plants. Plants identified as highly susceptible through these inoculation studies should be examined more closely than those that appeared to have a low level of susceptibility or those that were not susceptible at all. However, it is possible that leaves of some plants may exhibit only small lesions, although the pathogen could sporulate heavily on the infected tissue, such as on leaves of the bay laurel trees in California. Such plants could be a source of inoculum that would cause the pathogen to be spread within the forest system even before being detected on highly susceptible host plants (2).

The use of sporangial inoculum to determine relative susceptibility of the plant species tested proved to be very reliable, and the inoculum levels used were high enough in both years than were needed to initiate lesions on susceptible plants, based on other studies comparing sporangial inoculum levels. Sporangial inoculum of the two isolates usually caused similarly sized lesions when comparisons were made the first year. While there was considerable variation in reaction to inoculation between species, that variation could easily have been due to the time of plant tissue collection, variation in edaphic and other environmental conditions, and even age of plants. Those factors have not been studied extensively, and we had no way of being totally consistent between the two years of the studies.

One can conclude from these studies that there are numerous plants in the Appalachian range that could be infected should *P. ramorum* be introduced. The susceptible plants occur in under-story, mid-story, and over-story plant species, so highly susceptible plants should be examined at all levels. This situation is comparable to our earlier findings (4,6) when inoculating a range of nursery plants, that there are species and cultivars within species that are highly susceptible that have not been infected naturally and are therefore not on the host list (16). We found in this study that a native *Acer rubrum* was not susceptible, but we also inoculated several commercial cultivars from arboreta and found some to be highly susceptible and others not (*unpublished data*). Thus, surveyors should expect that there can be considerable variation in susceptibility of plants within the varied forest ecosystems.

Acknowledgment and Disclaimers

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