Susceptibility of *Rhododendron* to Azalea Lace Bug, *Stephanitis pyrioides* (Scott)[1]

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─────────────────────────── Abstract ───────────────────────────

The invasive azalea lace bug, *Stephanitis pyrioides* (Scott), is one of the most damaging insect pests of the genus *Rhododendron*, especially azaleas. Feeding by nymphs and adults reduces the aesthetic value of infested plants. *Rhododendron* spp. (L.) and cultivars are a major component of public and private landscapes in the Pacific Northwest, as well as other parts of the U.S.. Variability in the susceptibility of *Rhododendron* to *S. pyrioides* has been previously examined but generally on a limited number of *Rhododendron* species and cultivars. We measured the susceptibility of 71 *Rhododendron* species, varieties, and cultivars over two years, selecting plants across the phylogenetic tree of *Rhododendron*, and quantified the proportion of leaf damage caused by *S. pyrioides*. We observed that trichome presence did not predict *S. pyrioides* damage. Plants from the subgenus *Azaleastrum* were the significantly most susceptible subgenus, and all sampled plants from this subgenus had measurable damage. In contrast, plants from the subgenus *Hymenanthes* were the significantly least susceptible, and most *Hymenanthes* plants had no measurable damage. This study provides a guideline for using host plant resistance to *S. pyrioides* in plant selection, and emphasizes the potential for *S. pyrioides* management if susceptible azaleas are to be used in the landscape.

Species used in this study: Azalea lace bug, *Stephanitis pyrioides*, *Rhododendron* spp.

Index words: Botanic gardens, horticultural entomology, host plant resistance.

Significance to the Horticulture Industry

The invasive azalea lace bug, *Stephanitis pyrioides* (Scott), is one of the most damaging insect pests of the genus *Rhododendron*, especially evergreen azaleas, an especially popular subgenus of *Rhododendron*. Feeding by nymphs and adults remove chlorophyll from leaves, causing stippling on the top of the leaf, which reduces the aesthetic value of infested plants, and severe infestations can result in plant death. *Rhododendron* species exhibit a remarkable range of diversity in form, foliage and flower, and are prized worldwide in the areas that they can be grown. They are extensively hybridized in cultivation, with >28,000 cultivars listed in the International Rhododendron Registry held by the Royal Horticultural Society. Given the extensive use of *Rhododendron* species and varieties in ornamental plantings in both public and private landscapes, identifying those plants that are susceptible to azalea lace bug will allow control measures to be more effectively targeted against this invasive pest. Identifying

resistant species or cultivars will assist breeding programs to develop *Rhododendron* plants that have desirable aesthetic qualities as well as resistance to azalea lace bug. Lastly, research focusing on resistant species and varieties may also shed light on the mechanisms of resistance in *Rhododendron* to azalea lace bug.

Introduction

The non-native, invasive azalea lace bug, *Stephanitis pyrioides* (Scott) (Tingidae: Hempitera), is one of the most damaging insect pests of the genus *Rhododendron*, especially azaleas (Schuh and Slater 1995, Shrewsbury and Smith-Fiola 2000, Klingeman et al. 2001, Nair and Braman 2012). Feeding by nymphs and adult *S. pyrioides* removes chlorophyll from leaves, reducing rates of photosynthesis, and transpiration of infested plants (Buntin et al. 1996). Chlorotic stippling appears on the top of the leaf (Johnson and Lyon 1994), which causes severe economic damage to landscapes and cultivated *Rhododendron* in areas where they are grown (Shrewsbury and Smith-Fiola 2000, Klingeman et al. 2001). *Stephanitis pyrioides* is native to Japan, and was first reported in the United States in New Jersey where it was thought to have been introduced on infested nursery stock from Japan (Weiss 1916). It is now present throughout the eastern United States where azaleas are grown (Weiss and Headlee 1918, Drake and Ruhoff 1965). It was first detected in Washington in 2007 (Looney et al. 2016), and it is now established in Oregon, Washington, and British Columbia (Rosetta 2013, Lee et al. 2019, Garrison and Tobin 2022).

The biology and life history of *S. pyrioides* have been previously studied (Nair and Braman 2012). Briefly, life stages of *S. pyrioides* are generally found on the underside of leaves. Eggs are oviposited in the leaf generally along the midrib or along larger side veins (Neal and Douglass 1988). Nymphs are colorless at hatching and turn darker after each successive molt, and there are five instars. It

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undergoes 2-3 generations in the Pacific Northwest (Lee et al. 2019, Garrison and Tobin 2022). Eggs diapause over the winter and hatch in spring following the accumulation of the required number of degree days (Garrison and Tobin 2022).

Prior reports describe the occurrence of *S. pyrioides* on species of *Rhododendron*, as well as other species within the Ericaceae (Drake 1965, Nair and Braman 2012). Ericaceae is a large family with approximately 4,250 known species across 124 genera. It is also an economically important family, with some species having edible berries (e.g., species within *Arctostaphylos*, *Gaylussacia*, and *Vaccinium*; Venturini et al. 2017) and others cultivated as ornamental plants (e.g., species within *Erica*, *Rhododendron*, *Arbutus*, and *Pieris*; Yu et al. 2009). There are also limited reports of *S. pyrioides* attacking hosts of plant families other than Ericaceae; for example, damage by *S. pyrioides* have been observed on *Eucryphia* sp. Gay and *Oemleria cerasiformis* (Torr. & A. Gray ex Hook & Arn.) Landon, but it is not known if it can complete its life cycle on these genera.

Natural history of Rhododendron. The genus *Rhododendron* contains >1,000 species of woody ornamentals mostly known for their showy flowers, but also for its ecological importance (Paul et al. 2005, United States Department of Agriculture 2011). It is widely cultivated in temperate regions, and in the wild they form important components of montane ecosystems. The genus *Rhododendron* exhibits a wide range of form, foliage and flower (Nelson 2001). The majority of wild *Rhododendron* species occur in the Himalayas and southeastern Tibet, or in the mountain ranges that form the backbones of the archipelago stretching between mainland Asia and Australia, the islands of Java, Sumatra, Borneo, New Guinea, and the Philippines (Irving and Hebda 1993). The remaining species are distributed over the northern hemisphere, occurring in pockets that are isolated from one another in Japan, northwestern North America, and the Appalachian and Caucasus Mountains (Irving and Hebda 1993).

The current and most accepted hypothesis of its geographic distribution is that *Rhododendron* was more widely and continuously distributed across North America and Eurasia during the milder climate of the Tertiary geologic era, approximately 65 million years ago (MYA) (Nelson 2001). Before the onset of the glacial periods during the late Tertiary, *Rhododendron* could have extended more or less continuously from North America to Europe, and eastward into China and northeastern Asia (Nelson 2001). However, as the climate changed and conditions became less favorable to *Rhododendron*, populations became isolated in only those regions where survival was possible (Nelson 2001). The collision of India with Asia about 40 MYA created a region favorable to *Rhododendron*, a "region of extreme relief" (Nelson 2001) where deep valleys are clustered close together (Irving and Hebda 1993). At the onset of hostile climate conditions, *Rhododendron* populations at the margins of this area were able to persist in this region of extreme relief, taking advantage of newly developed and amiable conditions (Nelson 2001). From here, plants within the *Rhododendron*

section Vireya are hypothesized to have spread into the high-island archipelago in the Indo-Malaysian region based upon a global genetic reconstruction of its phylogeny supporting this northeast Asian origin of *Rhododendron* (Shrestha et al. 2018). This work also suggests that *Rhododendron* first dispersed out of northeast Asia into North America in the mid-Eocene, followed by dispersal to South Asia and the Malay Archipelago in the late Eocene (Shrestha et al. 2018). Multiple dispersal events were inferred throughout the late Eocene and Oligocene between northeast Asia and the other regions, leading to paraphyletic assemblages in these regions (Shrestha et al. 2018). The ancestor of the Australian species likely occurred in the Malay Archipelago first, and dispersed to Australia approximately 10 MYA (Shrestha et al. 2018).

The genus *Rhododendron* can be separated into two categories: lepidote and elepidote. Lepidote species have specialized trichome structures on their leaves and elepidote species lack these structures. These structures in large part regulate the water supply of the plant and thus enable *Rhododendron* to withstand climatic extremes (Cowan 1950). Trichomes often take the form of scales or dense hairy layers called indumentum, but the architecture of the trichome varies within the genus nearly as widely as it does within the plant kingdom (Cowan 1950). Beyond the separation of lepidote and elepidote, further subdivision of *Rhododendron* has been, and still is, a complex and evolving process. In 1753, Linnaeus proposed two genera, *Rhododendron* and *Azalea*, into which 9 recognized species were placed (Cox and Cox 1997). Subsequent botanists such as Salisbury and Tate began to question the distinction between *Azalea* (which has 5 stamens) and *Rhododendron* (10 stamens), and in 1834, *Azalea* was incorporated into the genus *Rhododendron* (Don 1831). Over the years many botanists have further reorganized the taxonomy of *Rhododendron*, including the prominent 19th and 20th century botanists G. Don, C. J. Maximovicz, J. D. Hooker, and T. Nakai (Cox and Cox 1997). From 1916 onward, the classification of the genus has been largely carried out by botanists working in the herbarium of the Royal Botanic Garden, Edinburgh, Scotland (Cox and Cox 1997). From 1916 to 1922 Sir Isaac Bayley Balfour developed the system of Series (Cox and Cox 1997). In this system, groups of related *Rhododendrons* were placed in Series, and one species in each Series was selected as typical of its associates (Sleumer et al. 1978). The series were conglomerations, some with large numbers of extremely diverse species, others with a few species differing from those in another series by nuance (Sleumer et al. 1978). Although Balfour acknowledged that his system of Series was more of an expedient than a scientific statement, they did provide a conveniently ordered array for the scores of species that came pouring into Europe from the expeditions of Forrest, Kingdon-Ward, Farrer and others in the early years of the 20th century (Sleumer et al. 1978, Cox and Cox 1997). Unfortunately, Balfour died before he could remedy the situation, and the authors' temporary stopgap measures became engraved in stone for many English-speaking horticulturalists. In 1937, Sleumer began to produce a

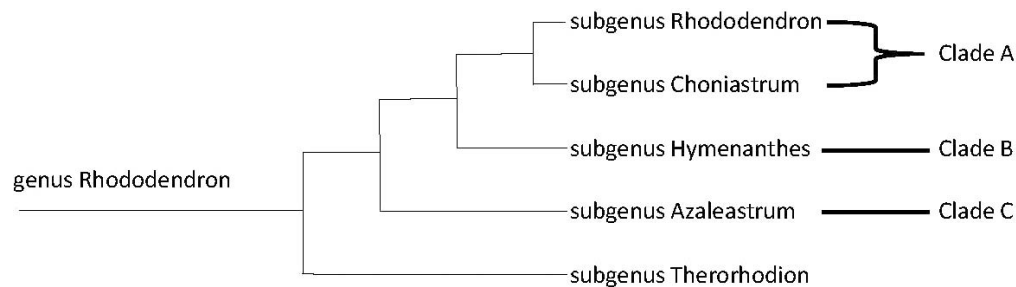J. Environ. Hort. 40(3):94–102. September 2022

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Fig. 1. Cladogram of the genus *Rhododendron* (Goetsch et al. 2005).

practical, comprehensive and cohesive classification of *Rhododendron*, based on the work of 19th century taxonomists and revised by his own research (Sleumer et al. 1978). Subsequently, the conclusions of a number of more narrowly focused morphological taxonomic studies (Sleumer 1966, Cullen 1980, Chamberlain 1982, Philipson and Philipson 1986, Judd and Kron 1995) were incorporated into an alternative *Rhododendron* classification (Chamberlain et al. 1996, Goetsch et al. 2005). This taxonomic system has been generally accepted by *Rhododendron* specialists (Cox and Cox 1997) because it embodies the findings of substantially all morphology-based *Rhododendron* systematic studies since 1980 (Goetsch et al. 2005).

Following the genetic analysis of Goetsch et al. (2005, 2011), these subgenera were further grouped into a higher level based on the discovery of three major clades (A, B, C) (Fig. 1). Subgenera *Rhododendron* and *Hymenanthes* are nested within clades A and B as monophyletic groups, respectively (Goetch et al. 2005). In contrast, subgenera *Azaleastrum* and *Pentanthera* were polyphyletic, and were divided between two clades. The four sections of *Pentanthera* were divided between clades B and C, with two each, while *Azaleastrum* had one section in each of A and C (Goetch et al. 2005). The subgenus Rhododendron was relatively untouched with regard to its three sections. Four other subgenera were eliminated and one new subgenus was created, leaving a total of five subgenera in all, compared to eight from Chamberlain et al. (1996). The discontinued subgenera are *Pentanthera*, *Tsutsusi*, *Candidastrum* and *Mumeazalea*, while a new subgenus was proposed by elevating section *Azaleastrum* section to subgenus rank (Goetch et al. 2005, 2011, Shrestha et al. 2018). In 2018, Shrestha et al. (2018) performed a phylogenetic reconstruction based on 423 species using 16 gene regions that fit well with the current understanding of the evolutionary relationships and time-scale of diversification of the genus (Goetsch et al. 2005, 2011).

Before 1860, the richness of the native flora of China was unsuspected and unknown; China was closed to Europeans and to foreign travel until the middle of the 19th century. Foreigners were generally not allowed to go more than a few kilometers outside Canton and Macao, the only ports open to Europeans; thus, it was impossible for early collectors from Europe and elsewhere to explore the country (Davidian 1996). At the conclusion of the second Opium War, China was opened to foreign travel (Davidian 1996), and this opening and the exposure to the richness of

the diversity of *Rhododendron* in the region of extreme relief led to the introduction and development of *Rhododendron* as "king of shrubs" (Nelson 2001). By the end of the 19th century, a period of plant collecting in China by amateurs, missionaries, travelers, merchants and diplomats was over, and a new period of the professional or horticultural collector with E. H. Wilson and George Forrest began (Davidian 1996). These early 20th century *Rhododendron* discoveries provided a vital link in the horticultural cultivation and dissemination of *Rhododendron* worldwide (Nelson 2001).

Susceptibility of Rhododendron species and cultivars to S. pyrioides. Differences in susceptibility of various *Rhododendron* species and cultivars to *S. pyrioides* have been previously studied. For example, deciduous *Rhododendron* spp. were found to be less suitable for adult feeding, oviposition, and nymphal development of *S. pyrioides* than the evergreen *R. mucronatum* variety 'Delaware Valley White'; however, all species supported adult activity and oviposition in no-choice and free-choice tests (Braman and Pendley 1992). Another study reported that plant physical characteristics, such as bloom color and abaxial leaf texture, were not associated with host plant acceptance by *S. pyrioides* (Schultz 1993). Several possible mechanisms of resistance to *S. pyrioides* have been investigated, including the role of epicuticular waxes (Balsdon et al. 1995, Wang et al. 1998, 1999, Chappell and Robacker 2006). As *S. pyrioides* rests on the abaxial leaf surface, it comes into contact with these waxes, which are hypothesized to directly inhibit feeding or otherwise give a chemical signal that reduces feeding. Resistant and susceptible deciduous cultivars differ in components of the leaf-surface lipids, identified as n-alkanes and triterpenoids, and these lipids were negatively associated with *S. pyrioides* behavior as measured by oviposition, egg and nymphal development, nymphal survivorship and leaf area damaged (Nair and Braman 2012). Leaf wax extracts from resistant genotypes, when applied to susceptible ones, resulted in resistance to feeding and oviposition by *S. pyrioides*; in contrast, wax extracts from susceptible genotypes when applied to resistant ones increased susceptibility, indicating that leaf wax plays a role in resistance to *S. pyrioides* (Clark 2000, Chappell et al. 2004, Chappell and Robacker 2005, 2006, Chappell 2007, Nair and Braman 2012).

Leaf pubescence (Schultz 1993, Wang et al.1998), stomatal character (Kirker et al. 2008), and leaf moisture

content (Wang et al.1998) have also been investigated. Because *S. pyrioides* feeds on the underside of the leaf, inserting their stylet through the stomata, leaf characteristics are hypothesized to affect the ability of *S. pyrioides* to insert its stylet to the palisade parenchyma where it feeds. In a study of 17 deciduous cultivars and one evergreen species, leaf water content and leaf hair density were not correlated with *S. pyrioides* damage, except in *Rhododendron canescens*, which had extremely high trichome density and was highly resistant to azalea lace bug feeding (Wang et al. 1998). Likewise, stomatal characters of 33 azalea cultivars were compared with their preference by *S. pyrioides* and, although stomata size differed significantly among the cultivars, they were not associated with *S. pyrioides* feeding preference (Kirker et al. 2008). However, across all these studies, a relatively few numbers of species and cultivars have been evaluated for their susceptibility to *S. pyrioides*.

The maritime climate of the Pacific Northwest is particularly well suited to growing *Rhododendron*, and most species, except the tropical section Vireya, can be grown outdoors in landscapes. During the winter months in the Pacific Northwest, mild temperatures, continual rainfall, and cloud cover are all conducive to the growing of *Rhododendron*, but during the summer months irrigation is often necessary (Nelson 2001). This beneficial climate and long history of *Rhododendron* cultivation in the Pacific Northwest provides an opportunity to study the damage caused by *S. pyrioides* in a wider variety of *Rhododendron* than has been done previously. In this paper, we sampled 71 *Rhododendron* species and cultivars over two years and quantified the damage done by *S. pyrioides*. Leaves were sampled during mid-summer when damage by *S. pyrioides* was generally the highest. Leaves were scanned after their collection, and the proportion of leaf area damaged was calculated for each sampled plant. Damage proportions were analyzed to determine if any patterns could be ascertained within the phylogenetic tree of *Rhododendron*, and we hypothesized that at some level of classification, differences in susceptibility would be detected.

Materials and Methods

Study site. This study was conducted at the Washington Park Arboretum in Seattle, Washington (47° 38′ 28.32″ N, 122° 17′ 36.996″ W), which contains >14,500 accessioned plant specimens including >4,000 different types of trees, shrubs and other plants native to 98 countries. Since the inception of the Washington Park Arboretum in 1936, *Rhododendron* spp. have been planted extensively, and there are >2,400 individual records for the genus *Rhododendron*. Plant records at the Washington Park Arboretum are georeferenced and maintained by the University of Washington Botanic Gardens.

Plant selection. *Rhododendron* plants to be sampled were first chosen by species or cultivars that had been measured in previous studies (e.g., Wang et al. 1998). *Rhododendron* spp. were also selected from available species and cultivars within the Washington Park Arboretum across the phylogenetic tree of *Rhododendron*, and

these plants were selected based on the phylogenetic analysis by Goetch et al. (2005); this selection procedure resulted in 26 species and cultivars. *Rhododendron* spp. and cultivars were also selected across the taxonomic family tree from Cox and Cox (1997). Lastly, 41 species and cultivars were selected due to their phylogenic proximity to those already included for sampling. The 71 species and cultivars selected for this study are listed in Table 1, and full methodological details are available in Garrison (2020).

Collection protocol. To quantify susceptibility of *Rhododendron* to *S. pyrioides*, a total of 80 leaves were collected from one plant of each of the 71 different *Rhododendron* species, varieties, and cultivars in August of 2018 and 2019. August was chosen so that at least one full generation of *S. pyrioides* would have been completed, with adults from the first generation and immatures from the second generation having the opportunity to feed on host plants (Garrison and Tobin 2022). Twenty leaves were collected from each plant once a week for 4 weeks. From each plant, four leaves were randomly collected from the north, south, east and west side of the plant, alternating from inner to outer canopy, and then four leaves were chosen at random from the plant. Leaves were placed in a re-sealable plastic bag, labeled, and stored at 4 C (39 F) for no more than one week to avoid leaf degradation. During preliminary data collection, it was observed that storage for one week at 4 C did not alter the samples, but that some leaves stored longer than two weeks became discolored, which could affect their analysis.

Processing of samples. Leaves were removed from the plastic bag, and placed between two pieces of clear polycarbonate sheets. Both sides of the leaves were scanned. The scans of the top sides of leaves were used to calculate damage from *S. pyrioides* for use in subsequent analyses. The scans of the undersides of the leaves were only used to confirm that damage was indeed caused by *S. pyrioides* based on the presence of frass and life stages (i.e., nymphs or adults). Leaves were scanned with an Epson Perfection V800 Photo scanner at 600 dpi using SilverFast SE 8 scanning software (https://www.silverfast.com/) and saved as a JPEG file. Files were named with the accession number and species name of each *Rhododendron* species or cultivar. Leaf damage by *S. pyrioides* was estimated using Assess 2.0: Image Analysis Software for Plant Disease Quantification (Lamari 2008). This program uses a threshold level of leaf coloration that separates the area of chlorosis caused by *S. pyrioides* damage from the total leaf area. The protocol for measuring leaf area and the area damaged was as follows. The program has two modes, 'Leaf' for measuring leaf area, and 'Lesion' for measuring disease (in this case chlorotic areas). We used the 'Leaf' mode to select the entire leaf, the 'Lesion' mode to select the chlorotic area, from which we could estimate the proportion of the leaf area damaged. Of the 80 leaves collected for each *Rhododendron* species, varieties, and cultivars in each year, a mean of 77 provided usable data.

Table 1. Mean proportion damage by *Rhododendron* species, cultivar, or variety in 2018 and 2019, and total number of leaves across both years. Also indicated for each is the subgenus, section, and clade, and whether or not trichomes are present (lepidote, L) or absent (elepidote, E). ND = No data collected.

Species, cultivar, or variety	Mean proportion damage		Total number of leaves	Subgenus	Section	Clade	L or E
	2018	2019					
Rhododendron spp.	0.0851	0.1156	163				
Rhododendron spp.	0.1206	0.1804	162				
R. (azalea) 'Anchorite'	0.2517	0.1693	162	Azaleastrum	Tsutsusi	C	E
R. (azalea) 'Atalanta'	0.0613	0.1167	144	Azaleastrum	Tsutsusi	C	E
R. (azalea) 'Carmel'	0.1404	0.1680	121	Azaleastrum	Tsutsusi	C	E
R. (azalea) 'Corsage'	0.2931	0.1949	162	Azaleastrum	Tsutsusi	C	E
R. (azalea) 'Daphne'	0.4500	0.1112	174	Azaleastrum	Tsutsusi	C	E
R. (azalea) 'Ladylove'	0.1615	0.1014	168	Azaleastrum	Tsutsusi	C	E
R. (azalea) 'Lustre'	0.1282	0.1334	165	Azaleastrum	Tsutsusi	C	E
R. (azalea) 'Maxwellii'	0.1345	0.0983	157	Azaleastrum	Tsutsusi	C	E
R. (azalea) 'Roberta'	0.1748	0.0991	167	Azaleastrum	Tsutsusi	C	E
R. (azalea) 'Trouper'	0.2705	0.1516	169	Azaleastrum	Tsutsusi	C	E
R. aberconwayi	0.0000	0.0000	159	Hymenanthes	Ponticum	B	E
R. adenopodum	0.0000	0.0000	167	Hymenanthes	Ponticum	B	E
R. aff. cuneatum	0.0120	0.0223	164	Rhododendron	Rhododendron	A	L
R. albrechtii	0.1042	0.1012	164	Azaleastrum	Sciadorhodion	C	E
R. amagianum	0.0133	0.0045	161	Azaleastrum	Tsutsusi	C	E
R. argyrophyllum	0.0000	0.0000	167	Hymenanthes	Ponticum	B	E
R. augustinii	0.1294	0.1302	189	Rhododendron	Rhododendron	A	L
R. auriculatum	0.0000	0.0000	163	Hymenanthes	Ponticum	B	E
R. barbatum	ND	0.0555	80	Hymenanthes	Ponticum	B	E
R. 'Big Yak'	0.0000	0.0000	154	Hymenanthes	Ponticum	B	E
R. breviperulatum	0.1272	0.0966	165	Azaleastrum	Tsutsusi	C	E
R. calendulaceum	0.0000	0.0000	167	Hymenanthes	Pentanthera	B	E
R. campanulatum ssp. aeruginosum	0.0000	0.0000	138	Hymenanthes	Ponticum	B	E
R. canescens	ND	0.0087	60	Hymenanthes	Pentanthera	B	E
R. coeloneuron	0.0000	0.0000	160	Hymenanthes	Ponticum	B	E
R. davidsonianum	0.0675	0.0492	160	Rhododendron	Rhododendron	A	L
R. degronianum ssp. heptamerum	0.0000	0.0000	157	Hymenanthes	Ponticum	B	E
R. degronianum ssp. yakushimanum	0.0000	0.0000	159	Hymenanthes	Ponticum	B	E
R. fortunei spp. discolor	0.0000	0.0000	149	Hymenanthes	Ponticum	B	E
R. floribundum	0.0000	ND	79	Hymenanthes	Ponticum	B	E
R. fortunei	0.0000	0.0000	122	Hymenanthes	Ponticum	B	E
R. 'Gill's Crimson'	0.0654	0.0678	150	Hymenanthes	Ponticum	B	E
R. 'Ginny Gee'	0.2490	0.3236	159	Rhododendron	Rhododendron	A	L
R. groenlandicum	0.0000	0.0000	163	Rhododendron	Rhododendron	A	L
R. hemitrichotum	0.0000	0.0000	175	Rhododendron	Rhododendron	A	L
R. hemsleyanum	0.0000	0.0000	162	Hymenanthes	Ponticum	B	E
R. cinnabarinum ssp. xanthocodon	0.0941	0.0805	165	Rhododendron	Rhododendron	A	L
R. indicum	0.2625	0.4276	187	Azaleastrum	Tsutsusi	C	E
R. irroratum	0.0006	0.0029	159	Hymenanthes	Ponticum	B	E
R. kaempheri	0.1319	0.0832	171	Azaleastrum	Tsutsusi	C	E
R. lutescens	0.0008	0.0039	163	Rhododendron	Rhododendron	A	L
R. luteum	0.0751	0.0273	164	Hymenanthes	Pentanthera	B	E
R. macabeanum	0.0000	0.0000	151	Hymenanthes	Ponticum	B	E
R. moupinense	0.0000	0.0000	144	Rhododendron	Rhododendron	A	L
R. mucronatum	0.0426	0.0300	121	Azaleastrum	Tsutsusi	C	E
R. mucronulatum	0.0491	0.0532	168	Rhododendron	Rhododendron	A	E
R. mucronulatum var. ciliatum	0.0899	0.0638	162	Rhododendron	Rhododendron	A	L
R. obtusum	0.2228	0.1284	184	Azaleastrum	Tsutsui	C	E
R. occidentale	0.0000	0.0000	166	Hymenanthes	Pentanthera	B	E
R. oreotrephes	0.0046	0.0009	162	Rhododendron	Rhododendron	A	L
R. ponticum	0.0000	0.0000	146	Hymenanthes	Ponticum	B	E
R. praevernum	0.0059	0.0056	154	Hymenanthes	Ponticum	B	E
R. racemosum	0.0000	0.0000	169	Rhododendron	Rhododendron	A	L
R. ririei	0.0019	0.0016	163	Hymenanthes	Ponticum	B	E
R. rubiginosum	0.1967	0.0994	141	Rhododendron	Rhododendron	A	L
R. sanguineum var.	0.0000	0.0000	162	Hymenanthes	Ponticum	B	E
R. scabrifolium	0.0000	0.0000	161	Rhododendron	Rhododendron	A	L
R. schlippenbachii	0.0420	0.0247	152	Azaleastrum	Sciadorhodion	C	E
R. searsiae	0.0883	0.0806	170	Rhododendron	Rhododendron	A	L
R. siderophyllum	0.0000	0.0000	143	Rhododendron	Rhododendron	A	L
R. simsii	0.0617	0.0346	162	Azaleastrum	Tsutsusi	C	E
R. sutchuenense var. geraldii	0.0000	0.0000	159	Hymenanthes	Ponticum	B	E
R. triflorum var. bauhiniiflorum	0.0000	0.0000	165	Rhododendron	Rhododendron	A	L

Table 1. Continued.

Species, cultivar, or variety	Mean proportion damage		Total number of leaves	Subgenus	Section	Clade	L or E
	2018	2019					
R. ungernii	0.0000	0.0000	162	Hymenanthes	Ponticum	B	E
R. vernicosum	0.0025	0.0153	158	Hymenanthes	Ponticum	B	E
R. viscosum	0.0325	0.0618	161	Hymenanthes	Pentanthera	B	E
R. wardii	0.0005	0.0010	162	Hymenanthes	Ponticum	B	E
R. williamsianum	0.0023	0.0002	162	Hymenanthes	Ponticum	B	E
R. x lochmium	0.0078	0.0041	171	Rhododendron	Rhododendron	A	L
R. yunnanense	0.2070	0.0472	154	Rhododendron	Rhododendron	A	L
R. yunnanense	0.1458	0.1079	167	Rhododendron	Rhododendron	A	L

Statistical analysis. For each *Rhododendron* species, cultivar or variety, we used the mean proportion of leaf area damaged by *S. pyrioides* as the response variable. Mean proportions were calculated from leaves sampled in 2018 and 2019, and were transformed according to log$_e$ (mean proportion / (1 - mean proportion)) to meet the assumption of normality in an Analysis of Variance (ANOVA). Prior to the transformations, means were adjusted by the addition of 0.001 to permit transformation of zero means. We tested the effect of trichome presence (lepidote) or absence (elepidote) on the transformed mean proportion of damage using ANOVA. We also examined the effect of *Rhododendron* subgenus and section on the transformed mean proportion of damage using ANOVA. Post-hoc tests, when appropriate, were done using Tukey's HSD (honestly significant difference). All statistical analyses were done in R (R Core Team 2018).

Results and Discussion

In 2018 and 2019, a total of 5,680 and 5,738 leaves were collected and scanned, respectively. The mean proportion of leaf area damaged for each *Rhododendron* species, cultivar or variety for both years is presented in Table 1. Overall, the proportion of the leaf damaged by *S. pyrioides* across all *Rhododendron* plants ranged from 0 to 0.3451. *Rhododendron calendulaceum*, and *R. canescens* were the only two species available in the Washington Park Arboretum that had been previously evaluated for their susceptibility to *S. pyrioides* (Wang et al. 1998). *Rhododendron calendulaceum* was described as less suitable for *S. pyrioides* feeding and oviposition by Braman and Pendley (1992). In this study, we observed similar findings as there was no measurable damage recorded on *R. calendulaceum*. *Rhododendron canescens* was described as resistant to *S. pyrioides* by Wang et al. (1998), and we observed a low proportion of leaf area damaged (<0.01) in this study.

Subgenus *Azaleastrum* (clade C, N=19 plants) had the highest overall level of damage with a mean proportion of 0.1408, and all samples were observed to have some level of stippling damage, which ranged from 0.0089 to 0.3451 (Table 1). The amount of leaf area damage was lowest in the subgenus *Hymenanthes* (clade B); samples from this subgenus (N=31 plants) had a mean proportion of leaf area damaged of 0.0080, and ranged from no damage to 0.0666 (Table 1). In addition, 20 of the species and cultivars from the subgenus *Hymenanthes* had no measurable damage.

Samples from the subgenus *Rhododendron* (clade A, N=21) had intermediate levels of damage caused by *S. pyrioides*; in this group, the mean proportion of leaf area damaged was 0.0573, and ranged from 0 (which was recorded from seven species) to 0.2863 (Table 1). The compiled phylogenic tree of *Rhododendron* with the species matched to the corresponding proportion of leaf area damaged is presented in Figure 2, which highlights the variation in susceptibility among and within subgenera.

When comparing between lepidote (e.g., with trichomes) and elepidote (e.g., without trichomes) species, there were no statistically significant differences in the proportion of leaf damage due to *S. pyrioides* ($F = 0.07$, df = 1,69, $P = 0.80$, Fig. 3A). However, there were significant differences in the proportion of leaf damage by *Rhododendron* subgenera ($F = 35.99$, df = 2, 68, $P < 0.01$). Post hoc tests revealed that among the subgenera, all were significantly different from each other, with *Azaleastrum* being the most susceptible and *Hymenanthes* being the least susceptible to *S. pyrioides* (Fig. 3B). We also observed significant differences in the proportion of leaf damage by *Rhododendron* section ($F = 19.39$, df = 4,66, $P < 0.01$), with *Tsutsusi* (evergreen azaleas) being the most susceptible to *S. pyrioides* (Fig. 3C).

Rhododendron species can be subdivided through the presence or absence of trichomes (i.e., lepidote or elepidote, respectively), after which it can be subdivided by a hierarchy of clade/subgenus, section, subsection, and species (Chamberlain 1996, Goetch et al. 2005). When comparing between lepidote and elepidote species, there were no differences in the proportion of leaf area damaged (Fig. 3A). Thus, among the species and cultivars tested in this study, trichome structures on *Rhododendron* leaves do not predict susceptibility to *S. pyrioides*, and their presence did not deter *S. pyrioides* life stages from feeding on host plants. However, differences were observed among *Rhododendron* subgenera (Fig. 3B). The two subgenera that are lepidote, subgenus *Azaleastum* (clade C) and subgenus *Hymenanthes* (clade B), were observed to have the highest and lowest levels of damage by *S. pyrioides*, respectively, which reinforces that the presence of trichomes is not a predictor of susceptibility to *S. pyrioides*. However, there can be considerable variation in the architecture of *Rhododendron* trichomes (Cowan 1950), and although trichome presence might not be a predictor of susceptibility, future work that considers, for example, details of morphological structure in trichomes, and their possible production of chemical defenses against herbivores, might
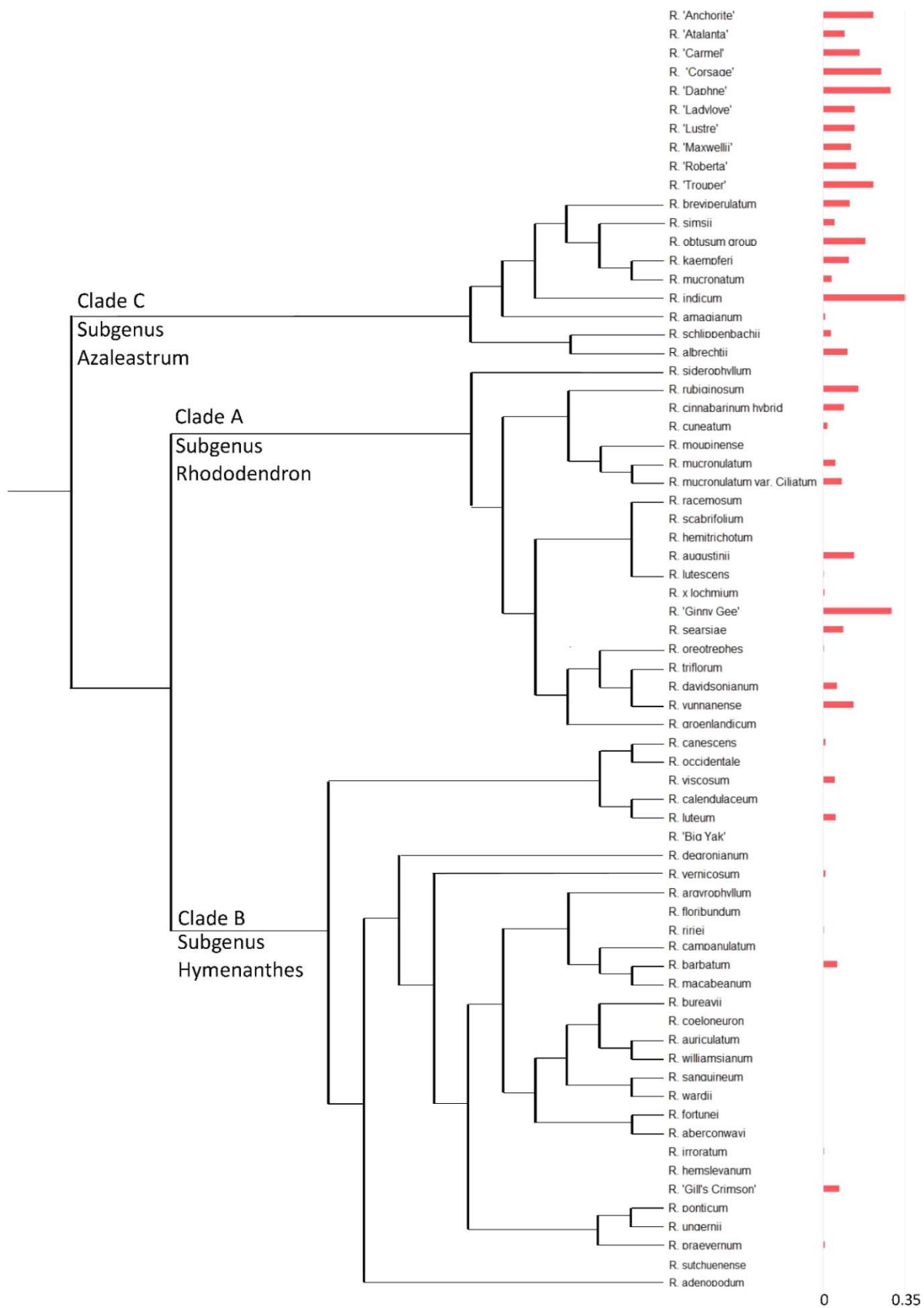
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Fig. 2. Compiled phylogenetic tree of *Rhododendron* spp. based upon phylogenetic analyses by Goetch et al. (2005) and Shrestha et al. (2018), and morphological taxonomic organization by Chamberlain (1996). The boxes indicate subgenera. The horizontal red bars represent the proportion of leaf area damaged.
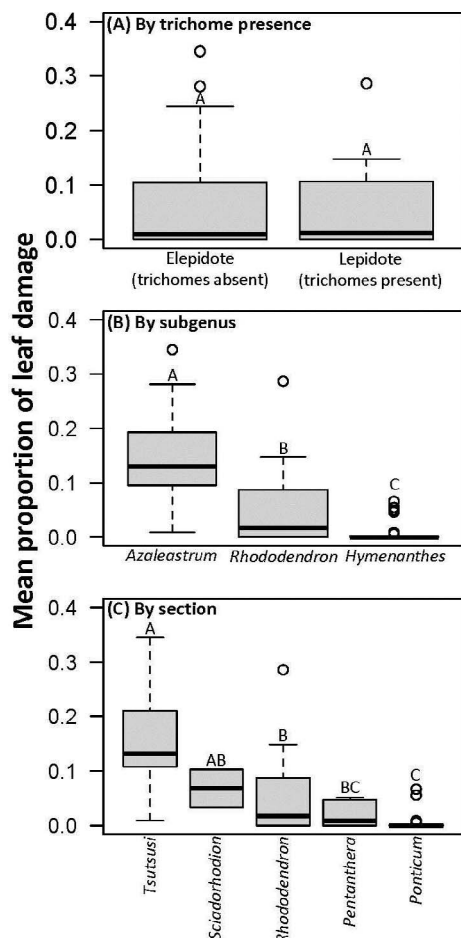
Fig. 3. Box-and-whiskers plot of the proportion of leaf damaged by *S. pyrioides* based upon the presence or absence of trichomes (A), *Rhododendron* subgenus (B), and *Rhododendron* section (C). In each plot, different letters indicate significant differences using Tukey's HSD ($\alpha = 0.05$).

provide greater insight into the role that trichomes could play in conferring resistance to *S. pyrioides*.

Although we did observe some *Rhododendron* species and cultivars that are highly susceptible to *S. pyrioides*, it is not trivial to suggest that growers simply avoid planting susceptible ones (e.g., evergreen azaleas from the section *Tsutsusi*) and plant instead resistant species or varieties (e.g., plants from the section *Ponicum*) (Fig. 3C). Evergreen azaleas, which are highly susceptible to *S. pyrioides*, are also highly valued for their generally small stature and year-round foliar interest, and exhibit a range of color and form that may not be available in other subgenera (Kobayashi 2013). They are also culturally important in Japan, Korea, and China (Lee 1978), and they are a featured commodity of historic landscapes in western Washington, such as Azalea Way in the Washington Park Arboretum. Also, species and cultivars within the subgenus *Azaleastrum* were the most susceptible to *S. pyrioides* and all tested plants within this subgenus had some level of stippling damage; however, they are noted for their diversity of form and flower and a common choice in landscaping. The results from this research suggest that if susceptible species and cultivars are desired, a management

program for *S. pyrioides* will be necessary. However, the identification of resistance species and cultivars, such as those within the subgenus *Hymenanthes* and the section *Ponicum*, also provide useful information for growers that might desire plants that require less management.

The identification of susceptible *Rhododendron* species and cultivars in botanical gardens can also be valuable for the preservation of *Rhododendron* that are threatened or endangered in their native range. Approximately 70% of *Rhododendron* species are classified as vulnerable, threatened, endangered or critically endangered, and about 25% of all *Rhododendron* taxa are under threat of extinction in the wild (Gibbs et al. 2011). Global trade is thought to have resulted in the introduction of *S. pyrioides* to many places outside of its native range (Nair and Braman 2012). Identification of susceptible species *ex situ* would allow susceptible populations to be monitored more intensively for *S. pyrioides* and to take measures to mitigate the introduction of *S. pyrioides* to areas in which *Rhododendron* species are endemic. Given the susceptibility of azaleas (subgenus *Azaleastrum*), and that *S. pyrioides* is already established in most areas of the United States where azaleas can be grown, careful consideration should be made when they are used. One possible avenue of future research is whether or not resistant species can be strategically planted among more susceptible species as a form of cultural control against *S. pyrioides* to reduce the overall damage in landscapes.

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