Optimizing Management Guidelines for the Non-Native Azalea Lace Bug on Rhododendron Species in Western Washington

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A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Science

University of Washington

2020

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Program Authorized to Offer Degree:

Environmental and Forest Sciences

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Abstract

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The non-native, invasive azalea lace bug, *Stephanitis pyrioides* (Scott), is one of the most serious insect pests of the genus *Rhododendron*, especially evergreen azaleas, an especially popular subgenus of Rhododendron. Feeding by nymphs and adults remove chlorophyll from leaves, reducing rates of photosynthesis and transpiration of infested plants, and causes stippling on the top of the leaf, which reduces the aesthetic value of infested plants. Severe infestations can lead to plant death. Introduced to the eastern U.S. from Japan in 1916, its presence in western Washington was confirmed in 2007. Research on azalea lace bug in the Pacific Northwest is extremely limited to date. In my thesis research, I investigated the seasonality of azalea lace bug in western Washington, and developed region-specific degree-day models to optimize sampling efforts and the timing of control measures. I also studied the susceptibility of *Rhododendron* spp. to azalea lace bug by assessing feeding damage in 71 different species and

cultivars. This research provides improved management guidelines for azalea lace bug in the Pacific Northwest, and provides a framework for using host plant resistance in the design of landscapes where *Rhododendron* spp. are desired.

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ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. Patrick Tobin, who graciously agreed to take me on as a graduate student following the passing my former advisor Dr. Sarah Reichard. He has been incredibly helpful and understanding through an amazing array of obstacles that popped up along the way.

I would also like to thank the members of my committee, Dr. Thomas M. Hinckley, Dr. Soo-Hyung Kim, and Dr. Helena Puche. They were always helpful, patient and gave excellent advice and assistance.

I would like to thank the University of Washington Botanic Gardens. The leadership and my supervisors were always supportive and understanding. They allowed me the flexibility in my work I needed, and I absolutely could not have maintained a full-time job and pursued a degree without their assistance. Thanks particularly to Director Fred Hoyt, Horticulture Manager David Zuckerman, and Horticulture Supervisor Roy Farrow. I’d also like to thank my fellow staff members at the Washington Park Arboretum, whose patience and support were very much appreciated.

I would like to thank the undergraduate employees who assisted with this project, Marisa Deluccia, Sabrina Gilmour, and Kendra Potoshnik. Their assistance counting bugs on thousands of leaves and scanning thousands of leaves was invaluable. The amount of data I was able to bring together for this project is due in no small part to their efforts.

Thank you to my lab mates Michael Bradshaw, Alex Pane, and Lila Westreich, for your company, patience, and support.

Thank you to the Horticultural Research Institute (Project # 26610180), the John A. Wott Fellowship in Plant Collection and Curatorship, the Elisabeth Carey Miller Scholarship in Horticulture, and the University of Washington for providing financial support for this project.

Lastly, I thank my wife, Deborah Ann Garrison. The list of things I would not have been able to do without her help is beyond counting. From start to finish, at every step she has been a constant light to lead me in the right direction. Achieving this degree is a testament to her encouragement and strength. Your love and patience are forever appreciated.

DEDICATION

This thesis is dedicated to Dr. Sarah Hayden Reichard, whose leadership, mentorship, and support was instrumental to my entry into graduate school..

CHAPTER 1. AZALEA LACE BUG (STEPHANITIS PYRIOIDES SCOTT) LIFE HISTORY, ACTIVITY IN THE PUGET SOUND, AND STUDY SUMMARY

The non-native, invasive azalea lace bug, *Stephanitis pyrioides* (Scott) (Tingidae: Hempitera), is one of the most serious insect pests of the genus *Rhododendron*, especially azaleas (Nair and Braman 2012). Within Hemiptera, species within Tingidae are known as economically important pests of ornamental trees and shrubs (Johnson and Lyon 1994). The family is distributed worldwide and is comprised of three subfamilies, Tinginae, Cantacaderinae, and Vianaidinae. The genus *Stephanitis* Stål (Hemiptera: Tingidae) comprises over 60 species, many of which are pests of fruit, ornamental trees, and shrubs in tropical and temperate regions of the world (Nair and Braman 2012). The azalea lace bug (*S. pyrioides*), the Andromeda lace bug (*S. takeyai* Drake and Maa) and the rhododendron lace bug (*S. rhododendroni* Horváth) are three non-native species that are established in North America. All three are known to attack woody ornamentals, especially azaleas, rhododendrons, and related plants of the family *Ericaceae* (Nair and Braman 2012, Schuh and Slater 1995); however, among them, *S. pyrioides* is especially noted for its economic damage (Shrewsbury and Smith-Fiola 2000, Klingeman et al. 2001, Nair and Braman 2012). Feeding by nymphs and adult *S. pyrioides* remove 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 was originally described by Scott (1874) as *Tingus pyrioides* from an insect collection from Japan. It was later renamed and described in detail by Dickerson and Weiss (1917), Weiss and Headlee (1918), and Shen et al. (1985). It 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). Since its introduction into the United States, *S. pyrioides* has spread throughout the eastern United States, extending to all areas where *Rhododendron* can be grown. (Weiss and Headlee 1918, Drake and Ruhoff 1965). It has also been reported in other countries in Asia, Europe, and South America (Nair and Braman 2012). It was first detected in Washington in 2007 through a pubic complaint of ornamental damage to the Washington State University extension service (Looney et al. 2016).

The biology and life history of *S. pyrioides* have been widely studied and reviewed (Nair and Braman 2012). All life stages of *S. pyrioides* are found on the underside of leaves. The adult males are 2.8-3.3 mm and adult females 2.9-3.3 mm in length (Shen et al. 1985). Both male and female adult *S. pyrioides* have transparent, areolate patterned wings and pronotum that are the origin of its common name. The wings have extensive venation, leading to the appearance of lacey wings, and are marked by black or brown patches (Fig. 2.1). Adults are weak fliers, and flight generally occurs in response to depletion of food (Neal and Schaefer 2000) or when disturbed (Nair and Braman 2012). Adults do not diapause (Neal and Douglass 1988) and can be active in winter in the east and west coast of the United States (Neal 1985, Lee et al. 2019). Adults are surprisingly long-lived for an insect of their size, and have been observed to survive > 100 days under optimal conditions (Neal and Douglass 1988). Winter oviposition has been recorded in

North Carolina (Nalepa and Baker 1994), but significantly more oviposition occurs in June and July relative to other months (Schultz 1993). Eggs are the overwintering stage and measure 0.36-0.43 mm (Shen et al. 1985). The eggs are flask shaped and capped with an oval operculum. Eggs are oviposited in the leaf generally along the midrib or along larger side veins, with the operculum extending slightly above the horizontal surface of the leaf (Neal 1988). After oviposition, females will cover the egg with a dark brownish adhesive liquid excrement, which quickly hardens to form a protective coating. Nymphs are colorless at hatching and turn darker after each successive molt (Figs. 2.2 & 2.3, Nair and Braman 2012). There are five instars, ranging in length from 0.1-1.8 mm, and wing pads are present in the last two instars (Shen et al. 1985).

Developmental time for *S. pyrioides* has been previously studied (Neal and Douglass 1988, Braman et al. 1992); specifically, egg-to-adult development required 22 days at 30°C and 97 days at 15°C, but was not successful at 33°C (Nair and Braman 2012). Four generations per year have been reported in Georgia, USA (Braman et al. 1992), between two and three generations in New England, and three generations are reported in Oregon (Lee et al. 2019).

Stephanitis pyrioides nymphs and adults have piercing-sucking mouthparts consisting of appressed mandibular and maxillary stylets that form a bundle lying in a groove in the labium, which is a universal characteristic of Hemiptera (Gullan et al. 2014). Both nymphs and adults cause feeding injury by inserting this stylet through stomata on the abaxial leaf surface and removing the chlorophyll content from the upper palisade parenchyma (Ishihara and Kawai 1981). Depletion of chlorophyll leads to chlorosis, resulting in stippled or bleached appearance of the upper surface of leaves, and severely damaged leaves may dry prematurely and abscise (Fig. 2.4, Nair and Braman 2012). Resulting chlorosis from even moderate levels of injury can reduce the aesthetic value of azalea (Klingeman et al 2001), and reduced CO_2 assimilation presumably would reduce the amount of photosynthates available for plant growth, flowering and possibly survival (Buntin et al 1996). This pattern of chlorosis on the top of the leaf, and the presence of the brown excrement and cast skins on the bottom can be used to identify damage from *S. pyrioides* (Fig. 2.5, Johnson and Lyon 1994).

Prior reports describe the occurrence of *S. pyrioides* on species of *Rhododendron*, or other species within Ericaceae (Drake and Ruhoff 1965, Nair and Braman 2012). *Rhododendron* has a broad geographic distribution in the wild throughout temperate and montane tropical ecosystems (Luteyn 1980). Species of *Rhododendron* are native to Asia, Australia, Europe and North America (Nelson 2001). *Rhododendron* contains > 1,000 species of woody ornamentals mostly known for their showy flowers (USDA 2011). In addition, there are > 28,000 named *Rhododendron* hybrids and cultivars (Leslie 2004). Azaleas (*Rhododendron* spp.) are native to Asia, Europe, and North America (Scariot et al. 2007) and comprise two of the subgenera of the genus (Chamberlain and Rae 1990, Chamberlain et al. 1996). Evergreen azaleas belong to subgenus *Azaleastrum,* and deciduous azaleas are divided between *Azaleastrum* (section *Tsutsusi*) and *Hymenanthes* (section *Pentanthera*) (Goetsch et al. 2011, 2015). *Rhododendron* and Azalea are among the most widely cultivated ornamental and landscape plants (Raupp and Noland 1984). Although all *Rhododendron* spp. are thought to be suitable hosts for *S. pyrioides* (Braman and Pendley 1992), prior work has observed that there is a considerable variation in susceptibility to *S. pyrioides* damage (Nair and Braman 2012).

The overwhelming majority of research to date on *S. pyrioides* has occurred in the eastern United States. Collectively, these studies include those that have examined its seasonality (e.g., Basldon et al. 1993, Neal and Douglass 1988, Braman et al. 1992), use of different chemical (e.g., Buss and Short 2001, Held and Parker 2011) and biological (e.g., Henry et al 1986, Basldon et al.

1996) control tactics, and its damage to *Rhododendron* spp. (e.g., Buntin et al 1996, Schultz 1993). This past work has limited applicability in Pacific Northwestern ecosystems, and at present, there is no research on how this insect will behave in western Washington. In fact, peer-reviewed research on *S. pyrioides* in the Pacific Northwest is limited to one recent study from Oregon (Lee et al. 2019). In my thesis research, I address this information gap by quantifying *S. pyrioides* seasonality in western Washington, and measuring the susceptibility of *Rhododendron* species in western Washington to *S. pyrioides*. This study was conducted at the Washington Park Arboretum (47° 38' 28.32" N, 122° 17' 36.996" W), Seattle, WA, USA.

The maritime climate of the Pacific Northwest is particularly well suited to growing *Rhododendron*, and most species, except tropical species, can be grown outdoors. Numerous non-native species of *Rhododendron* have been introduced from numerous gardeners, collectors and hybridizers, and it is a staple of private and public landscapes throughout the region (Nelson, 2001). The Washington Park Arboretum is a public park and arboretum, managed jointly by the City of Seattle and the University of Washington. The Washington Park Arboretum is located in the Puget Sound Basin with downtown Seattle directly southwest, the University of Washington to the northwest, and Lake Washington directly east. The 0.93 km^2 park occupies most of a long narrow glacial ravine running north and south with the northern most tip touching Union Bay (Ion, 2003). The park was officially set aside as a botanical garden and arboretum in 1924, and in 1936 James Frederick Dawson completed a design for the Olmsted Brothers design firm (Ion 2003). *Rhododendron* plants were planted early and often in the arboretum, and several gardens contained therein feature *Rhododendron* prominently (e.g., Azalea Way, Rhododendron Glen, Puget Sound Rhododendron Hybrid Garden). This variety of available *Rhododendron* species and hybrids growing together presents a unique opportunity to study both the range of susceptibility of

Rhododendron to *S. pyrioides* damage, and to develop predictive models of *S. pyrioides* seasonality in the Pacific Northwest.

This thesis is divided into two primary chapters (Chapters 2 and 3), each of which is written as a stand-alone manuscript. In Chapter 2, I measured the phenology of *S. pyrioides* in western Washington from infested leaves that were collected from *Rhododendron* from April to October in both 2018 and 2019. This allowed me to develop degree-day models of its seasonality, which can be used to improve the timing of management interventions and sampling efforts. Degree-day models are an established tool in plant sciences, pest management, and ecology and are useful in understanding insect and plant phenology, driving computer simulation models, and predicting pest status (Pruess 1983, Higley et al. 1986).

In Chapter 3, I quantified the susceptibility of *Rhododendron* to *S. pyrioides* from 2018 to 2019. During mid-summer when damage from *S. pyrioides* was at its peak, I collected 80 leaves from 71 different *Rhododendron* species, varieties, and cultivars. Leaves were scanned and analyzed using Assess 2.0: Image Analysis Software for Plant Disease Quantification (Lamari 2008) to quantify damage from *S. pyrioides*. Damage proportions were analyzed to determine if any patterns could be ascertained within the phylogenetic tree of *Rhododendron*. Damage proportions were also analyzed to determine their relationship within the taxonomic hierarchy of *Rhododendron* leaf trichome presence (lepidote/elepidote), clade, and section.

By developing a region specific degree-day model and identifying patterns of susceptibility I hope to address this invasive pest on both a local and global level. Degree-day based control recommendations allow more effective control measures to be taken in the Puget Sound region, while identifying susceptible species and varieties will allow scouting and control measures to be more effectively targeted wherever *Rhododendron* is threatened by *S. pyrioides*.

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CHAPTER 2. SEASONALITY OF AZALEA LACE BUG IN WESTERN WASHINGTON

2.1 ABSTRACT

The non-native, invasive azalea lace bug, *Stephanitis pyrioides* (Scott), is one of the most serious insect pests of the genus *Rhododendron*, especially azaleas, an especially popular subgenus of *Rhododendron.* Feeding by nymphs and adult *S. pyrioides* remove chlorophyll from leaves, reducing rates of photosynthesis and transpiration of infested plants, and causes stippling on the top of the leaf, which reduces the aesthetic value of infested plants. Rhododendron is a major component of public and private landscapes in the Pacific Northwest. Previous studies on the seasonality of *S. pyrioides* in North America are largely from the southeastern United States and thus could have limited applicability in the Pacific Northwest. To quantify seasonality in western Washington, ~200 leaves from 18 *Rhododendron* plants were destructively sampled 1-2 times per week from April to October in 2018 and 2019, from which the number of *S. pyrioides* eggs, early instars, late instars, and adults were microscopically counted. Degree-day models were developed for each of these stages for the first generation, and used to estimate voltinism in the region. Five and 50% of hatch from overwintering eggs occurred at 93.2 and 172.7 accumulated degree-days (base threshold = 10.2°C) from 1 January. The mean degree-days required for egg-to-adult development was 229.6. The seasonality of *S. pyrioides* life stages suggests that it undergoes two generations with a partial third generation in western Washington. The use of region-specific degree-day models optimizes sampling efforts and the timing of effective control measures in western Washington.

2.2 INTRODUCTION

The non-native, invasive azalea lace bug, *Stephanitis pyrioides* (Scott) (Tingidae: Hempitera), is one of the most serious insect pests of the genus *Rhododendron*, especially azaleas (Nair and Braman 2012). Within Hemiptera, species within Tingidae are known as economically important pests of ornamental trees and shrubs (Johnson and Lyon 1994). Within this family, the azalea lace bug (*S. pyrioides*), the Andromeda lace bug (*Stephanitis takeyai* Drake and Maa) and the rhododendron lace bug (*Stephanitis rhododendroni* Horváth) are three non-native species that are established in North America. All three are known to attack woody ornamentals, especially the genus *Rhododendron* and related plants of the family *Ericaceae* (Nair and Braman 2012, Schuh and Slater 1995); however, among them, *S. pyrioides* is especially noted for its economic damage (Shrewsbury and Smith-Fiola 2000, Klingeman et al. 2001, Nair and Braman 2012). Feeding by nymphs and adult *S. pyrioides* remove 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). Since its introduction into the United States, *S. pyrioides* has spread throughout the eastern United States, extending to all areas where *Rhododendron* can be grown. (Weiss and Headlee 1918, Drake and Ruhoff 1965). It has also been reported in other countries in Asia, Europe, and South America (Nair and Braman 2012). It was first detected in Washington in 2007 through a pubic complaint of ornamental damage to the Washington State University extension service

(Looney et al. 2016). It is now widely established in western Oregon, Washington, and British Columbia (Rosetta 2013, Lee et al. 2019).

The biology and life history of *S. pyrioides* have been previously studied (Nair and Braman 2012). Briefly, the adults are relatively small with males ~2.8-3.3 mm and females ~2.9-3.3 mm in length (Fig. 2.1; Shen et al. 1985). All life stages of *S. pyrioides* are found on the underside of leaves. Both male and female adult *S. pyrioides* have transparent, areolate patterned wings and pronotum that are the origin of its common name. Adults are weak fliers, and flight generally occurs in response to depletion of food (Neal and Schaefer 2000) or when disturbed (Nair and Braman, 2012). Eggs are the overwintering stage, and are oviposited in the leaf generally along the midrib or along larger side veins (Neal 1988). Nymphs are colorless at hatching and turn darker after each successive molt (Fig. 2.2, 2.3; Nair and Braman 2012). There are five instars, ranging in length from 0.1-1.8 mm, and wing pads are present in the last two instars (Shen et al. 1985). Adults do not diapause (Neal and Douglass 1988) and have been observed to be active during winter in the east and west coast of the United States (Neal 1985, Lee et al. 2019). At present, hatch from overwintering eggs is thought to be degree-day driven, but the cues that initiate diapause induction are not known.

The principle of using temperature to describe poikilothermic development has been recognized for >200 years (Taylor 1981, Higley et al. 1986). Poikilothermic development depends on the rates of various biochemical reactions, and as these reactions occur through time, development proceeds (Higley et al. 1986). These reactions are slowed or stopped below and above certain base temperature thresholds. For example, immature insect development stops (or is extremely slowed) at temperatures below some species-specific threshold (e.g., base temperature threshold). Because of the relationship between temperature and development, degree-day models can be developed and used to predict the occurrence of life stages, and thus are valuable tools in integrated pest management programs (Pruess 1983, Braman et al. 1992). Briefly, a degree-day is a measure of the amount of heat that accumulates above (and below) base temperature thresholds, and often are estimated over a 24-hour period (Arnold 1959, Herms 2004). One degree-day accumulates for each degree the mean temperature remains above the minimum base temperature threshold and below the maximum base temperature threshold. A degree-day model is then developed by measuring the life stages present at a particular point of time and linking those counts to the accumulated degree-days at that time.

Prior work on the developmental time for *S. pyrioides* from the southeastern U.S. showed that egg-to-adult development required 22 days at 30°C and 97 days at 15°C, but was not successful at 33°C (Nair and Braman 2012); this suggests that the maximum base temperature threshold for *S. pyrioides* is no more than 33°C. Developmental studies at constant temperatures of 15, 18, 21, 24, 27, 30, and 33 were reported by Braman et al. (1992), who estimated a minimum base temperature threshold of 10.2°C. First generation egg hatch, and development from egg-to-adult using this minimum base temperature threshold was estimated to be 211.1 and 398.4 accumulated degree-days, respectively (Braman et al. 1992). The authors also reported that ~50% of overwintered eggs in Georgia, USA had hatched by Day 75 in 1989 and 1990, and development of the first generation was largely completed by Day 120 (Braman et al. 1992). However, it is not known if these past observations from Georgia apply to the much different climate of the Pacific Northwest. For example, a recent study by Lee et al. (2019) in the Willamette Valley in Oregon suggested that *S. pyrioides* egg hatch might occur at ~150 accumulated degree-days (instead of 211.1), but also at much later calendar day (mid-May instead of mid-March). These differences highlight the limitations of current models from the southeastern United States and the need for refined models for predicting seasonality in western Washington.

Past studies have also reported on its voltinism (Dickerson and Weiss 1917, Bailey 1951, Neal and Douglass 1988, Braman et al. 1992). Up to four generations per year have been reported in Georgia, USA (Braman et al. 1992), between two and three generations in New England, and three generations are reported in Oregon (Lee et al. 2019). Moreover, insects can display variation in the distinctness of generational cycles within a year; some species exhibit very distinct generations, whereas others, following diapause (which generally synchronizes initial emergence) exhibit overlapping generations with multiple life stages present simultaneously (Bjørnstad et al. 2016). *Stephanitis pyrioides* seems to exhibit some degree of synchrony in hatch from overwintering eggs, but in subsequent generations, several different life stages may be present on infested plants at any given time (Trumbule et al. 1995). This 'generational smearing' (Bjørnstad et al. 2016) can complicate management efforts since many insect control tactics are most effective against only certain insect stages.

Indeed, management of *S. pyrioides* has shown to be difficult since its introduction to North America, and several factors contribute to its status as a primary pest of azaleas (Trumbule et al. 1995). Accurate prediction of insect development and emergence is essential for effective pest management, and pesticide applications must be timed precisely to maximize effectiveness and minimize the number of applications (Herms 2004). Contact insecticidal control measures taken before egg hatch or after adults appear and begin laying eggs can be ineffective. Nymphs, and especially early nymphs, are also more susceptible to insecticides than adults (Neal and Schaefer 2000, Sparks et al. 2000, Nair and Braman 2012). Systemic insecticides, such as imidacloprid can be effective against *S. pyrioides* and due to its residual effect, the timing of application is not as critical as with contact insecticides; however, there are concerns about their lethal and sub-lethal effects on pollinators (Villa et al. 2000).

In this chapter, I sampled life stages of *S. pyrioides* over two years at six locations at the Washington Park Arboretum. Approximately 200 leaf samples from 18 *Rhododendron* plants were collected 1-2 times per week from April to October in 2018 and 2019. Counts were then linked to degree-day accumulations to develop a predictive development model of *S. pyrioides* in western Washington.

2.3 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). The plant collections include over 14,500 accessioned specimens in the collections; over 4,000 different types of trees, shrubs and other plants native to 98 countries, which serve as vital resources for scientific study (UWBG 2018). Since the inception of the Washington Park Arboretum in 1936, *Rhododendron* spp. have been planted extensively. The University of Washington Botanic Gardens maintains the plant records at the Washington Park Arboretum in BG-BASE (O'Neal 2019), a database application designed to manage information on biological (primarily botanical) collections, and the plants are mapped using ArcGIS Desktop 10.7.1 (ESRI 2019). The Arboretum currently has > 2,400 individual records for the genus *Rhododendron*. Using the map records, *Rhododendron* density was determined using the 'Euclidean Distance' tool in ArcMap to highlight concentrations of *Rhododendron* plants (Fig. 2.6). *Rhododendron* spp. have been historically planted in distinct beds, separated by turf and plantings of other species. Six sites were chosen based on an observation of a variety of species or cultivars and a range of susceptibility to *S. pyrioides* damage (Fig. 2.6). At each site, three groups of *Rhododendron* plants were chosen based on prior observations of susceptibility to *S. pyrioides*: (A) one group that seemed to be highly susceptible to *S. pyrioides*; (B) one group that seemed to be moderately susceptible; and (C) one group that seemed to be resistant to *S. pyrioides* attack. Susceptibility of *Rhododendron* plants to *S. pyrioides* was estimated based on amount of stippling and amount of frass (Johnson and Lyon 1994). Groups were composed of plants of the same accession. When plants are acquired by the Washington Park Arboretum, they are given a unique accession number that includes the acquisition year and a sequentially assigned number. Plants from the same source (e.g., seedlot, cuttings, and tissue culture) are included in the same accession. Soil moisture was periodically monitored and irrigation was supplied when needed to minimize drought stress to *Rhododendron* plants.

Collection Protocol

Infested leaves were collected from Rh*ododendron* plants from April to October in 2018 and 2019. In 2018, at each sampling interval, 12 leaves were collected from each site (1-6) and plant group (A-C), for a total of 216 leaves. Samples were collected twice a week (Monday and Thursday) from April to September, and once per week (Thursday) in October, for a total of 35 sampling intervals. In 2019, sampling was repeated but only from groups A and C, and over a total of 31 sampling intervals. Infested leaves were collected alternately from the top, middle, and bottom of the plant, while also alternating from outer to inner canopy to account for light and temperature variation within the plant. Leaves were removed by grasping the petiole and pulling down to remove the leaf. This was done to avoid crushing nymphs and adults, and to disturb the leaf as little as possible to avoid dispersing adults. Adults rarely fled the leaf when it was removed gently.

Processing of samples

Leaves were placed in 100 x 15 mm polystyrene petri dishes, which were sealed with Parafilm and placed at -20°C to kill all life stages until counting could be done. The number of unhatched eggs, early instars (1st-3rd instars), late instars (4th and 5th instars), and adults were counted under a microscope at 3.5X-180X. Unhatched eggs were identified by the presence of an intact operculum. Early instars were differentiated from late based on size, color, appearance and wing pad presence (Drake and Ruhoff 1965, Shen et al. 1985). Differentiating instars based on the number of eye facets was also successfully attempted (Shen et al. 1985), but deemed impractical for this study given the sample size and the reliability of using the presence of wing pads in late instars (which are absent in earlier instars). After counting all life stages, frass was removed from leaves by a combination of washing with warm water and then removing any remaining frass covering eggs with a needle point tool while counting using a backlit microscope (Braman et al. 1992). Although not the focus of this study, leaves were casually inspected for the presence of *S. pyrioides* natural enemies that are established in the U.S. These include the egg parasitoid, *Anagrus takeyanus* Gordh (Hymenoptera: Mymaridae) (Balsdon et al. 1996), and a predatory true bug, *Stethoconus japonicas* Schumacher (Heteroptera: Miridae) (Henry et al. 1986).

Degree-day modeling

A weather station located at the University of Washington Center for Urban Horticulture (47°39'26.4"N 122°17'20.4"W), located 2.1 km from collection sites, was used to estimate daily degree-days (Agweathernet 2020). Hourly data was collected for each year, beginning on 1 January. Prior information on the minimum base temperature threshold (10.2°C) and maximum base temperature threshold (31°C) (Braman et al. 1992) was used to estimate degree-days. Degree-day accumulation from 1 January for each year was estimated using the trapezoidal method of integration applied to each hourly interval (Tobin et al. 2003). A visual inspection of the numbers of eggs, early instars, late instars, and adults over calendar day from both 2018 and 2019 (Figs. 2.7A and 2.7B), and these numbers over accumulated degree-days for each year (Figs. 2.8A and 2.8B), suggested that the first generation of egg hatch, early instars, late instars, and adults occurred within 300, 400, 500, and 600 accumulated degree-days, respectively. We were able to confirm the estimation of these durations by examining the cumulative proportions of development and identifying the degree-day where the graph appears to level off at the end of generational development (Fig. 2.9). Using this information, we then modeled the proportion of the first-generation egg hatch, early instar, late instar, and adults, P, over accumulated degree-days, DD, using a Gompertz function,

$$P = \exp(\exp(-rDD + b)) \text{ ,} \qquad [1]$$

in which r and b are the rate of increase and lag, respectively, and P and DD are predicted proportion of life stages and accumulated degree-days, respectively (Brown and Mayer 1988). Nonlinear convergence was based on the Marquardt algorithm (Marquardt 1963) in R (R Core Team 2018).

Predicting seasonality

To provide management guidelines that link the degree-day model to calendar dates, temperature data from the University of Washington Center for Urban Horticulture from 2015 to

2019, which reflect recent climatic trends, was used to predict the occurrence of 1st generation *S. pyrioides* egg hatch, early instars, late instars, and adults. We calculated the mean degree day accumulations from hourly measurements for each day over the last 5 years (Fig 2.11). We used Eq. 1 to predict the degree-day requirements for egg hatch, early instars, late instars, and adults at specific percentiles (5, 25, 50, 75, and 95%). These requirements were then linked back to calendar day to predict the earliest, mean, and latest date at which 5, 25, 50, 75, and 95% of each life stage is predicted to occur in our study region.

2.4 RESULTS

Across both years, the total number of eggs, early instars, late instars, and adults sampled was 14941, 1489, 2466, and 652, respectively. The first hatched eggs (early instars) were observed at 121.6 DD or Day 131 (11 May) in 2018, and at 116.1 DD or Day 126 (6 May) in 2019. The first adults were observed at 300 DD or Day 165 (14 June) in 2018 and at 286.6 DD or Day 157 (6 June) in 2019. The first generation of early instars began in early May, between 100 and 200 DD or Day 130-145 (Fig. 2.7), followed by the first generation of late instars ranging from late May to mid-June between 200-300 DD or Day 145-175 (Fig. 2.7). The first generation of adults appeared in mid-June, around 300-400 DD or Day 170, and adults were found consistently throughout the rest of the sampling period. We observed an increase in the numbers of early and late instars, and adults, in late August, around 900- 1000 DD or Day 240, corresponding to a second generation (Fig. 2.7). This second generation of adults will then lay the eggs of a partial third generation. This third generation may or may not hatch, depending on the cue that induces diapause (Tauber and Tauber 1976, Denlinger 2002). There is no perceivable increase of instars or adults to indicate that there is a full third generation.

Model parameters for the degree-day model for *S pyrioides* first generation egg hatch, early instars, late instars, and adults are presented in Table 2.1, and the predicted accumulations by degree-day using this model is presented in Figure 2.10. The degree-day accumulation at which 5, 25, 50, 75, and 95% of each life stage is predicted to occur is presented in Table 2.2. Based on temperature data from the last 5 years, the calendar day and date that 5, 25, 50, 75, and 95% of each life stage is predicted to have occurred is presented in Tables 2.3 and 2.4. According to these estimates the mean day of egg hatch (50% hatched or 172.7 DD) is day 136. The earliest that egg hatch has occurred (5% hatched or 93.2 DD) is day 107 (17 Apr) and the latest is day 139 (19 May). The mean day of adult development is 402.3 DD or day 174.8 (23 Jun). The earliest day that adults would have appeared (5% developed or 320 DD) is 156 (5 Jun) and the latest is day 175 (24 Jun).

2.5 DISCUSSION

Successful management of *S. pyrioides* requires the use of an integrated pest management (IPM) program. IPM is a science-based, sustainable decision-making process that uses information on pest biology, environmental data, and technology to minimize economic and ecological damage in a way that minimizes the economic costs and risks to people, property, and the environment (USDA 2018). Prior studies on *S. pyrioides* that compared IPM with traditional pest control approaches in landscapes found that pesticide volume was reduced by an average of 85.3%, and reduced the overall cost of plant care without compromising aesthetic quality of the landscape (Smith and Raupp 1986, Stewart et al. 2002, Nair and Braman 2012). Accurate timing of scouting and treatments are extremely critical to the success of IPM. Degree-day models can be a keystone element in developing IPM programs for any pest insect. The results from this study should assist in the development of management programs against of *S. pyrioides* in the Pacific Northwest.

Although initial hatch from overwintering eggs is somewhat synchronized, the subsequent occurrence of life stages is not as discrete (Fig. 2.7). Because of this generational smearing, and the higher susceptibility of early instars to insecticides, relative to later instars and adults, we concentrated on modeling the first generation from egg to adult. This will allow scouting and treatment to be targeted when control efforts are the most effective. We designated a control window between mean egg hatch (50%) and the first appearance of adults (5%). On an average year, the most effective time to take control measures for *S. pyrioides* in the Pacific Northwest is between Day 136 to 164 (Table 2.3). This control window extends for 18 days on an average year. However, even within the most recent 5 years, this window could be as early as Day 127 or as late as Day 175 (Table 2.3).

A graphical comparison of the counts of eggs and early instars from 2018 and 2019 by either degree-day accumulations or calendar day is shown in Figure 2.12. Calendar day predictions are usually preferred by general audiences due to their simplicity, even though degree-day models are more accurate for predicting insect seasonality. Predicted proportions of life stages tend to occur at approximately the same degree-day totals each year, but degree-day accumulations can vary considerably from year-to-year. I hypothesized that the occurrence of eggs and early instars in both years would be more similar when graphing against accumulated degree-days and vary more when graphed against calendar day. However, there seemed to be very little variation between the two approaches (Fig. 2.12), perhaps due to the relatively little variation between both of the study years in the Puget Sound region, which is furthermore tempered by the maritime climate. We would expect more variation between years that were more climactically variable. Thus, the degree-day model developed in this chapter should perform well in the relatively stable climate of the Puget Sound region, but more work is needed to determine its utility in more climactically variable regions of western Washington (e.g. the foothills of the Cascade Range).

Neither of the natural enemies that established elsewhere in the eastern U.S. (*A. takeyanus* and *S. japonicas*) were found during the sampling efforts. These species were also not found during a recent study in Oregon (Lee et al. 2019), and might not yet be established in the Pacific Northwest. Generalist predators such as spiders and green lacewings (*Chrysoperla* sp.) were occasionally found on collected leaves. By targeting the most effective treatment window, additional sprays can hopefully be avoided and risk to these non-target organisms will be minimized, which is a goal of IPM programs.

We predicted that *S. pyrioides* has at least two generations and a partial third generation per year in western Washington. A large 1st generation of adults is seen to mature at between 400 to 500 DD (Day 160), and a smaller generation of nymphs and adults is seen about 1000 DD (Day 250) (Fig. 2.8). Because *S. pyrioides* overwinters in the egg stage, at some point after this second generation there is likely a cue that induces eggs to diapause and not hatch into a new generation. Environmental cues that can trigger this are photoperiod, temperature, food quality, moisture, or other chemical cues (Gullan et al. 2014). Research to determine this cue for diapause in *S. pyrioides* would allow for a more accurate estimation of the number of generations per year possible in western Washington.

By tracking the accumulation of degree-days, it is possible to predict the exact day to begin scouting and implementing control measures for *S. pyrioides*. Horticultural and pest management professionals will find this an effective tool, but unfortunately residential gardeners or non-professionals may not be familiar with degree–day modeling and tracking, and will likely turn to calendar-based scheduling for pest control. The western Washington calendar-based recommendations presented in this research will be much more useful than those based on observations from Georgia, USA, where egg hatch can occur over a month before Washington (Braman et al. 1992, Table 2.4). Using the control window specific to western Washington, whether degree-day or calendar based, identified in this research optimizes sampling efforts and the timing of effective control measures. This window is between 172.7 and 320 accumulated degree-days, which, on an average year in western Washington, occurs between 16 May and 12 June.

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CHAPTER 3: SUSCEPTIBILITY OF RHODODENDRON TO AZALEA LACE BUG S. PYRIOIDES (SCOTT) DAMAGE

3.1 ABSTRACT

The non-native, invasive azalea lace bug, *Stephanitis pyrioides* (Scott), is one of the most serious insect pests of the genus *Rhododendron*, especially azaleas, an especially popular subgenus of *Rhododendron.* Feeding by nymphs and adult *S. pyrioides* remove chlorophyll from leaves, reducing rates of photosynthesis and transpiration of infested plants, and causes stippling on the top of the leaf, which reduces the aesthetic value of infested plants. *Rhododendron* spp. and cultivars are a major component of public and private landscapes in the Pacific Northwest. The variability in susceptibility of *Rhododendron* to *S. pyrioides* has been examined in the past but these previous studies considered only a very limited number of *Rhododendron* species and cultivars. We measured the susceptibility of Rhododendrons to determine if any patterns could be ascertained within the phylogenetic tree of *Rhododendron*. To measure susceptibility of *Rhododendron* to S. pyrioides we collected leaves from 71 *Rhododendron* species and cultivars in August of 2018 and 2019. These leaves were scanned and analyzed to determine the proportion of the leaf damage caused by *S. pyrioides* feeding. Damage proportions were examined across *Rhododendron* phylogeny and different hierarchies of taxonomy. We observed that the presence of leaf trichomes did not predict *S. pyrioides* damage. We also observed that plants from the subgenus *Azaleastrum* were the significantly most susceptible subgenus, and all the sampled plants within this subgenus (N=19) had some measurable damage. Sampled plants within the subgenus *Rhododendron* (N=20) was the next susceptible subgenus, and many plants (7 of 21) were seemingly free of damage. In contrast, plants from the subgenus *Hymenanthes* (N=31) were the

significantly least susceptible, and most plants (20 of 31) had no measurable damage. This study provides a guideline for using host plant resistance to *S. pyrioides* in plant selection, and emphasizes that if azaleas are to be used in the landscape, management programs for *S. pyrioides* will need to be implemented.

3.2 INTRODUCTION

The non-native, invasive azalea lace bug, *Stephanitis pyrioides* (Scott) (Tingidae: Hempitera), is one of the most serious insect pests of the genus *Rhododendron*, especially azaleas (Nair and Braman 2012). *Stephanitis* pyrioides is known to attack woody ornamentals, especially the Genus *Rhododendron* and related plants of the family *Ericaceae* (Nair and Braman 2012, Schuh and Slater 1995), and is especially noted for its economic damage (Shrewsbury and Smith-Fiola 2000, Klingeman et al. 2001, Nair and Braman 2012). Feeding by nymphs and adult *S. pyrioides* remove 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). Since its introduction into the United States, *S. pyrioides* has spread throughout the eastern United States extending to all areas were azaleas are able to be grown (Weiss and Headlee 1918, Drake and Ruhoff 1965). It has also been reported in other countries in Asia, Europe, and South America (Nair and Braman 2012). It was first detected in Washington in 2007 (Looney et al. 2016), and it is now widely established in western Oregon, Washington, and British Columbia (Rosetta 2013, Lee et al. 2019).

The biology and life history of *S. pyrioides* have been previously studied (Nair and Braman 2012). Briefly, the adults are relatively small with males ~2.8-3.3 mm and females ~2.9-3.3 mm in length (Fig. 2.1; Shen et al. 1985). All life stages of *S. pyrioides* are found on the underside of leaves. Both male and female adult S. pyrioides have transparent, areolate patterned wings and pronotum that are the origin of its common name. Eggs are the overwintering stage, and 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 (Figs. 2.2, 2.3; Nair and Braman 2012). There are five instars, ranging in length from 0.1-1.8 mm, and wing pads are present in the last two instars (Shen et al. 1985). Adults do not diapause (Neal and Douglass 1988) and have been observed to be active during winter in the east and west coast of the United States (Neal 1985, Lee et al. 2019). At present, hatch from overwintering eggs is thought to be degree-day driven, but the cues that initiate diapause induction are not known.

Prior reports describe the occurrence of *S. pyrioides* on species of *Rhododendron*, or other species within Ericaceae (Drake 1965, Nair and Braman 2012). Commonly known as the heath or heather family, *Ericaceae* is a large family with approximately 4250 known species across 124 genera. It is an economically important family, and some have edible berries, e.g., species within *Arctostaphylos*, *Gaylussacia*, and *Vaccinium* (Venturini et al. 2017). In addition, many species are cultivated as ornamentals, including those within *Erica*, *Rhododendron*, *Arbutus*, and *Pieris* (Yu et al. 2009). They are found most commonly in acid and infertile growing conditions, and like many other stress tolerant plants, many have mycorrhizal fungi to assist with extracting nutrients from infertile soils, as well as evergreen foliage to conserve absorbed nutrients (Cairney et al.

2003, Keddy 2007). In a preliminary visual assessment of *S. pyrioides* in western Washington, I have observed life stages feeding on species of the Ericaceous genera *Rhododendron*, *Gaultheria*, and *Pieris*. Moreover, there are limited reports of *S. pyrioides* attacking hosts of plant families other than Ericaceae (Nair and Braman 2012). Adults and their characteristic stippling and frass 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.

The genus *Rhododendron* contains >1000 species of woody ornamentals mostly known for their showy flowers (USDA 2011). In addition to its beautiful flowers, they have ecological significance and economic importance (Paul et al. 2005). It is widely cultivated in temperate regions, and in the wild they form important components of montane ecosystems. The genus *Rhododendron* exhibits an incredibly wide range of form, foliage and flower (Nelson 2001). From magnificent trees to sturdy understory shrubs to delicate groundcovers, *Rhododendron* plays innumerable roles in the garden by their form alone (Nelson 2001). The overwhelming majority of *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, although far fewer in number, are distributed much more widely 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, ~ 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). Nowhere else on Earth are there so many very deep valleys clustered so closely together (Irving and Hebda 1993). The hypothesis is that at the onset of the hostile climate conditions, *Rhododendron* populations at the margins of this area were able to enter the region of extreme relief and take advantage of newly developing 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. This hypothesis has support from Shrestha et al. (2018) in their study on global patterns of *Rhododendron* diversity. The ancestral area reconstruction based on both S-DIVA and DEC methods supports a northeast Asian origin of *Rhododendron* (Shrestha et al. 2018). The genus appears to have 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 ~ 10 Mya (Shrestha et al. 2018).

The genus *Rhododendron* can be separated into two categories: lepidote (with scales) and elepidote (without scales). 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 the 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 finally 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 grab-bags, some with large numbers of species incomprehensibly diverse; others with a few, differing from those in another series only by shadowy nuances (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 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. 3.1). 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 completely closed to Europeans and to foreign travel until the middle of the 19th century. No foreigners were allowed to go more than a few miles 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 completely opened to foreign travel (Davidian 1996). 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 cultivation of *Rhododendron* in the Pacific Northwest United States (Nelson 2001). *S. pyrioides* was introduced into the Pacific Northwest sometime near the turn of the 21st century, and was first detected in Washington in 2007 (Loony et al. 2016). By 2015 damage by *S. pyrioides* to *Rhododendrons* in private as well as public landscapes such as the Washington Park Arboretum became a serious problem, with widespread damage and plant death in susceptible varieties.

Differences in susceptibility of various *Rhododendron* species and cultivars to *S. pyrioides* have been previously studied. In one evaluation, 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 in azaleas 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, and they 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 both feeding and oviposition by *S. pyrioides* in the treated susceptible genotypes, and wax extracts from susceptible genotypes applied on resistant ones caused susceptibility, indicating that leaf wax has a definite role in *S. pyrioides* resistance in azaleas (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. *S. pyrioides* feed on the bottom of the leaf, inserting their stylet through the stomata. These leaf characteristics are hypothesized to somehow physically inhibit the ability of *S. pyrioides* to get 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, in another study, 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). Among all these studies, however, 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 Vireya species, can be grown outdoors. In Washington, the narrow coastal area bordering Puget Sound falls in USDA Zone 8b, with the rest of western Washington up to the foothills of the Cascade Mountains in Zone 8a, with an average annual minimum temperature of -9.4 and -12.2 °C, respectively (Nelson 2001, USDA 2012). 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 chapter, we sampled 71 *Rhododendron* species and cultivars and quantified the damage done by *S. pyrioides*. Leaves were sampled during mid-summer when damage by *S. pyrioides* is generally the highest (RRG, personal observations). Leaves were scanned after their collection, and the proportion of leave area damaged was calculated for each sampled *Rhododendron*. Damage proportions were analyzed to determine if any patterns could be ascertained within the phylogenetic tree of *Rhododendron*. It was our hypothesis that at some level of classification, a difference in susceptibility would be significantly different. To test this, damage proportions were analyzed to determine their relationship within the taxonomic hierarchy of *Rhododendron* leaf trichome presence (lepidote/elepidote), clade, and section.

3.3 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). The plant collections include over 14,500 accessioned specimens in the collections; over 4,000 different types of trees, shrubs and other plants native to 98 countries, which serve as vital resources for scientific study (UWBG 2018). Since the inception of the Washington Park Arboretum in 1936, *Rhododendron* spp. have been planted extensively. The University of Washington Botanic Gardens maintains the plant records at the Washington Park Arboretum in BG-BASE (O'Neal 2019), a database application designed to manage information on biological (primarily botanical) collections, and the plants are mapped using ArcGIS Desktop 10.7.1 (ESRI 2019). The Arboretum currently has > 2,400 individual records for the genus *Rhododendron*.

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, Balsdon et al. 1995); this selection resulted in 4 species and cultivars. *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 species and cultivars selected for this study are listed in Table 3.1.

Collection Protocol

To quantify susceptibility of *Rhododendron* to *S. pyrioides* feeding damage, 80 leaves were collected from 71 different *Rhododendron* species, varieties, and cultivars in August of 2018 and 2019. August was chosen so that the first generation of *S. pyrioides* would have been completed (Chapter 2), such that adults from the first generation and immatures from the second generation would have had the opportunity to feed on host plants. 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 with the species name, accession number, and date. Samples were stored at 4 °C for no more than a 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 underside scans also revealed any presence of frass, and *S. pyrioides* instars or adults. Leaves were scanned with an Epson Perfection V800 Photo scanner at 600 dpi using SilverFast SE 8 scanning software 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). After loading the image into Assess 2.0, color thresholds were adjusted in 'Leaf' mode so that the entire leaf area can be selected (Fig. 3.2). When the 'Area' button is pressed the leaf area is calculated and saved into the program's internal database. The 'Leaf' button was pressed to switch to 'Lesion' mode. Color thresholds were adjusted so that the chlorotic area was selected (Fig 3.2). The 'Area' button was pressed to calculate lesion area, values were saved into the program's internal database. Leaf area and damaged area were calculated to provide a value for proportion of leaf area damaged.

Statistical Analysis

The sampling unit for analysis was each *Rhododendron* plant species, cultivar or variety. The raw response variable was the mean proportion of leaf area damaged by *S. pyrioides*. Means were calculated from the total number of leaves sampled in 2018 and 2019, during which an average of 77 and 78 leaves were collected from each plant, respectively. The means were transformed using a logit transformation according to $\log_e$ (mean proportion / (1 - mean proportion)) to meet the assumption of normality in an Analysis of Variance (ANOVA). Prior to the logit transformations, means were adjusted by the addition of 0.001 to permit transformation of zero means. The effect of trichome presence or absence on the transformed mean proportion of damage was analyzed 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).

3.4 RESULTS

Across both 2018 and 2019, ~160 leaves from 71 *Rhododendron* species and cultivars were collected and scanned. A total of 5680 and 5738 leaves were collected and scanned in 2018 and 2019, respectively. Species sampled and their corresponding trichome presence, clade, subgenera, section, and mean proportion of leaf area damaged is presented in Table 3.1. Overall, the proportion of the leaf damaged by *S. pyrioides* across all *Rhododendron* species and cultivars ranged from 0 to 0.3451 (Fig. 3.3). Subgenus *Azaleastrum* (clade C) had the highest overall level of damage, and all samples were observed to have some level of stippling damage. Samples from the subgenus *Azaleastrum* (N=19) had a mean proportion of leaf area damaged of 0.1407, and damage ranged from .0089 to 0.3451 (Fig. 3.3). The amount of leaf area damage was lowest in the subgenus *Hymenanthes* (clade B); samples from this subgenus (N=29), had a mean proportion of leaf area damaged of 0.0080, and ranged from no damage to 0.0666. 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. The compiled phylogenic tree of *Rhododendron* with the species matched to the corresponding proportion of leaf area damaged is presented in Figure 3.3.

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* = 0.068; df = 1, 69; *P* = 0.796). However, there were significant differences in the proportion of leaf damage by *Rhododendron* subgenera (*F* = 35.99; df = 2, 68; *P* < 0.01) and *Rhododendron* section (*F* = 19.39; df = 4, 66; *P* < 0.01). The proportion of leaf area damaged by *S. pyrioides* by the presence or absence of trichomes, by subgenus, and by section is presented in Figures 3.4, 3.5, and 3.6, respectively.

Post hoc tests using Tukey's HSD revealed that among the subgenera analyzed (*Rhododendron*, *Hymenanthes*, and *Azaleastrum*), all were significantly different from each other (Fig. 3.5). Among sections, the sections *Tsutsusi* and *Sciadorhodion,* both from subgenus *Azaleastrum*, did not differ significantly (Fig. 3.6). *Section Ponicum* and *Pentanthera*, both from subgenus *Hymenanthes*, also did not differ significantly (Fig. 3.6). Finally, section *Tsutsusi* (evergreen azaleas) differed significantly from all the other sections except *Sciadorhodion* (Asian deciduous azaleas) (Fig. 6.3).

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, there was no measurable damage recorded (Fig. 3.3). *Rhododendron canescens* was described as resistant to *S. pyrioides* by Wang et al. (1998), and we observed a low proportion leaf area damaged (< 0.0087) in this study (Fig. 3.3).

3.5 DISCUSSION

Rhododendron species can be most easily subdivided through the presence or absence of trichomes (i.e., lepidote or elepidote, respectively), after which this genus is 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 statistically significant differences between group means of proportion of leaf area damaged. Thus, among the species and cultivars tested in this study, trichome structures on *Rhododendron* leaves did not predict susceptibility to *S. pyrioides* and did not seem to deter *S. pyrioides* life stages from feeding on host plants with trichomes. However, differences were observed among all *Rhododendron* subgenera (Fig. 3.5). 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 alone is not a predictor of susceptibility to *S. pyrioides.* Indeed, these two groups seemed to cancel each other out in the analysis of susceptibility between lepidote and elepidote *Rhododendron*. Past studies have reported on the incredible variation in the architecture of *Rhododendron* trichomes (Cowan 1950). Thus, although the presence of trichomes alone 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 provide greater insight into the role that trichomes play in conferring resistance to *S. pyrioides* feeding. Leaf wax and leaf chemical content of resistant versus susceptible species could also be investigated for their role in host plant resistance.

Although this study did highlight susceptible *Rhododendron* species and cultivars, it is not trivial to suggest that growers that desire to include *Rhododendron* spp. in landscape plans 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*). Evergreen azaleas are highly valued for their generally small stature and year-round foliar interest. They exhibit a range of color and form that may not be available in other subgenera (Kobayashi 2013). Evergreen azaleas also have an important place in the culture of 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. Such plants cannot be simply or easily replaced with other *Rhododendron* varieties. The variety of form and flower existing in the *Rhododendron* outside the subgenus *Azaleastrum* may not be as wide, but if resistant *Rhododendron* species or cultivars are desired, then these species and cultivars should be considered. The finding that species in the subgenus *Azaleastrum* were the most susceptible to *S. pyrioides*, from which all tested plants within this subgenus had some level of stippling damage, indicate that if one is determined to grow these azaleas, then a management program for *S. pyrioides* will be necessary.

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 natural range. Approximately 70% of *Rhododendron* species are classified as vulnerable, threatened, endangered or critically endangered, and ~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 where azaleas can be grown, careful consideration should be made if and when they are used. One possible avenue of future research is whether or not resistant species can be strategically planted to reduce the overall damage by *S. pyrioides* in landscapes.

The presence of this invasive insect and cultural, ecological, and horticultural importance of the wide diversity of both resistant and susceptible azalea and rhododendron species, control of the insect becomes critical. A greater understanding of the patterns of susceptibility to *S. pyrioides* feeding in *Rhododendron* will allow scouting and control resources to be more effective in protecting the health of this beloved genus.

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TABLES

Table 2.1 Parameter estimates of the exponential model (P=exp (exp (-*r*DD+*b*)), Eq. 1) fit to the cumulative proportion of first generation egg hatch, early instars, late instars, and adults.

Phenological event	Parameter estimates (±SE)	
	r	*B*
Egg hatch	0.0184 (0.0009)	2.8115 (0.1634)
Early instars	0.0143 (0.0007)	2.6295 (0.1656)
Late instars	0.0192 (0.0009)	5.1188 (0.2603)
Adults	0.0178 (0.0006)	6.7936 (0.2607)

Table 2.2 Accumulated degree-days at which 5, 25, 50, 75, and 95% of each life stage of *S. pyrioides* is predicted to occur using Eq. 1 and parameters from Table 1.

percent	egg hatch	early instars	late instars	adult
5	93.2	107.2	209.5	320
25	135	161	249.6	363.3
50	172.7	209.5	285.7	402.3
75	220.5	271	331.5	451.7
95	314.2	391.6	421.3	548.5

Table 2.3 The mean, earliest, and latest calendar day at which 5, 25, 50, 75, and 95% of each phenological event is predicted to occur using temperature data from our study region from 2015 to 2019.

	egg hatch			early instars			late instars			adult appearance		
%	earliest	average	latest	earliest	average	latest	earliest	average	latest	earliest	average	latest
5	107	120.2	139	111	123.2	141	133	143.6	157	156	163.8	175
25	120	129.8	145	124	134.2	148	143	151.6	163	160	168.4	181
50	127	136.8	150	133	143.6	157	152	158.6	170	166	174.8	186
75	135	145.6	158	149	155.8	168	157	164.8	176	172	181.6	191
95	156	163.6	175	165	173	184	169	177.6	187	181	192.8	203

Table 2.4 The mean, earliest, and latest date at which 5, 25, 50, 75, and 95% of each phenological event is predicted to occur using temperature data from our study region from 2015 to 2019.

	egg hatch			early instars			late instars			adult appearance		
%	earliest	average	latest	earliest	average	latest	earliest	average	latest	earliest	average	latest
5	17-Apr	30-Apr	19-May	21-Apr	3-May	21-May	13-May	23-May	6-Jun	5-Jun	12-Jun	24-Jun
25	30-Apr	9-May	25-May	4-May	14-May	28-May	23-May	31-May	12-Jun	9-Jun	17-Jun	30-Jun
50	7-May	16-May	30-May	13-May	23-May	6-Jun	1-Jun	7-Jun	19-Jun	15-Jun	23-Jun	5-Jul
75	15-May	25-May	7-Jun	8-Jun	4-Jun	17-Jun	6-Jun	13-Jun	25-Jun	21-Jun	30-Jun	10-Jul
95	5-Jun	12-Jun	24-Jun	14-Jun	22-Jun	3-Jul	18-Jun	26-Jun	6-Jul	30-Jun	11-Jul	22-Jul

Table 3.1 Species sampled and their corresponding trichome presence, clade, subgenera, section, and mean proportion of leaf area damaged.

Accession #	Name	(n) 2018	mean damage 2018	(n) 2019	2019	(n) total	mean proportion damaged	Lepidote/ Elepidote	subgenus	section	Clade	
4C	Rhododedron sp.	81	0.0851	82	0.1156	163	0.1003					
4A	Rhododedron sp.	82	0.1206	80	0.1804	162	0.1505					
544-42	Rhododendron (azalea) 'Anchorite'	79	0.2517	83	0.1693	162	0.2105	E	Azaleastrum	Tsutsusi	C	
741-56	Rhododendron (azalea) 'Atalanta'	61	0.0613	83	0.1167	144	0.0890	E	Azaleastrum	Tsutsusi	C	
532-42	Rhododendron (azalea) 'Carmel'	81	0.1404	40	0.1680	121	0.1542	E	Azaleastrum	Tsutsusi	C	
1039-57	Rhododendron (azalea) 'Corsage'	82	0.2931	80	0.1949	162	0.2440	E	Azaleastrum	Tsutsusi	C	
2219-41	Rhododendron (azalea) 'Daphne'	89	0.4500	85	0.1112	174	0.2806	E	Azaleastrum	Tsutsusi	C	
735-49	Rhododendron (azalea) 'Ladylove'	79	0.1615	89	0.1014	168	0.1314	E	Azaleastrum	Tsutsusi	C	
807-48	Rhododendron (azalea) 'Lustre'	81	0.1282	84	0.1334	165	0.1308	E	Azaleastrum	Tsutsusi	C	
499-58	Rhododendron (azalea) 'Maxwellii'	84	0.1345	73	0.0983	157	0.1164	E	Azaleastrum	Tsutsusi	C	
183-82	Rhododendron (azalea) 'Roberta'	81	0.1748	86	0.0991	167	0.1370	E	Azaleastrum	Tsutsusi	C	
733-49	Rhododendron (azalea) 'Trouper'	82	0.2705	87	0.1516	169	0.2111	E	Azaleastrum	Tsutsusi	C	
105-56	Rhododendron aberconwayi	80	0.0000	79	0.0000	159	0.0000	E	Hymenanthes	Ponticum	B	
442-41	Rhododendron adenopodum	87	0.0000	80	0.0000	167	0.0000	E	Hymenanthes	Ponticum	B	
1479-38	Rhododendron aff. cuneatum	83	0.0120	81	0.0223	164	0.0171	L	Rhododendron	Rhododendron	A	
1521-40	Rhododendron albrechtii	81	0.1042	83	0.1012	164	0.1027	E	Azaleastrum	Sciadorhodion	C	
196-51	Rhododendron amagianum	80	0.0133	81	0.0045	161	0.0089	E	Azaleastrum	Tsutsusi	C	
1520-41	Rhododendron argyrophyllum	85	0.0000	82	0.0000	167	0.0000	E	Hymenanthes	Ponticum	B	
117-56	Rhododendron augustinii	104	0.1294	85	0.1302	189	0.1298	L	Rhododendron	Rhododendron	A	
2352-38	Rhododendron auriculatum	84	0.0000	79	0.0000	163	0.0000	E	Hymenanthes	Ponticum	B	
90-10 & 89-10	Rhododendron barbatum	0	NDA	80	0.0555	80	0.0555	E	Hymenanthes	Ponticum	B	
12-98	Rhododendron 'Big Yak'	73	0.0000	81	0.0000	154	0.0000	E	Hymenanthes	Ponticum	B	
495-58	Rhododendron breviperulatum	83	0.1272	82	0.0966	165	0.1119	E	Azaleastrum	Tsutsusi	C	
172-44	Rhododendron calendulaceum	85	0.0000	82	0.0000	167	0.0000	E	Hymenanthes	Pentanthera	B	
188-14&189-14	Rhododendron campanulatum ssp. aeruginosum	57	0.0000	81	0.0000	138	0.0000	E	Hymenanthes	Ponticum	B	
269-64	Rhododendron canescens	0	NDA	60	0.0087	60	0.0087	E	Hymenanthes	Pentanthera	B	
23-03	Rhododendron coeloneuron	80	0.0000	80	0.0000	160	0.0000	E	Hymenanthes	Ponticum	B	
6-64*A	Rhododendron davidsonianum	81	0.0675	79	0.0492	160	0.0584	L	Rhododendron	Rhododendron	A	
X-177	Rhododendron degronianum ssp. heptamerum	80	0.0000	77	0.0000	157	0.0000	E	Hymenanthes	Ponticum	B	
565-60	Rhododendron degronianum ssp. yakushimanum	79	0.0000	80	0.0000	159	0.0000	E	Hymenanthes	Ponticum	B	
1154-56	Rhododendron fortunei spp. discolor	77	0.0000	72	0.0000	149	0.0000	E	Hymenanthes	Ponticum	B	
x-x	Rhododendron floribundum	79	0.0000	0	NDA	79	0.0000	E	Hymenanthes	Ponticum	B	
462-41 & 261-49	Rhododendron fortunei	41	0.0000	81	0.0000	122	0.0000	E	Hymenanthes	Ponticum	B	
163-68	Rhododendron 'Gill's Crimson'	70	0.0654	80	0.0678	150	0.0666	E	Hymenanthes	Ponticum	B	
108-89	Rhododendron 'Ginny Gee'	81	0.2490	78	0.3236	159	0.2863	L	Rhododendron	Rhododendron	A	
1120-48	Rhododendron groenlandicum	81	0.0000	82	0.0000	163	0.0000	L	Rhododendron	Rhododendron	A	
1508-37	Rhododendron hemitrichotum	87	0.0000	88	0.0000	175	0.0000	L	Rhododendron	Rhododendron	A	
800-47	Rhododendron hemsleyanum	81	0.0000	81	0.0000	162	0.0000	E	Hymenanthes	Ponticum	B	
606-67	Rhododendron cinnabarinum ssp xanthocodon	82	0.0941	83	0.0805	165	0.0873	L	Rhododendron	Rhododendron	A	
2217-41	Rhododendron indicum	83	0.2625	104	0.4276	187	0.3451	E	Azaleastrum	Tsutsusi	C	
186-56 & 184-56	Rhododendron irroratum	87	0.0006	72	0.0029	159	0.0018	E	Hymenanthes	Ponticum	B	
767-38	Rhododendron kaempheri	85	0.1319	86	0.0832	171	0.1076	E	Azaleastrum	Tsutsusi	C	
94-10 & 93-10	Rhododendron lutescens	81	0.0008	82	0.0039	163	0.0023	L	Rhododendron	Rhododendron	A	
746-40	Rhododendron luteum	81	0.0751	83	0.0273	164	0.0512	E	Hymenanthes	Pentanthera	B	
505-69	Rhododendron macabeanum	71	0.0000	80	0.0000	151	0.0000	E	Hymenanthes	Ponticum	B	
30-03	Rhododendron moupinense	84	0.0000	60	0.0000	144	0.0000	L	Rhododendron	Rhododendron	A	
707-40	Rhododendron mucronatum	80	0.0426	41	0.0300	121	0.0363	E	Azaleastrum	Tsutsusi	C	
31-03	Rhododendron mucronulatum	85	0.0491	83	0.0532	168	0.0511	E	Rhododendron	Rhododendron	A	
310-69	Rhododendron mucronulatum var. ciliatum	81	0.0899	81	0.0638	162	0.0768	L	Rhododendron	Rhododendron	A	
646-58	Rhododendron obtusum	82	0.2228	102	0.1284	184	0.1756	E	Azaleastrum	Tsutsui	C	
54-07	Rhododendron occidentale	81	0.0000	85	0.0000	166	0.0000	E	Hymenanthes	Pentanthera	B	
113-56	Rhododendron oreotrephes	80	0.0046	82	0.0009	162	0.0027	L	Rhododendron	Rhododendron	A	
127-53	Rhododendron ponticum	66	0.0000	80	0.0000	146	0.0000	E	Hymenanthes	Ponticum	B	
278-44	Rhododendron praevernum	79	0.0059	75	0.0056	154	0.0057	E	Hymenanthes	Ponticum	B	
1531-45	Rhododendron racemosum	85	0.0000	84	0.0000	169	0.0000	L	Rhododendron	Rhododendron	A	
75-56	Rhododendron ririei	81	0.0019	82	0.0016	163	0.0017	E	Hymenanthes	Ponticum	B	
116-48	Rhododendron rubiginosum	60	0.1967	81	0.0994	141	0.1480	L	Rhododendron	Rhododendron	A	
1031-40	Rhododendron sanguineum var.	80	0.0000	82	0.0000	162	0.0000	E	Hymenanthes	Ponticum	B	
192-95	Rhododendron scabrifolium	80	0.0000	81	0.0000	161	0.0000	L	Rhododendron	Rhododendron	A	
245-45	Rhododendron schlippenbachii	80	0.0420	72	0.0247	152	0.0333	E	Azaleastrum	Sciadorhodion	C	
198-56	Rhododendron searsiae	89	0.0883	81	0.0806	170	0.0845	L	Rhododendron	Rhododendron	A	
183-56	Rhododendron siderophyllum	61	0.0000	82	0.0000	143	0.0000	L	Rhododendron	Rhododendron	A	
1117-45	Rhododendron simsii	82	0.0617	80	0.0346	162	0.0482	E	Azaleastrum	Tsutsusi	C	
712-38	Rhododendron sutchuenense var. geraldii	77	0.0000	82	0.0000	159	0.0000	E	Hymenanthes	Ponticum	B	
124-56 & 23-13	Rhododendron triflorum var. bauhiniiflorum	83	0.0000	82	0.0000	165	0.0000	L	Rhododendron	Rhododendron	A	
475-41	Rhododendron ungernii	80	0.0000	82	0.0000	162	0.0000	E	Hymenanthes	Ponticum	B	
176-56	Rhododendron vernicosum	97	0.0025	61	0.0153	158	0.0089	E	Hymenanthes	Ponticum	B	
1386-50	Rhododendron viscosum	80	0.0325	81	0.0618	161	0.0472	E	Hymenanthes	Pentanthera	B	
246-44	Rhododendron wardii	80	0.0005	82	0.0010	162	0.0007	E	Hymenanthes	Ponticum	B	
X-112	Rhododendron williamsianum	80	0.0023	82	0.0002	162	0.0013	E	Hymenanthes	Ponticum	B	
188-56	Rhododendron x lochmium	90	0.0078	81	0.0041	171	0.0059	L	Rhododendron	Rhododendron	A	
2383-38	Rhododendron yunnanense	80	0.2070	74	0.0472	154	0.1271	L	Rhododendron	Rhododendron	A	
X-144	Rhododendron yunnanense	86	0.1458	81	0.1079	167	0.1269	L	Rhododendron	Rhododendron	A	

FIGURES

Figure 2.1 Adult *S. pyrioides* (Photo credit: Ryan R. Garrison).

Figure 2.2 Second instar *S. pyrioides*. Early instars are differentiated from late instars by lighter color, lack of wing pads, and smaller spines (Photo credit: Ryan R. Garrison).

Figure 2.3 Late instar *S. pyrioides*; note the presence of wing pads, darker body, and longer and darker spines (Photo credit: Ryan R. Garrison).

Figure 2.4 Clorotic stippling and bleached leaves on a *Rhododendron* sp., characteristic of *S. pyrioides* feeding (Photo credit: Ryan R. Garrison).

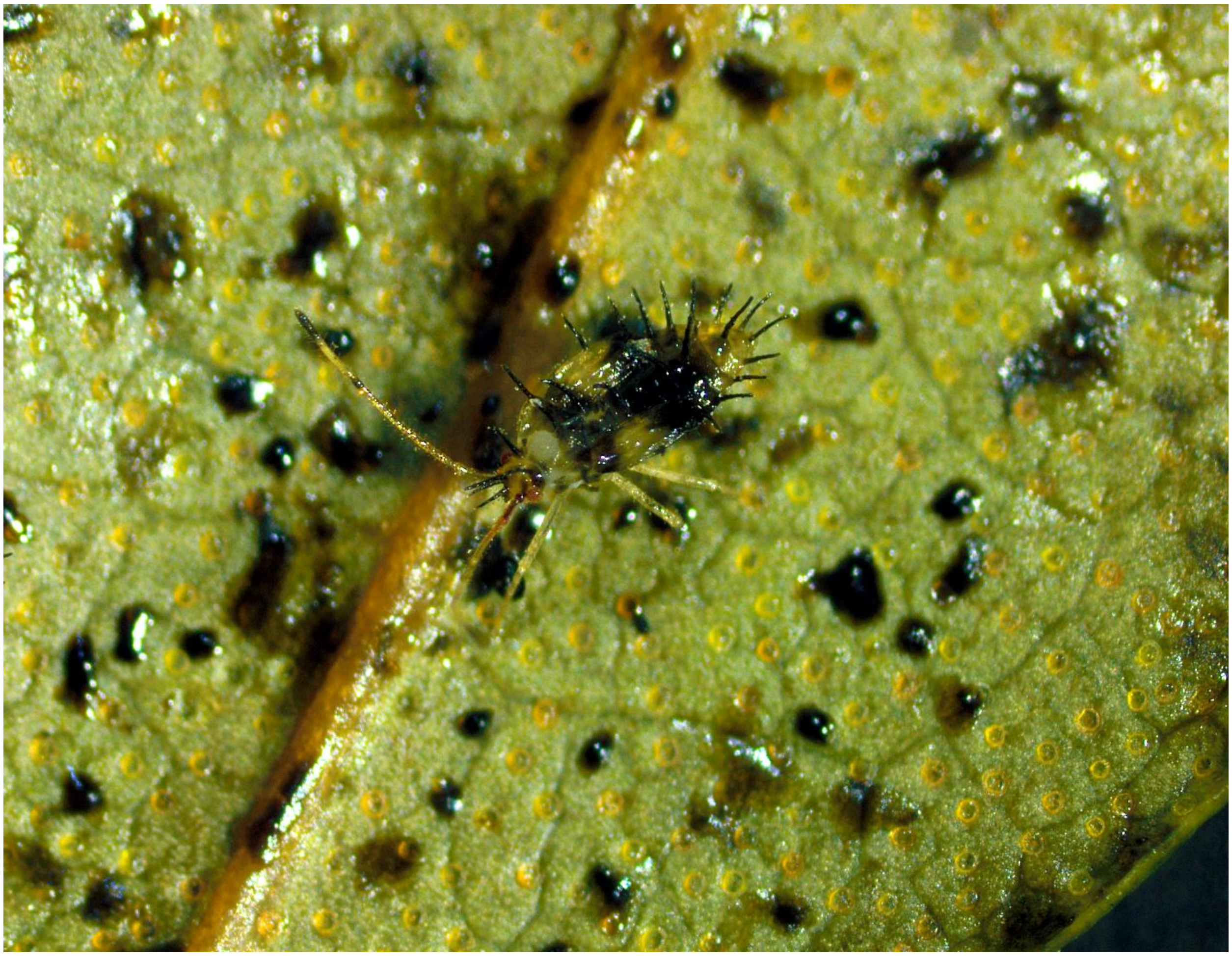
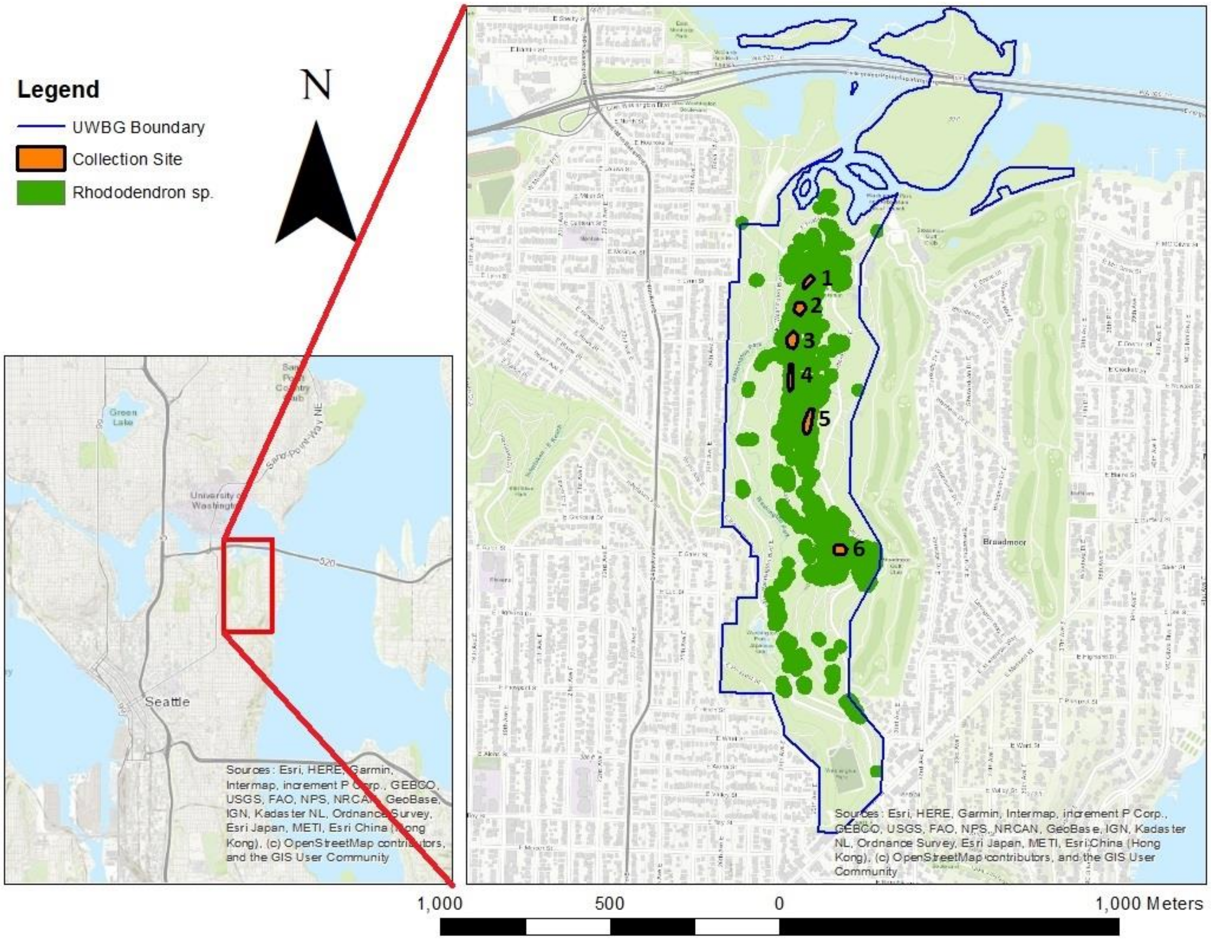


Figure 2.5 Late instar *S. pyrioides* with frass deposits (Photo credit: Ryan R. Garrison).

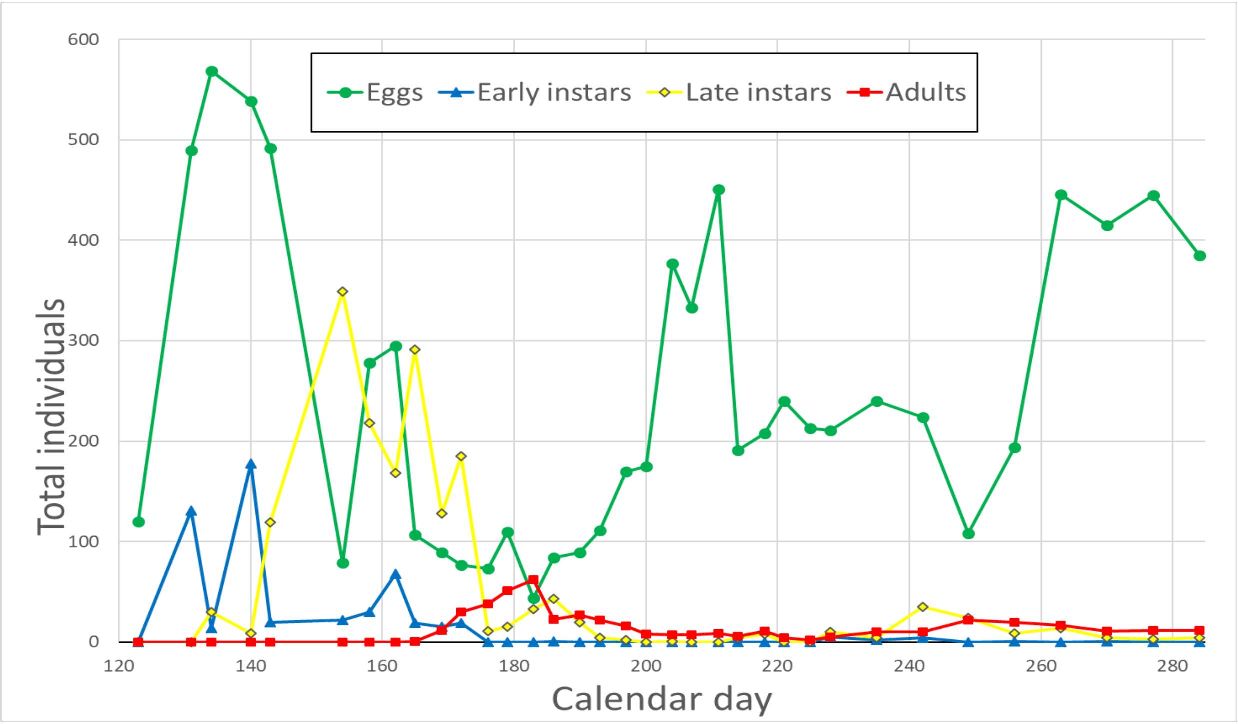


Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

Sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), (c) OpenStreetMap contributors, and the GIS User Community

Figure 2.6 Concentrations of *Rhododendron* spp. in the Washington Park Arboretum, Seattle, WA, are noted in green and were used to select collection sites. Collection sites are represented by the orange polygons.

A



B

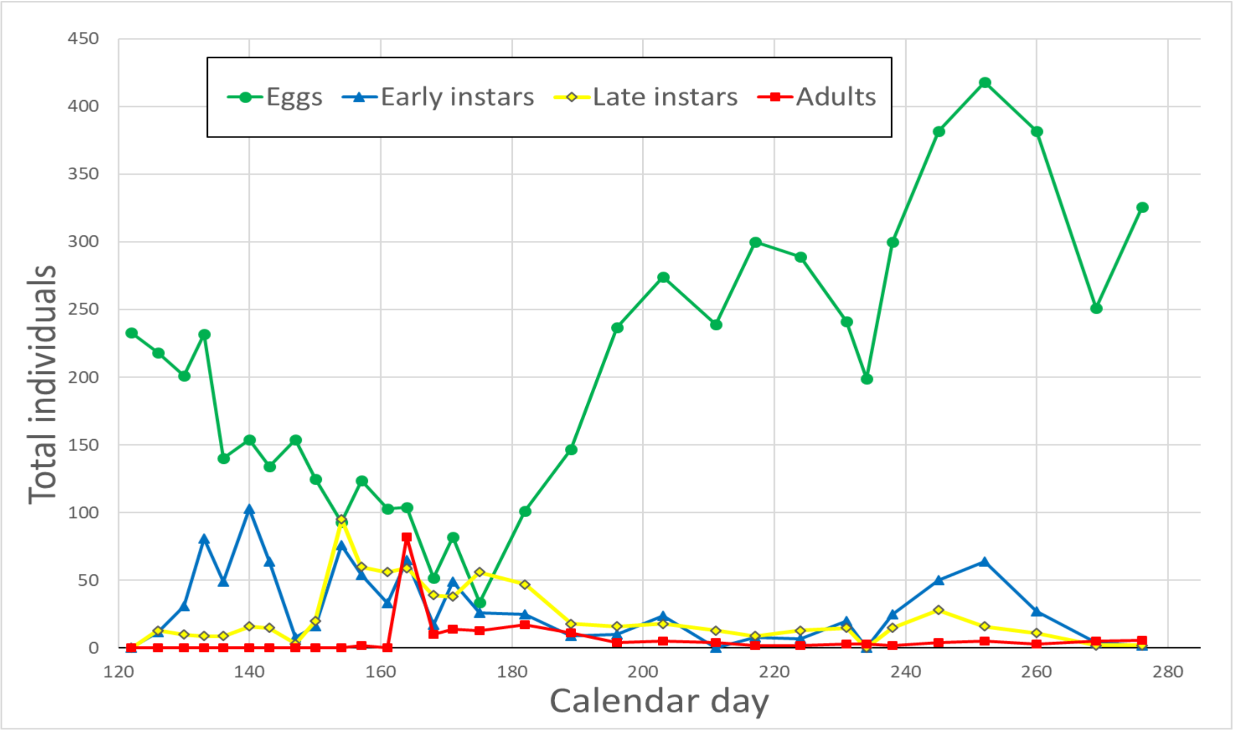
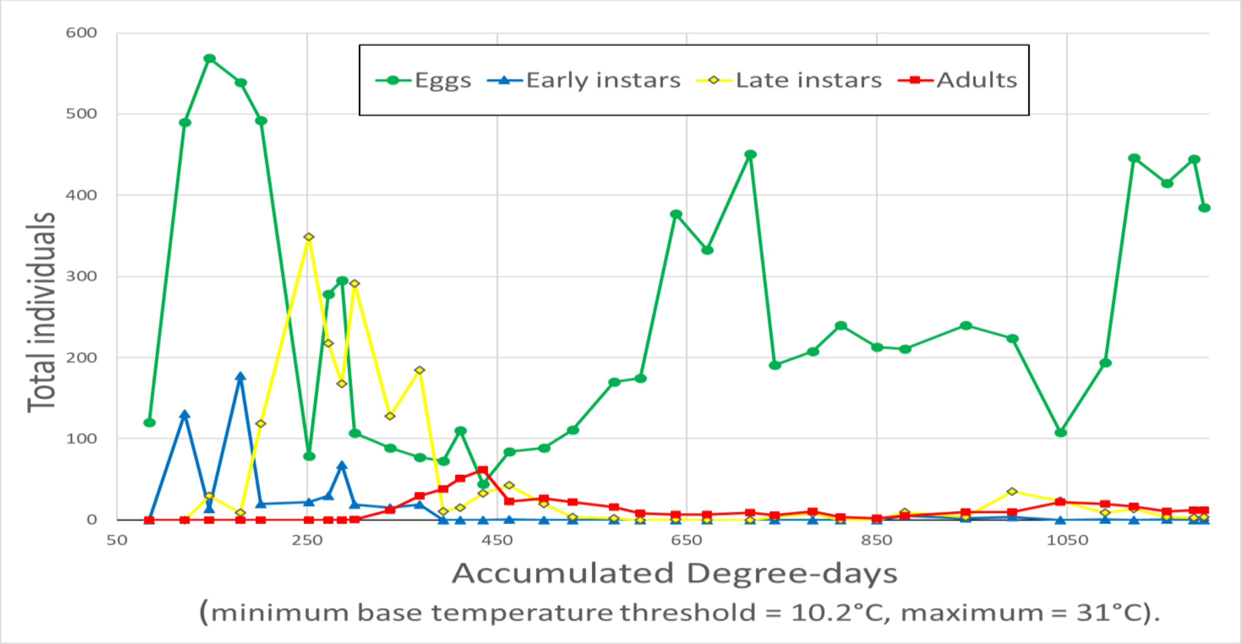


Figure 2.7 Number of *S. pyrioides* in 2018 (A) and 2019 (B) by calendar day.

A



B

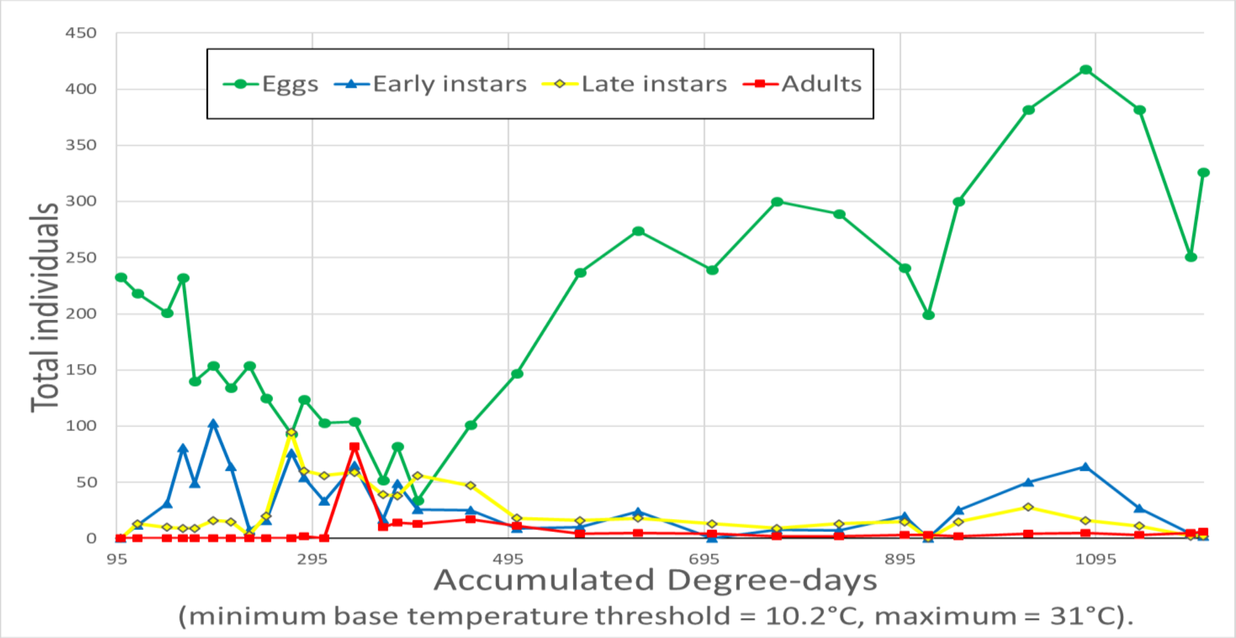


Figure 2.8 Number of *S. pyrioides* in 2018 (A) and 2019 (B) by accumulated degree-days from January 1; Base temperatures thresholds = 10.2°C (lower) and 31.0°C (upper). Based on data from 2018-2019, *S. pyrioides* likely undergoes two and a partial third generations in western Washington. First generation (from overwintering eggs to adults) occurs within the first 600 degree-days from January 1.

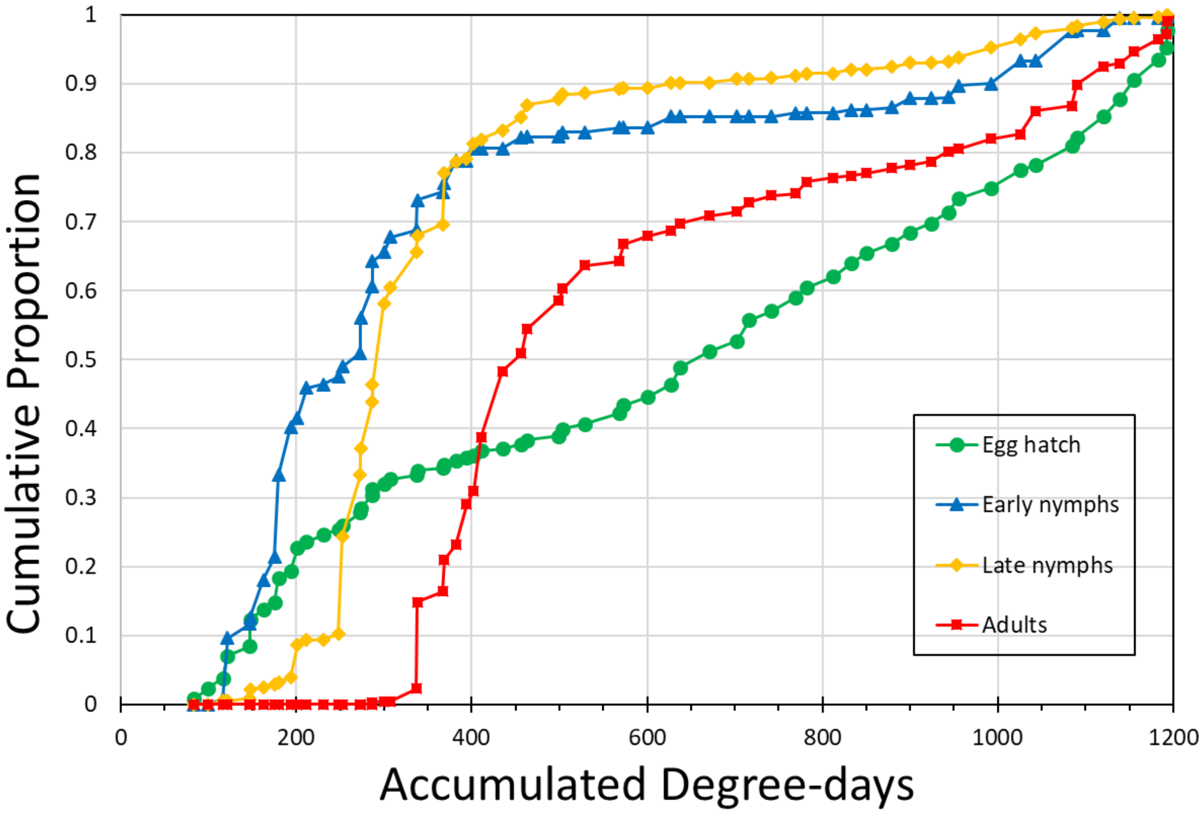


Figure 2.9 Cumulative proportion of *S. pyrioides* life stages, pooled across 2018 and 2019, over accumulated degree days from January 1; Base temperatures thresholds = 10.2°C (lower) and 31.0°C (upper).

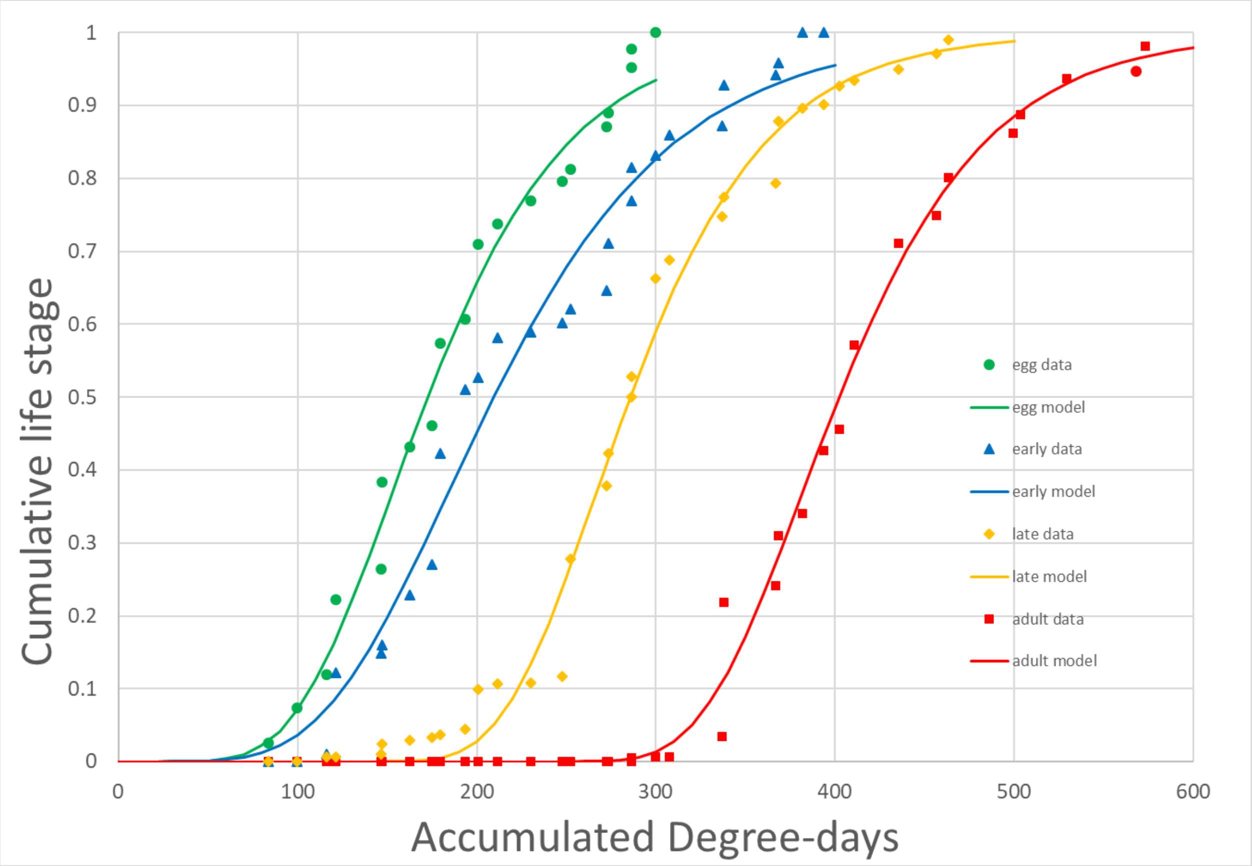


Figure 2.10 Cumulative proportion of first generation egg hatch, early instars (1-3), late instar (4-5), and adult *S. pyrioides* over degree-day accumulation from January 1; Base temperatures thresholds = 10.2°C (lower) and 31.0°C (upper).

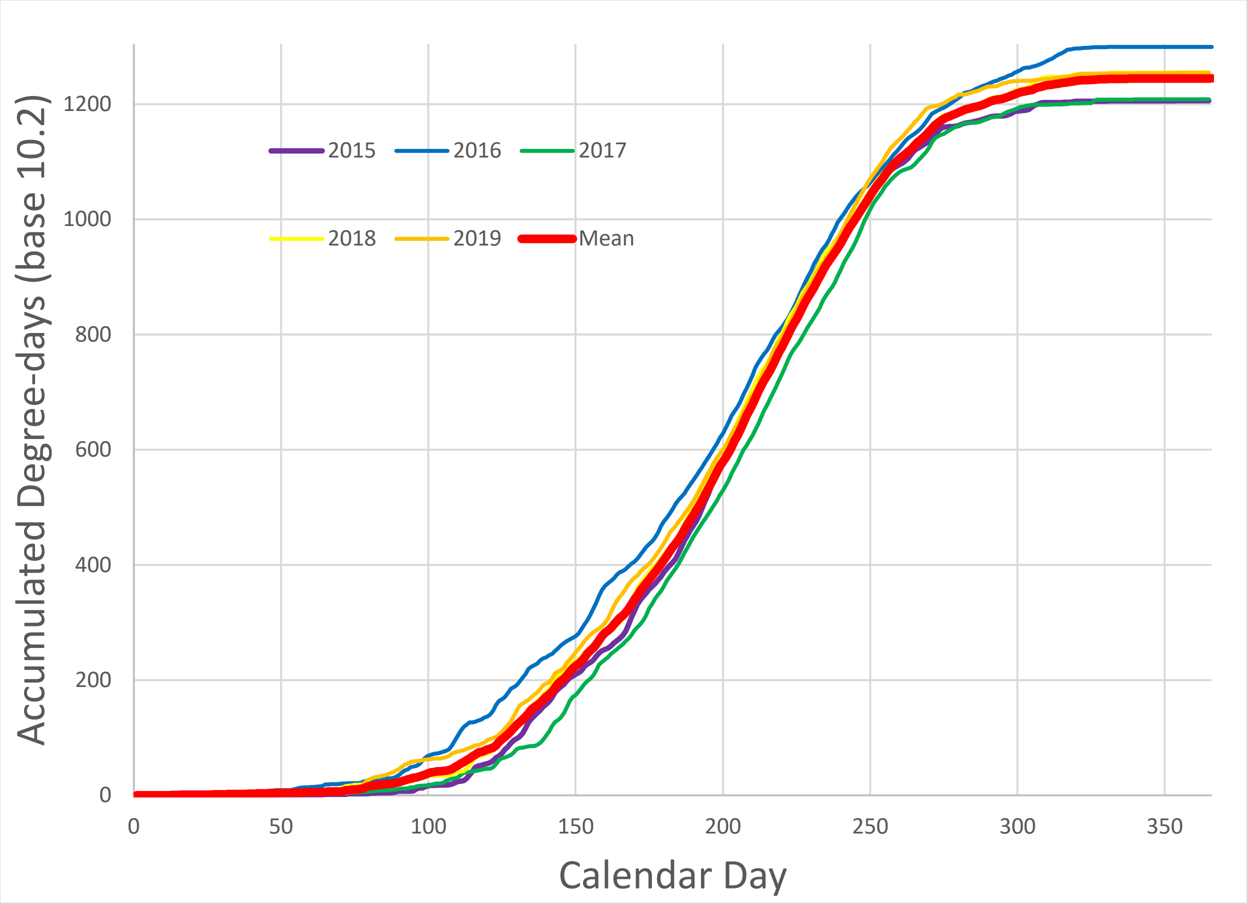
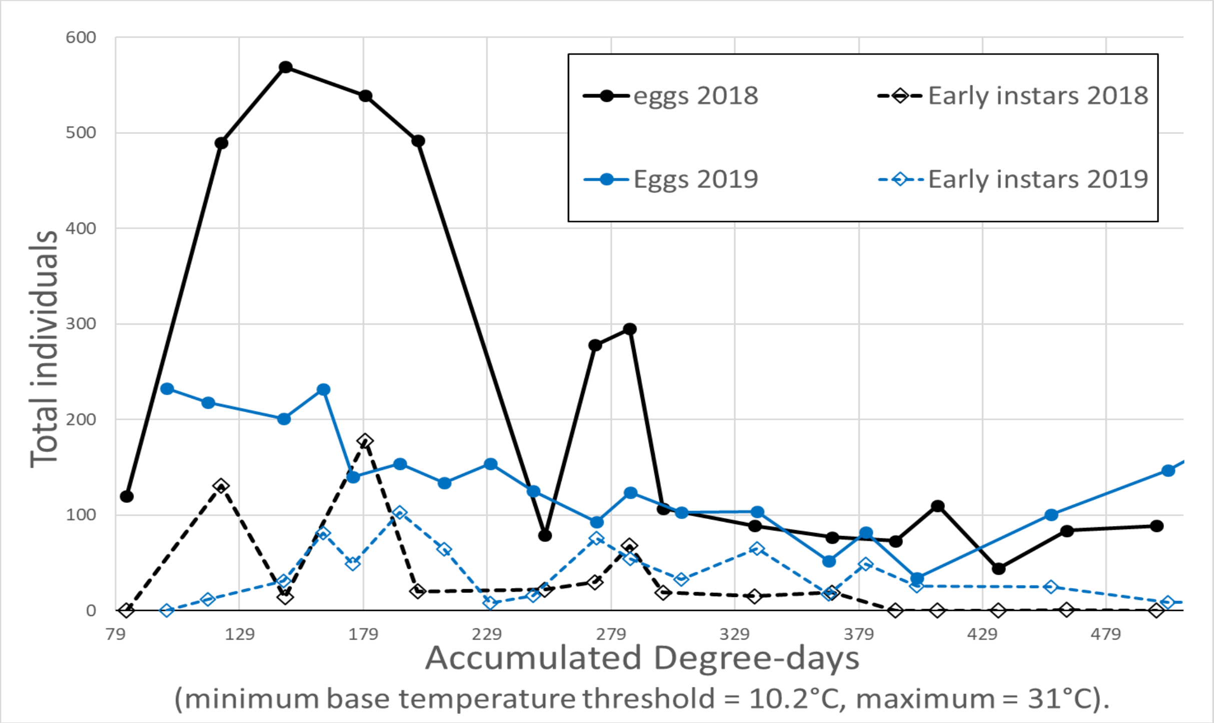


Figure 2.11 Accumulated Degree-days by calendar day from 2015 to 2019 from the Washington Park Arboretum when using a minimum base temperature threshold of 10.2°C, and the mean of those years. Generally, ~1,200 degree-days accumulate each year.

A



B

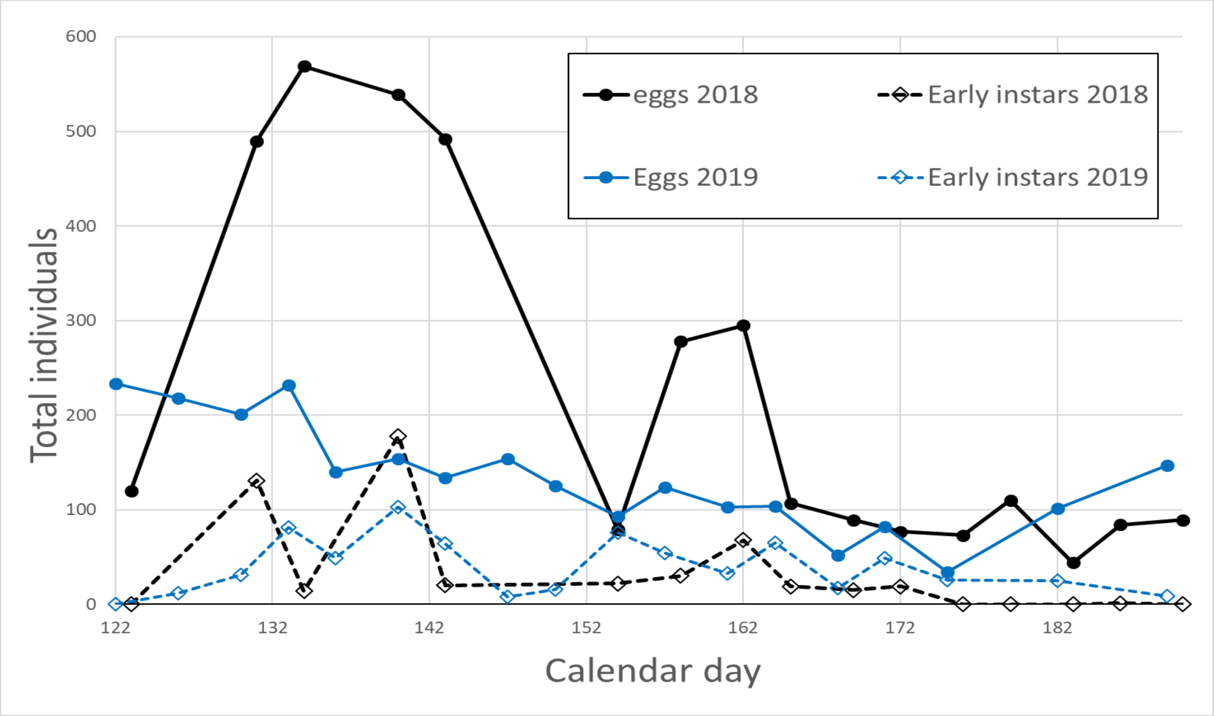


Figure 2.12 Eggs and early instar counts by accumulated degree-days (A) and calendar day (B) in 2018 and 2019.

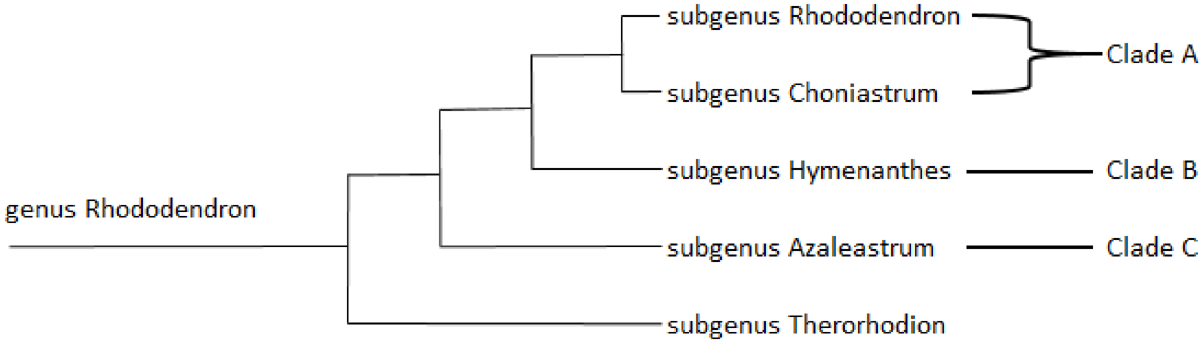
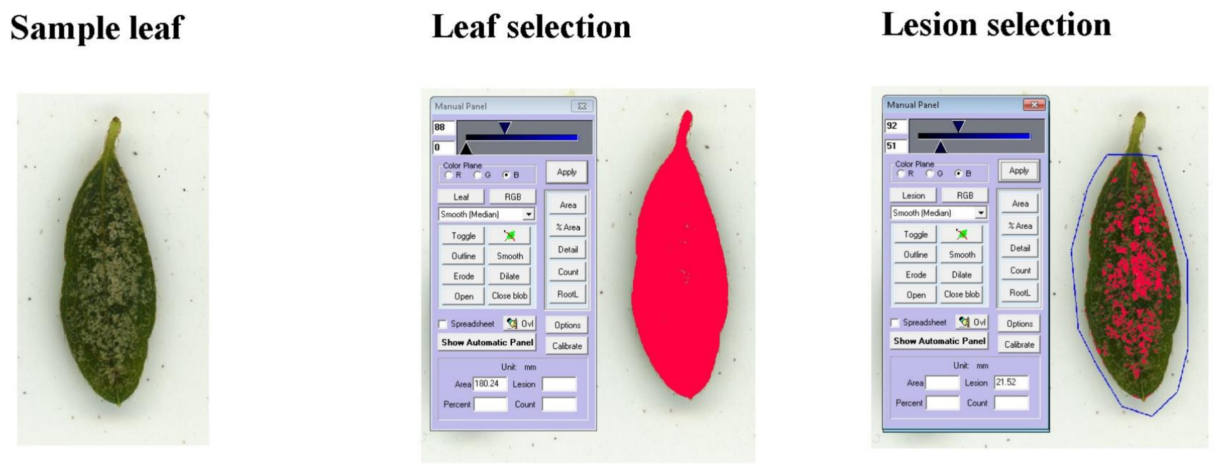


Figure 3.1 Cladogram of the Genus *Rhododendron* according to Goetsch et al. (2005).



Leaf selection: 88, 0; Smooth (Median); Unit: mm; Area 180.24

Lesion selection: 92, 51; Smooth (Median); Unit: mm; Lesion 21.52

Figure 3.2 Example of leaf processing using Assess 2.0 (Lamari 2006). From a damaged leaf, the software estimates both the total leaf area and the area affected by *S. pyrioides* feeding.

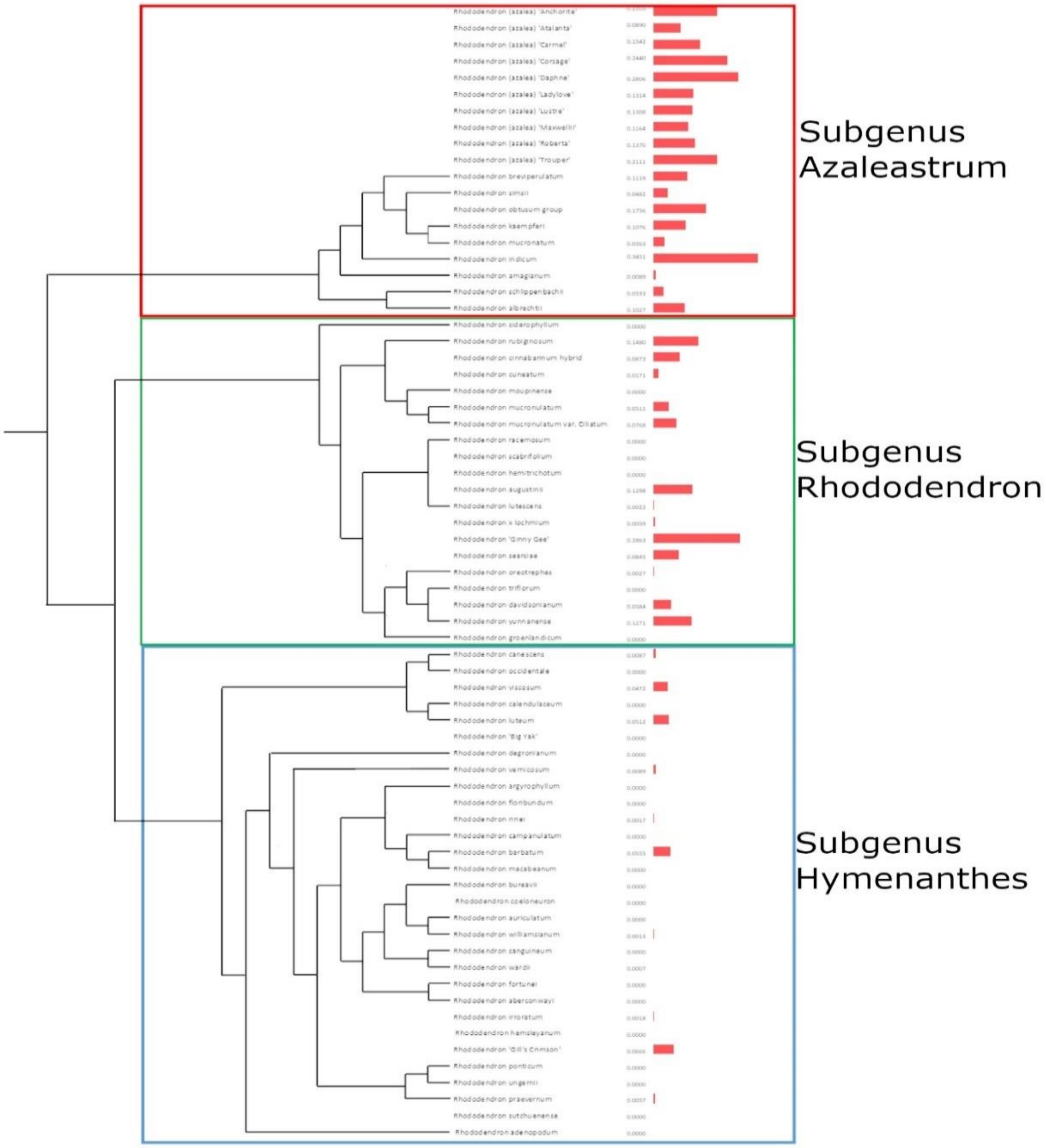


Figure 3.3 Compiled phylogenetic tree of *Rhododendron* spp. based on the phylogenetic analysis done by Goetch et al. (2005), Shrestha et al. (2018), and morphological taxonomic organization by Chamberlain (1996). The boxes indicate subgenera. The horizontal red bars represent the proportion of leaf area damaged.

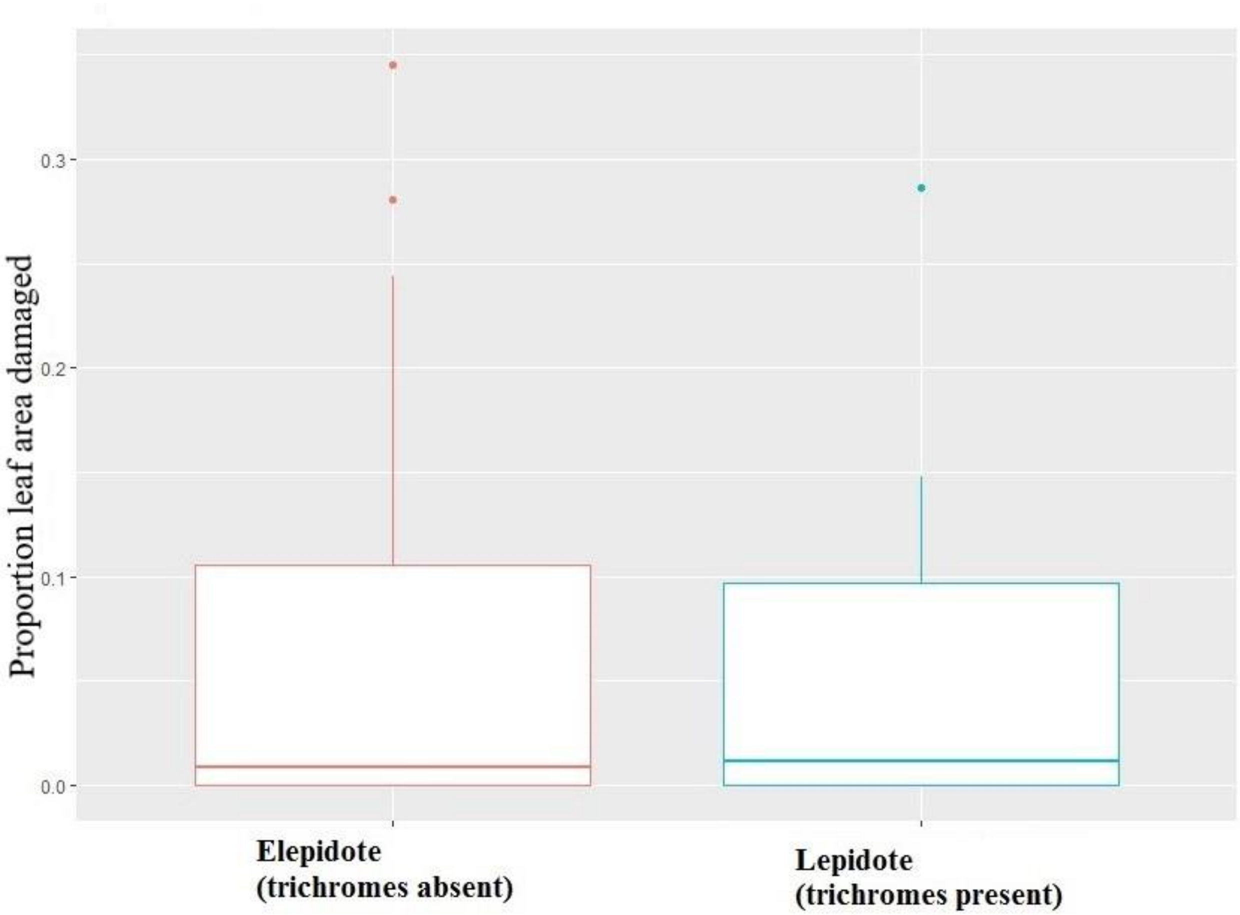


Figure 3.4 Boxplot of the proportion of leaf damaged by *S. pyrioides* from *Rhododendron* spp. with (N=21) and without (N=50) trichomes.

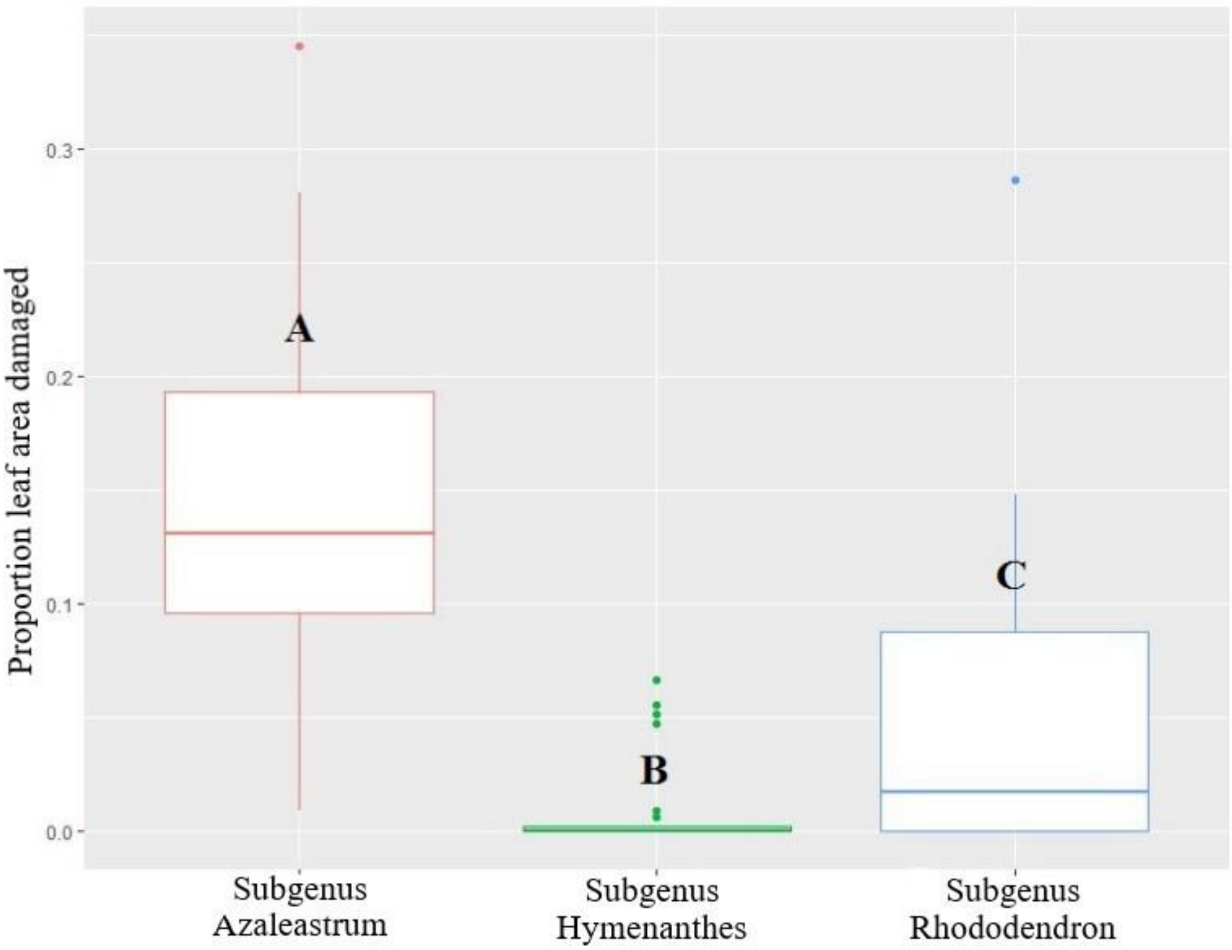


Figure 3.5 Boxplot of the proportion of leaf damaged by *S. pyrioides* by *Rhododendron* subgenus; different letters indicate significant differences using Tukey's HSD (p = 0.05).

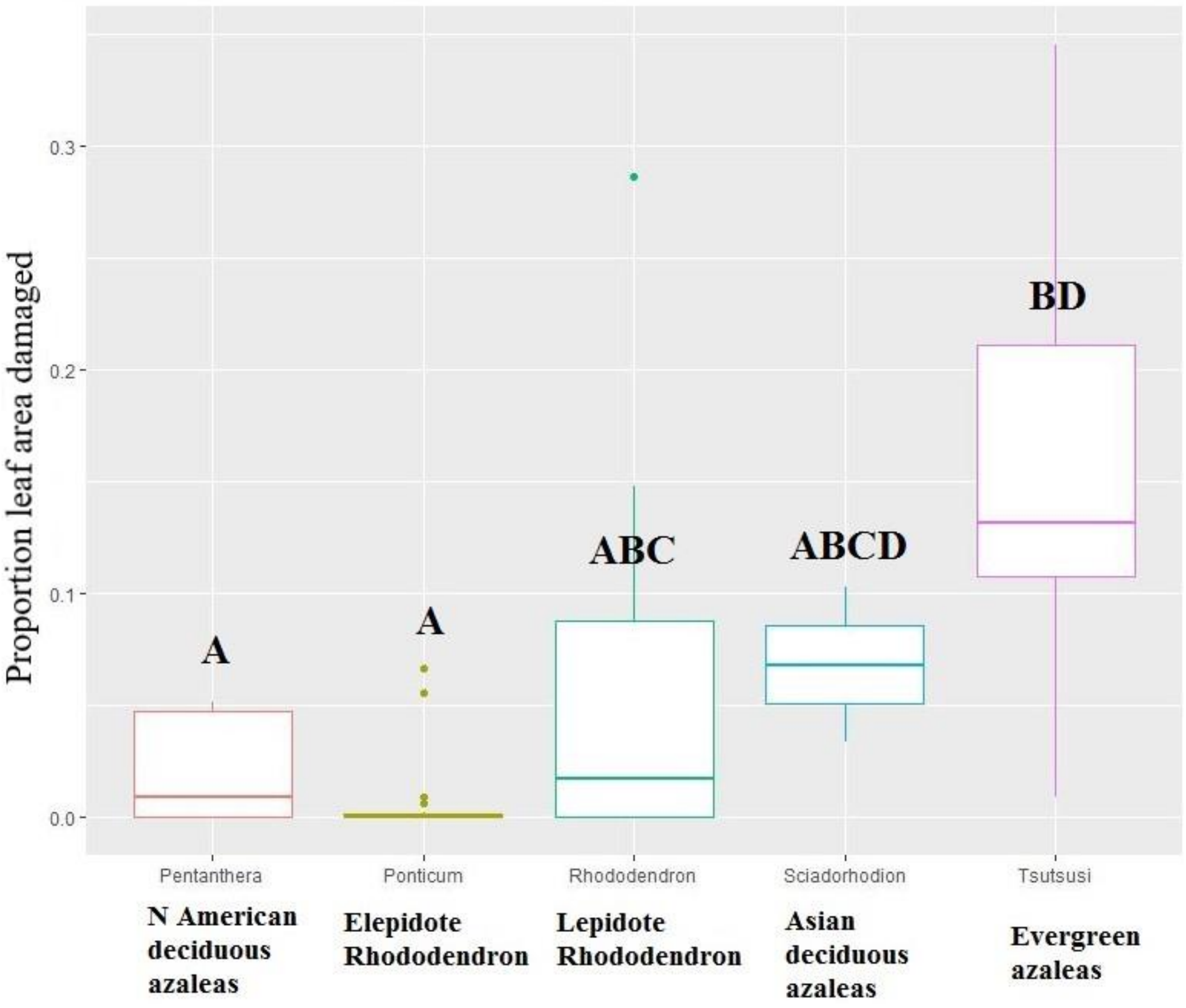


Figure 3.6 Boxplot of the proportion of leaf damaged by *S. pyrioides* by *Rhododendron* section; different letters indicate significant differences using Tukey's HSD ($p = 0.05$).