

# Susceptibility of *Viburnum* Species and Cultivars to *Phytophthora ramorum*<sup>1</sup>

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## Abstract

*Phytophthora ramorum* causes ramorum blight on *Viburnum* species, which are commonly grown as ornamentals. This study evaluated 24 different species or cultivars for susceptibility to *P. ramorum*. Whole plants were inoculated with an NA1 isolate of *P. ramorum* and placed in dew chambers at 20C (68F). After 5 days, the percentage of necrotic leaves for each plant and the percentage of necrotic area for each leaf were calculated. The percentage of necrotic leaves per plant ranged from 96.1% (*V. tinus*) to 7.9% (*V. opulus* 'Notcutt') and the percentage of necrotic leaf area ranged from 73.0% (*V. × carlcephalum* 'Cayuga') to 2.4% (*V. trilobum* 'Wentworth'). In addition, six species or cultivars were evaluated for their susceptibility to a EU1 isolate of *P. ramorum*. The isolate had a significant effect on three of the six species or cultivars tested, although one isolate did not always yield the greatest necrosis. Evergreen species and cultivars had a higher percentage of necrotic leaves and higher percentage of necrotic leaf area than semi-evergreen or deciduous species and cultivars. All *Viburnum* species and cultivars showed some degree of susceptibility to *P. ramorum*.

**Index words:** host range, ornamental, ramorum blight, sudden oak death.

**Species used in this study:** Allegany lantanaphyllum viburnum (*V. × rhytidophylloides* 'Allegany'); arrowwood viburnum (*V. dentatum*); blue muffin arrowwood viburnum (*V. dentatum* 'Blue Muffin'); Burkwood viburnum (*V. × burkwoodii*); Cayuga hybrid viburnum (*Viburnum × carlcephalum* 'Cayuga'); Chesapeake service viburnum (*V. utile* 'Chesapeake'); Conoy Burkwood viburnum (*V. × burkwoodii* 'Conoy'); Cree leatherleaf viburnum (*V. rhytidophyllum* 'Cree'); Cunningham's white rhododendron (*Rhododendron* 'Cunningham's White'); dwarf Koreanspice viburnum (*V. carlesii* 'Compactum'); Erie linden viburnum (*V. dilatatum* 'Erie'); European snowball viburnum (*V. opulus* 'Roseum'); fragrant snowball viburnum (*V. × carlcephalum*); Judd viburnum (*V. × juddii*); laurustinus viburnum (*V. tinus*); linden viburnum (*V. dilatatum*); Maries doublefile viburnum (*V. plicatum* var. *tomentosum* 'Mariesii'); Mohawk Burkwood viburnum (*V. × burkwoodii* 'Mohawk'); Notcutt European cranberrybush viburnum (*V. opulus* 'Notcutt'); Prague viburnum (*V. × pragense*); Sarcoxii hybrid viburnum (*V. × burkwoodii* 'Sarcoxii'); Shasta doublefile viburnum (*V. plicatum* var. *tomentosum* 'Shasta'); summer snowflake doublefile viburnum (*V. plicatum* var. *tomentosum* 'Summer Snowflake'); Wentworth American cranberry viburnum (*V. trilobum* 'Wentworth'); willowwood lantanaphyllum viburnum (*V. × rhytidophylloides* 'Willowwood').

## Significance to the Nursery Industry

Viburnums are important landscaping shrubs to the nursery industry. While they are considered relatively pest-free, they are in a high risk host group for *P. ramorum*, the causal agent of sudden oak death. However, variations in susceptibility are expected since there are more than 160 *Viburnum* species. Whole plants of 24 commercially available *Viburnum* species or cultivars were tested by inoculation followed by evaluation of the number of symptomatic leaves and area of symptomatic tissue on each leaf. All plants tested exhibited some symptoms; however, some had more severe symptoms than others. Based upon the two evaluation criteria, the most susceptible species and cultivars were *V. tinus* and *V. × carlcephalum* 'Cayuga', while the most tolerant were *V. opulus* 'Notcutt' and *V. × rhytidophylloides* 'Willowwood'. This research is important as it increases our knowledge on which plants should be avoided in pathogen infested areas and it identifies more tolerant or resistant species and cultivars that can be used in a breeding program to develop new

tolerant varieties, helping reduce the risk of long distance spread of this pathogen.

## Introduction

*Viburnum* species (Dipsacales) are one of the most popular flowering landscape shrubs in the United States. In 2006, sales of broadleaf evergreens and deciduous shrubs, including viburnums, were \$839 and \$648 million, respectively, in the United States (29). Viburnums are relatively easy to grow and have a variety of attractive characteristics including constant leaves in evergreen species, fragrant flowers that attract butterflies and bees, and production of berries that may be consumed by birds and other animals. They also can be grown in a wide range of climates, having centers of species diversity in eastern Asia and Latin America (32).

Most viburnums are relatively pest-free and have few serious foliar diseases. However, with the discovery of *Phytophthora ramorum* causing ramorum blight or ramorum shoot dieback on different *Viburnum* spp. and cultivars (19, 30), there has been interest in the role that viburnums may play in the epidemiology of this pathogen. *Phytophthora ramorum* is also the causal agent of sudden oak death, causing mortality of oak and tanoak trees in forests of the western United States. Ornamentals are believed to play an important role in the long distance spread of this pathogen (13). The entire *Viburnum* genus has been placed on the *P. ramorum* host list (27), having been grouped into one of the five high risk genera that includes *Camellia*, *Kalmia*, *Pieris*, and *Rhododendron* (28). However, since there are approximately 160 *Viburnum* species, a range of susceptibility is expected. Therefore, it is important to identify which individual spe-

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cies or cultivars are more resistant to this disease in order to minimize risk of pathogen dispersal on container-grown nursery stock and could be utilized in a breeding program.

Previous studies regarding the susceptibility of some *Viburnum* species and cultivars to *P. ramorum* have been conducted using detached leaves (9, 10, 18). Detached leaf bioassays can generally indicate host susceptibility, but the method is highly artificial and of doubtful accuracy (4, 20). The objective of this study was to compare differences in susceptibility to *P. ramorum* among *Viburnum* species and cultivars using whole plants rather than detached leaves. Inoculation of whole plants of different *Viburnum* species and cultivars was done using *P. ramorum* zoospores. The percentage of leaves per individual plant that became necrotic and the percentage of necrotic leaf tissue area for each necrotic leaf were evaluated, allowing species and cultivars to be compared for susceptibility to *P. ramorum* using two different criteria.

## Materials and Methods

**Plant materials.** Twenty-four *Viburnum* species or cultivars were selected for this study based upon availability and taxonomic relatedness. *Viburnum* species and cultivars were obtained from greenhouse production plants at Angelica Nurseries, Inc. (Kennedyville, MD) or from cuttings from the United States National Arboretum (Washington, DC). The species or cultivars used are listed in Table 1. All plants were maintained in the greenhouse in 10 cm (4 in) pots. For deciduous species or cultivars, the plants were used only after the leaves were fully expanded and allowing them some time to mature (minimum of 1 month time). For evergreen or semi-evergreen species or cultivars, plants were tested after the new growth flush to allow fully expanded leaves time to mature (minimum of 1 month). Plants selected for testing

were uniform in size. If not uniform, plants were pruned to 10 to 20 total leaves. Uniform *Rhododendron* 'Cunningham's White' rooted cuttings in 5 cm (2 in) pots, obtained from a commercial nursery, were trimmed to leave approximately 10 to 20 leaves per plant and used as a positive control because its susceptibility to *P. ramorum* is well documented (25).

**Pathogen isolates.** *Phytophthora ramorum* isolates WSDA-1772 [lineage NA1 (8)] originally isolated from *Viburnum plicatum* var. *tomentosum* 'Mariesii' in Oregon, and PRN-1 [lineage EU1 (8)], originally isolated from *Rhododendron* sp. in The Netherlands, were used in this study. These isolates were selected since they had been used in a previous study (31) with consistent results and infection. The isolates, which were maintained on 20% clarified V8 agar at 20C (68F), were occasionally re-isolated from infected plant tissue by surface-sterilizing the inoculated leaves and plating onto PARPH+V8 selective medium (6).

Zoospores were used as inoculum and prepared as described by Widmer (31). Five 4-mm plugs of each isolate were added to individual 60-mm plates containing sterile 20% V8 broth. Cultures were incubated 3 days in the dark at 20C (68F). The resulting mycelium was rinsed three times in sterile 0.1 mM 2-[N-morpholino]ethanesulfonic acid (MES) buffer, pH 6.2 (herein referred to as MES buffer) one day before inoculation and maintained overnight at 20C (68F) in the dark. Zoospores were induced to release from sporangia by placing the cultures at 4C (39F) for 30 min, followed by incubation at room temperature. After 30 to 45 min, released zoospores were filtered through four layers of cheesecloth into a beaker. The concentration of the zoospore suspension was determined by diluting the suspension in MES buffer, vortexing to induce encystment, and counting encysted zoospores on a hemacytometer. The inoculum suspension was adjusted to a final concentration of 50,000 zoospores/ml by

**Table 1.** *Viburnum* species and cultivars evaluated for susceptibility to *Phytophthora ramorum*.

Species	Cultivar	Section	Foliage characteristic	Isolate tested
<i>V. × burkwoodii</i>	—	<i>Lantana</i>	Semi-evergreen	WSDA-1772
<i>V. × burkwoodii</i> ( <i>V. × burkwoodii</i> × <i>V. carlesii</i> )	Mohawk	<i>Lantana</i>	Evergreen	WSDA-1772
<i>V. × burkwoodii</i> ( <i>V. × burkwoodii</i> × <i>V. carlesii</i> )	Sarcoxie	<i>Lantana</i>	Semi-evergreen	WSDA-1772; PRN-1
<i>V. × burkwoodii</i> ( <i>V. × burkwoodii</i> × <i>V. utile</i> )	Conoy	<i>Lantana</i>	Evergreen	WSDA-1772
<i>V. × carlecephalum</i> ( <i>V. × carlecephalum</i> × <i>V. carlesii</i> )	Cayuga	<i>Lantana</i>	Deciduous	WSDA-1772
<i>V. × carlecephalum</i> ( <i>V. carlesii</i> × <i>V. macrocephalum</i> )	—	<i>Lantana</i>	Deciduous	WSDA-1772
<i>V. carlesii</i>	Compactum	<i>Lantana</i>	Deciduous	WSDA-1772
<i>V. dentatum</i>	—	NW <i>Odontotinus</i>	Deciduous	WSDA-1772; PRN-1
<i>V. dentatum</i>	Blue Muffin	NW <i>Odontotinus</i>	Deciduous	WSDA-1772
<i>V. dilatatum</i>	—	OW <i>Odontotinus</i>	Deciduous	WSDA-1772
<i>V. dilatatum</i>	Erie	OW <i>Odontotinus</i>	Deciduous	WSDA-1772
<i>V. × juddi</i> ( <i>V. carlesii</i> × <i>V. bitchiuense</i> )	—	<i>Lantana</i>	Deciduous	WSDA-1772
<i>V. opulus</i>	Notcutt	<i>Opulus</i>	Deciduous	WSDA-1772
<i>V. opulus</i>	Roseum	<i>Opulus</i>	Deciduous	WSDA-1772
<i>V. plicatum</i>	Summer Snowflake	<i>Pseudopulus</i>	Deciduous	WSDA-1772
<i>V. plicatum</i> var. <i>tomentosum</i>	Mariesii	<i>Pseudopulus</i>	Deciduous	WSDA-1772; PRN-1
<i>V. plicatum</i> var. <i>tomentosum</i> ( <i>V. plicatum</i> var. <i>tomentosum</i> × <i>V. plicatum</i> var. <i>tomentosum</i> 'Mariesii')	Shasta	<i>Pseudopulus</i>	Deciduous	WSDA-1772
<i>V. × pragense</i> ( <i>V. rhytidophyllum</i> × <i>V. utile</i> )	—	<i>Lantana</i>	Evergreen	WSDA-1772
<i>V. × rhytidophylloides</i> ( <i>V. rhytidophyllum</i> × <i>V. rhytidophylloides</i> )	Allegany	<i>Lantana</i>	Semi-evergreen	WSDA-1772; PRN-1
<i>V. × rhytidophylloides</i> ( <i>V. rhytidophyllum</i> × <i>V. rhytidophylloides</i> )	Willowwood	<i>Lantana</i>	Deciduous	WSDA-1772; PRN-1
<i>V. rhytidophyllum</i>	Cree	<i>Lantana</i>	Evergreen	WSDA-1772
<i>V. trilobum</i>	Wentworth	<i>Opulus</i>	Deciduous	WSDA-1772
<i>V. tinus</i>	—	<i>Viburnum</i>	Evergreen	WSDA-1772; PRN-1
<i>V. utile</i>	Chesapeake	<i>Lantana</i>	Evergreen	WSDA-1772

pipetting a specific amount in MES buffer. At least 200 ml of inoculum was prepared, depending upon the number of plants to be inoculated.

**Viburnum susceptibility.** All *Viburnum* species and cultivars were inoculated with *P. ramorum* isolate WSDA-1772 in a randomized complete design. Due to limited space in the dew chambers, plants were inoculated in groups consisting of at least two species or cultivars, which were randomly selected. Based upon instrument recordings, it was assumed that temperature and dew were not variable between experimental runs or dew chamber used. The use of these dew chambers for susceptibility studies has been validated in past tests (23, 25). For each group, four plants of each species or cultivar and one rhododendron were inoculated with the zoospore suspension until run-off using a hand-pump sprayer. Inoculum was applied to the whole plant and both leaf surfaces were sprayed as uniformly as possible. One non-inoculated plant of each species or cultivar was treated with MES buffer alone as a negative control.

Inoculated plants were placed immediately in a dew chamber maintained at 100% relative humidity at 20C (68F) in the dark. After 5 days, the plants were removed and evaluated for symptoms. Quantification of necrosis was done by detaching the leaves from each plant and scanning them using an HP Scanjet 5500C model flatbed scanner (Hewlett-Packard; Palo Alto, CA). The percentage of necrotic leaf surface area for each leaf, including the controls, was calculated using ASSESS software (APS Press; St. Paul, MN). For the inoculated plants, a leaf was considered positive for necrosis if the leaf had a higher percentage of necrotic leaf surface area than the control average for that particular species or cultivar. Each species or cultivar was tested in three different groups for a total of 12 plants each.

**P. ramorum isolate comparison.** Sufficient plant material was available to test only *V. dentatum*, *V. × burkwoodii* (*V. × burkwoodii* × *V. carlesii*) ‘Sarcoxie’, *V. × rhytidophylloides* ‘Allegany’, *V. × rhytidophylloides* ‘Willowwood’, *V. plicatum* var. *tomentosum* ‘Mariesii’, and *V. tinus* for susceptibility to *P. ramorum* isolate PRN-1. The inoculation trials were set-up and analyzed as described above.

**Statistical analyses.** To avoid pseudo-replication, the mean percentage of necrotic leaf area for each plant was used as the subsample (5). Data for the percentage of leaves per plant that were necrotic and the percentage of necrotic area of each leaf failed the normality test based on the Shapiro-Wilk test ( $P \geq 0.05$ ) and were transformed using arcsine square root (7). Transformed data for the percentage of leaves per plant that were necrotic passed the subsequent Shapiro-Wilk test for normality and were analyzed further using PROC MIX in SAS for Windows (version 9.1). Cultivar data were compared using Duncan’s multiple range test ( $\alpha = 0.05$ ). However, the transformed data for the percentage of necrotic leaf area of each leaf also failed the Shapiro-Wilk test. Therefore, the non-parametric Kruskal-Wallis test using PROC NPAR1WAY in SAS for Windows was conducted on the ranked values (21). A post hoc non-parametric multiple comparison was performed according to Dunn’s method as listed in Hollander and Wolfe (12) using a SAS macro (14). Non-transformed data are presented for clarity in the presentation.

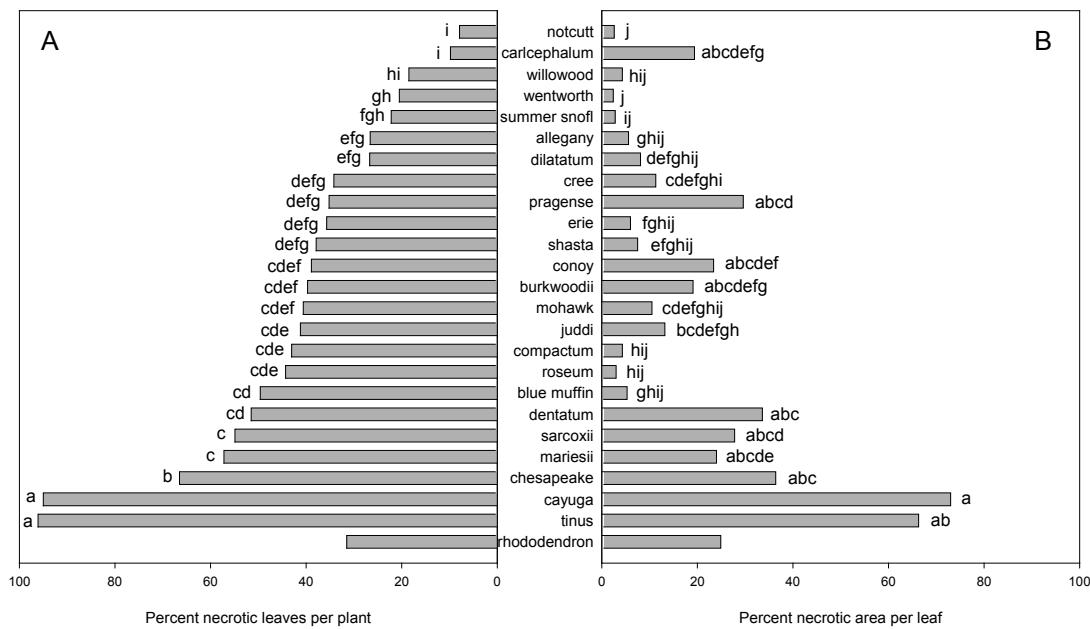
## Results and Discussion

In this study, viburnum susceptibility was evaluated only by inoculating the above-ground plant parts. Although *P. ramorum* does have an important soil phase (22), it is generally considered an above-ground pathogen. As such, susceptibility studies are generally conducted on leaves or inner bark (e.g. 3, 9, 11, 18, 23, 24, 25). For all plants tested, necrosis was observed only on the foliage and never on stems. However, data were collected only 5 days after inoculation, which may not have been enough time for necrotic symptoms to develop on the stems (16).

Results demonstrated a wide range of susceptibility of the *Viburnum* spp. or cultivars to *P. ramorum* (Figs 1A & 1B). Considering both percentage of necrotic leaves per plant and percentage of necrotic leaf area, the most susceptible species or cultivars tested were *V. × carlcephalum* ‘Cayuga’ and *V. tinus*, while the most tolerant were *V. × rhytidophylloides* ‘Willowwood’ and *V. opulus* ‘Notcutt’. In general, the wide range of susceptibility to *P. ramorum* among *Viburnum* species or cultivars was similar to that reported by Grunwald et al. (10) using detached leaves. However, different reactions among commonly tested species or cultivars were noted between the two studies. Results from this study highlight the importance of doing whole plant testing for assessing susceptibility rather than relying on necrosis observed on a detached leaf. For example, *V. × carlcephalum* exhibited a high necrotic leaf area but was statistically included in the group with the fewest number of necrotic leaves (Figs. 1A & 1B). Some cultivars or species, such as *V. dentatum* ‘Blue Muffin’, *V. opulus* ‘Roseum’, and *V. carlesii* ‘Compactum’ had a comparatively high percentage of necrotic leaves, but had among the lowest percentage of necrotic leaf area. These results are very important because lesion size alone has not been correlated with quantity of spore production (17, 26), which has epidemiological implications. Therefore, percentage of necrotic leaves becomes an important component that would be missed in a study where disease is assessed only on detached leaves in evaluating the susceptibility of a species or cultivar.

Results of the test comparing two different *P. ramorum* isolates on six different *Viburnum* species and cultivars were not consistent (Table 2). For *V. dentatum* ( $H = 5.326$ , 1 d.f.,  $P = 0.0210$ ), isolate WSDA-1772 had a higher percentage of necrotic leaf area, while isolate PRN-1 caused higher necrosis in leaves of *V. × burkwoodii* (*V. × burkwoodii* × *V. carlesii*) ‘Sarcoxie’ ( $H = 13.186$ , 1 d.f.,  $P = 0.0003$ ) and *V. × rhytidophylloides* ‘Allegany’ ( $H = 4.364$ , 1 d.f.,  $P = 0.0367$ ). No differences were observed among *V. × rhytidophylloides* ‘Willowwood’ ( $H = 1.524$ , 1 d.f.,  $P = 0.217$ ), *V. plicatum* var. *tomentosum* ‘Mariesii’ ( $H = 1.095$ , 1 d.f.,  $P = 0.295$ ), and *V. tinus* ( $H = 3.203$ , 1 d.f.,  $P = 0.0735$ ). Based upon these results and from other studies that compared differences in lesion development between European and North American isolates (2, 10, 15), it is difficult to conclude if there are differences in pathogenicity among different pathogen lineages.

When data were analyzed after grouping plants according their leaf shedding pattern (evergreen, semi-evergreen, or deciduous), this trait significantly affected the percentage of necrotic leaves per plant ( $P < 0.0001$ ) and the percentage of necrotic area per leaf ( $H = 42.57$ , 2 d.f.,  $P < 0.0001$ ). The data are graphically displayed in Fig. 2. Using both of the above criteria, the evergreen species or cultivars were more susceptible to *P. ramorum*. Evergreen species or cultivars consisted



**Fig. 1.** (A) The mean percentage of necrotic leaves per plant and (B) the mean percentage of necrotic leaf area for each leaf of tested *Viburnum* spp. and cultivars. Bars with the same letter were not significantly different according to (A) Duncan's multiple range test ( $\alpha = 0.05$ ) or (B) Kruskal-Wallis nonparametric test ( $\alpha = 0.05$ ) followed by a nonparametric multiple comparison. Values for Rhododendron 'Cunningham's White' are added for comparison purposes only. Abbreviations correspond to species or cultivar tested and are as follows: notcutt = *V. opulus* 'Notcutt'; caricephalum = *V. × caricephalum* (*V. carlesii* × *V. macrocephalum*); willowood = *V. × rhytidophylloides* (*V. rhytidophyllum* × *V. × rhytidophylloides*) 'Willowwood'; wentworth = *V. trilobum* 'Wentworth'; summer snofl = *V. plicatum* 'Summer Snowflake'; dilatatum = *V. dilatatum*; allegany = *V. × rhytidophylloides* (*V. rhytidophyllum* × *V. × rhytidophylloides*) 'Allegany'; cree = *V. rhytidophyllum* 'Cree'; pragense = *V. × pragense* (*V. rhytidophyllum* × *V. utile*); erie = *V. dilatatum* 'Erie'; shasta = *V. plicatum* var. *tomentosum* (*V. plicatum* var. *tomentosum* × *V. plicatum* var. *tomentosum*) 'Mariesii'; shasta = *V. plicatum* var. *tomentosum* 'Shasta'; conoy = *V. × burkwoodii* (*V. × burkwoodii* × *V. utile*) 'Conoy'; burkwoodii = *V. × burkwoodii*; mohawk = *V. × burkwoodii* (*V. × burkwoodii* × *V. carlesii*) 'Mohawk'; juddi = *V. × juddi* (*V. carlesii* × *V. bitchiuense*); compactum = *V. carlesii* 'Compactum'; roseum = *V. opulus* 'Roseum'; dentatum = *V. dentatum*; blue muffin = *V. dentatum* 'Blue Muffin'; mariesii = *V. plicatum* var. *tomentosum* 'Mariesii'; chesapeake = *V. utile* 'Chesapeake'; sarcoxii: *V. × burkwoodii* (*V. × burkwoodii* × *V. carlesii*) 'Sarcoxii'; tinus = *V. tinus*; cayuga = *V. × caricephalum* (*V. × caricephalum* × *V. carlesii*) 'Cayuga'; rhododendron = Rhododendron 'Cunningham's White' (control).

of mainly mature (> 1 year old) leaves with some (< 20%) new growth. These results are in contrast to the report by Tjosvold et al. (24), who observed that deciduous azaleas were generally more susceptible than evergreen azaleas. According to previous studies involving different plant species (3, 11), younger leaf tissue was found to be more susceptible to *P. ramorum* than older tissue. However, *Viburnum* species do not appear to follow this pattern. The deciduous species or cultivars have younger overall leaves than the mixed leaf ages

found on evergreen or semi-evergreen species, which should have been more susceptible. Although Balci et al. (1) found that younger tissue of *Quercus* spp. was more susceptible to different *Phytophthora* spp., they also found that susceptibility of evergreen species remained the same whether young or mature leaves were inoculated. In the present study, individual leaves on each evergreen or semi-evergreen plant were not tagged to indicate whether they were older mature leaves or from the present growing season.

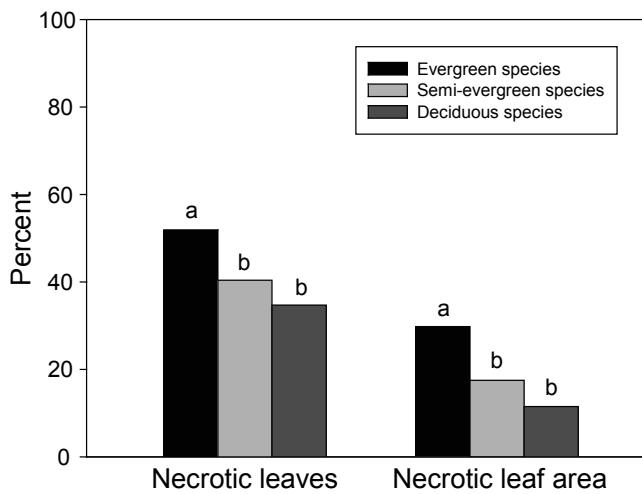
**Table 2.** Affect of *Phytophthora ramorum* isolate on selected *Viburnum* species and cultivars.

<i>Viburnum</i> spp. or cultivar	Necrotic leaves per plant (%) <sup>y</sup>		Necrotic leaf area (%) <sup>y</sup>	
	Isolate <sup>x</sup>		Isolate	
	PRN-1	WSDA-1772	PRN-1	WSDA-1772
<i>V. × rhytidophylloides</i> 'Allegany'	52.9A	26.6B	13.8*	5.6
<i>V. dentatum</i>	44.0A	51.5A	6.1	33.6*
<i>V. plicatum</i> var. <i>tomentosum</i> 'Mariesii'	73.4A	57.2A	20.5	24.0
<i>V. × burkwoodii</i> 'Sarcoxii'	80.3A	54.9B	59.0*	27.8
<i>V. tinus</i>	93.1A	96.1A	47.4	66.3
<i>V. × rhytidophylloides</i> 'Willowwood'	36.7A	12.4B	12.9	4.9

<sup>x</sup>Mean percentage of necrotic leaves per plant. Numbers followed by the same letter for each *Viburnum* species or cultivar is not significantly different according to Duncan's multiple range test ( $\alpha = 0.05$ ).

<sup>y</sup>Mean percentage of necrotic leaf area per leaf. Numbers followed by \* are significantly higher than the comparable isolate for each *Viburnum* species or cultivar according to Kruskal-Wallis nonparametric test ( $\alpha = 0.05$ ).

<sup>\*</sup>*Phytophthora ramorum* isolate PRN-1 (lineage: EU1) or WSDA-1772 (lineage: NA1) used to inoculate the selected *Viburnum* species or cultivar.



**Fig. 2.** The effect of viburnum leaf shedding pattern on the mean percentage of necrotic leaves per plant and the mean percentage of necrotic leaf area for each leaf. Bars with the same letter were not significantly different according to Duncan's multiple range test ( $\alpha = 0.05$ ) for the mean percentage of necrotic leaves per plant or to Kruskal-Wallis nonparametric test ( $\alpha = 0.05$ ) followed by a nonparametric multiple comparison for the mean percentage of necrotic leaf area for each leaf.

The specific growth chambers used in the experiment did not have a significant impact on the percentage of necrotic leaves per plant ( $P = 0.291$ ). The rhododendron positive controls showed results (Figs. 1A & 1B) comparable with those obtained from previous studies (23, 25), thereby validating the efficacy of the pathogen over the course of the study.

Results from this study demonstrate that all *Viburnum* spp. and cultivars tested were susceptible to some degree to *P. ramorum* infection. This study also demonstrates the importance of using whole plants in susceptibility studies. In addition, this research provides a better understanding of possible resistance or tolerance in *Viburnum* species and cultivars that might be used in areas infested now, or in the future with *P. ramorum*, as well as cultivars that might function in limiting the spread of this invasive pathogen.

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