AN ABSTRACT OF THE THESIS OF

Salvador M. Flores for the degree of Master of Science in Horticulture presented on June 10, 2016.

Title: Phenology, Host-Plant Resistance, and Biological Control of *Stephanitis pyrioides* in Oregon

Abstract approved: _____

Jana C. Lee

The azalea lace bug (*Stephanitis pyrioides* Scott) is a recently detected invasive pest to the Pacific Northwest and has become a major concern in Oregon. It feeds on azaleas as well as rhododendrons causing stippling on the top side of leaves. The damage is aesthetically displeasing and affects plant vigor and photosynthetic capabilities, and, in cases with severe damage, can kill plants. The objectives of this work were to: 1) monitor for the presence of potential natural enemies and life stages of *S. pyrioides* from 2014 to 2016 through sampling in the Willamette Valley in Oregon, 2) observe any resistant cultivars that may be present, and 3) examine biological control efficacy of *S. pyrioides* using a predator and pathogen. These three objectives were looked at to provide information on what natural enemies are present and what time of the year *S. pyrioides* control would be most effective, find potential plant characteristics that may confer resistance, and determine if biological control is a viable option to reduce *S. pyrioides* pest abundance. Two specialist natural enemies of *S. pyrioides*, *Anagrus takeyanus* Gordh and *Stethoconus japonicus* Schumacher were not found and only about

8% of all arthropods captured in sweep nets and 4% in sticky traps could potentially prey on *S. pyrioides* as generalist predators. From shake sampling and leaf collections, adults and eggs were found year round and a total of 8,780 *S. pyrioides* were collected for all stages. Over half of all collected adults were females. Nymphs were found almost exclusively during the spring and summer months. Roughly 3.5 generations, based on a total development time of 394 degree-days (DD) are estimated to have occurred using weather data from Salem, Oregon. Secondly, cultivar observations to evaluate natural resistance by assessing for infestation or damage provided a list of approximately 75 species and cultivars that had no evidence of azalea lace bug feeding. Of these 75, four cultivars, species and hybrids were tested in a laboratory for feeding and fecal deposition beside a susceptible cultivar. All four resistant rhododendrons had no feeding and resulted in mortality rates over 90%. Third, evaluations of biological control, specifically predation by a green lacewing, *Chrysoperla rufilabris* Burmeister, significantly reduced *S. pyrioides* per leaf in a nursery setting when combined with methyl salicylate in 2014. However, larger follow up studies in 2015 did not reflect these results. The pathogen *Metarhizium anisopliae* had a high mortality rate when sprayed, but only caused a marginally higher mortality rate when compared to water sprays. Overall, this work presents novel and valuable information on azaleas and rhododendrons and *S. pyrioides* in the Pacific Northwest. This work provides the framework of phenology, a mode of resistance and valuable information on biological control to consider and incorporate into future management of *S. pyrioides* in Oregon.

Phenology, Host-Plant Resistance, and Biological Control of *Stephanitis pyrioides* in Oregon

by
Salvador M. Flores

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented June 10, 2016
Commencement June 2017

Master of Science thesis of Salvador M. Flores presented on June 10, 2016

APPROVED:

Major Professor, representing Horticulture

Head of the Department Horticulture

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Salvador M. Flores, Author

ACKNOWLEDGEMENTS

I will start by thanking my major professor and mentor, Jana Lee for accepting me as her student. I will say I was lucky to get chosen by her, when graduate school seemed out of reach that year, she chose me to be her graduate student and for that I am very grateful. I would also like to thank Jana for her insight, guidance in my project, and for what she's taught me over the past two years that I couldn't have done on my own. And for allowing me to visit home several times. I would also like to thank my committee members: Robin Rosetta, James R. LaBonte, and SueAnn Bottoms for being able to serve on my committee and their input and time into my project.

This research would have been much harder to complete had it not been for help in the lab and field that I received. Adam Cave and Amanda Lake looked after my colonies while I went back home to visit and helped in field observations and collecting. Megan Woltz with field work and in editing my writing. I'd also like to thank my colleague Victoria Skillman for field and school work assistance as well. I'd like to thank all the other fellow graduate students I came to know, especially in entomology, as it would have been a much lonelier time without them at graduate social events, since I came here not knowing a single soul. I'd also like to thank the parks, gardens and nurseries where my field research was conducted. My experiments couldn't have been completed without their cooperation.

Outside of work and school, I'd like to thank the friends I made, mostly through playing soccer, Elijah Umek, Jose Valderrama, and Ilya Pylikhin, it wouldn't have been as fun in or around Corvallis without spending time with them.

A special thanks to my family: the support my parents, grandparents and siblings gave me helped me get through a higher education means a lot to me, even though it was hard living far away from them it showed me that they are very important in my life. Last but not least a very special thanks to my girlfriend Maricela Lopez, I know it was hard to support my goals for 2 years and I'm glad you were with me every step of the way. I couldn't have done this without you and your guidance, my love.

CONTRIBUTION OF AUTHORS

Dr. Jana Lee oversaw the planning, design, and set up of this project as well as editing of this paper and assisted with data interpretation. Dr. Megan Woltz helped edit Chapter 1 and also assisted with data interpretation. Jim LaBonte assisted with plans on the voltinism survey in Chapter 2. We had a collaborative project where he examined the wide host range of the lace bug and I specifically examined susceptibility among rhododendron cultivars in Chapter 3. Robin Rosetta assisted with plans on biological control in Chapter 4. Both Jim and Robin were instrumental in directing me to suitable sites for studies.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

DEDICATION

To my Mom and Dad and my amazing girlfriend, Maricela Lopez. I love you.

Chapter 1:

Literature review on the Azalea Lace Bug

Salvador M. Flores, Megan Woltz, and Jana Lee

Lace bugs (Hemiptera: Tingidae) in the genus *Stephanitis* feed on various host plants via piercing and sucking mouthparts common among all insects in this order. The azalea lace bug, *Stephanitis pyrioides* Scott, has become a major pest of azaleas and rhododendrons in the Pacific Northwest. It has become one of the most important and common problems affecting evergreen azaleas (Balsdon et al., 1996; Nair & Braman, 2012b; Rinehart & Boyd, 2006). While it does adversely affect evergreen azaleas, it was shown to have little effect on deciduous azaleas early in the season when first generation *S. pyrioides* emerge (Braman & Pendley, 1992; Braman & Beshear, 1994). A native of Japan (Chappell & Robacker, 2006), *S. pyrioides* is now invasive within the United States. Within the United States, *S. pyrioides* was first reported in New Jersey in 1916, and has since spread to several other states, including Pennsylvania, Washington D.C., New York, Missouri, Georgia, Maryland, Connecticut and Massachusetts (Braman et al., 1992; Neal & Haldemann, 1992; Nielsen, 1997; Shrewsbury & Smith-Fiola, 2000; Torres-Miller, 1989). It has also been reported in Virginia, Alabama, Texas and California (CABI 2005). After being present in the eastern U.S for almost a century, *S. pyrioides* was recently discovered in western states. In 2008, it was confirmed in Washington and by 2009 it was found in Oregon (Rosetta, 2013).

Stephanitis pyrioides are small, (2.8 mm-3.3mm) transparent-winged hemipterans with a lace-like pattern and two dark distinct bands on their wings. These insects are relatively weak fliers and mostly disperse when disturbed or when food is exhausted (Nair & Braman, 2012b). Its development and life history elsewhere have been documented in previous studies. In the eastern U.S., four generations per year have been reported in

Maryland and Georgia and around two to three generations per year in the New England states (Braman et al., 1992; Stewart et al., 2002). Neal and Douglass (1988) reported that adults do not diapause, the eggs are the overwintering stage, and the overwintered eggs are the first generation in each year. Eggs are usually oviposited along the midrib of the leaf or along a main leaf vein, covered with excrement and hatch during spring (Braman et al., 1992; Nair & Braman, 2012b). There are five instar or nymphal stages and under rearing conditions that best suit *S. pyrioides*, the adults can live up to 240 days (Nair & Braman, 2012b). Its average development times at various temperatures have been observed by Braman et al. (1992) and Neal and Douglass (1988) and development at room temperature is shown in Table 1.1. It has been noted that development is not successful at 33°C (Braman et al., 1992). However, its generation time is not yet known in the Pacific Northwest (Rosetta, 2013), which leads to uncertainty about its phenology in this region. Chapter 2 includes an empirical survey of *S. pyrioides* life stages to determine their presence throughout the year and estimate generations in the Mid-Willamette Valley of Oregon to compare the estimated generations to previous reports in the eastern United States.

Azaleas and rhododendrons, in the genus *Rhododendron*, are important landscape plans and a widely planted flowering shrub in the United States. However, it is a pest-prone genera and any slight aesthetic injuries make consumers unwilling to purchase an injured plant (Balsdon et al., 1996; Braman & Beshear, 1994; Klingeman et al., 2000; Klingeman et al., 2001; Shrewsbury & Raupp, 2000). All life stages and both sexes of *S. pyrioides* feed on azaleas and have been observed feeding on rhododendrons (Nair & Braman,

2012b). *Stephanitis pyrioides* feed by inserting their stylets on the underside of the plant's leaves, entering through the stomata and removing the chlorophyll within the leaves. Feeding damage is evident as stippling damage from the top of the leaf, and dark brown feces and exuviae on the underside of the leaf (Balsdon et al., 1996; Nair & Braman, 2012b; Rinehart & Boyd, 2006). Heavy feeding on the leaves can cause chlorosis, an insufficient amount of chlorophyll present in the leaf, which gives the leaf a distinct yellow-white or pale coloration. This removal of chlorophyll reduces rates of photosynthesis and transpiration (Nair & Braman, 2012b). Heavy feeding can lead to reduced plant vigor and, in extreme feeding damage, death of the plant. Previous studies have shown that *S. pyrioides* causes more damage in plants placed in the sun than plants placed in shade, in spite of the fact that this insect has higher oviposition, fecundity and survival on shade-grown plants in the absence of natural enemies (Nair & Braman, 2012b; Trumbule et al., 1995; Trumbule & Denno, 1995). When natural enemies are not excluded, *S. pyrioides* survival was higher on plants in the sun than the shade, suggesting that predation, parasitism, or pathogens in shade habitats may lower *S. pyrioides* survival and reduce feeding damage (Nair & Braman, 2012b; Trumbule & Denno, 1995).

Because excessive *S. pyrioides* feeding reduces plant quality and can result in the death of the plant, many studies have investigated methods of *S. pyrioides* control to prevent plant damage. Pesticides generally have been shown to have greater effects on nymphs than other life stages, as they are most vulnerable due to their fragility compared to adults (Nair & Braman, 2012b). Some pesticides such as acephate have been shown to sufficiently control all life stages and therefore can reduce the population of the next

generation. However, acephate is toxic to honey bees and other beneficial insects (EPA, 2001). Because azaleas and rhododendrons are landscape plants and are often in urban settings, the use of chemicals to control *S. pyrioides* can harm other beneficial insects and animals. Other insecticides aimed at controlling *S. pyrioides* have been tested (Balsdon et al., 1993; Braman et al., 2000; Held & Parker, 2011; Nair & Braman, 2012a; Shrewsbury & Smith-Fiola, 2000). Some studies examined low toxicity chemicals such as M-pede and Volck oil but these studies have also examined organophosphates, neonicotinoids, bendiocarbs, pyrethroids, which had varying degrees of success. Some of the four latter named categories of chemicals used in *S. pyrioides* studies can be toxic to humans or other vertebrates and are also highly and acutely toxic to bees and other beneficial insects (EPA, 1999, 2012; Held & Parker, 2011; Cornell, 1994, 1995a, 1995b). Commercial use of these chemicals to control *S. pyrioides* may contribute to declining bee populations if used on residential or public landscapes while plants are in bloom, where azaleas and rhododendrons are common. Application of pesticides can affect predator-prey interactions and have been shown to disrupt complex ecological processes, which in turn can have the opposite of the desired effect, potentially leading to pest outbreaks (Shrewsbury et al., 2004). Therefore, control tactics that have the lowest impact on beneficial insects and other non-target insects are sought after for the control of this pest.

A less chemically based alternative is to manipulate the habitat to increase natural enemies and directly add commercially available natural enemies. Manipulating the habitat could also help keep released natural enemies in the area. While it was shown that differences in host plant quality was not a strong bottom-up force influencing

patterns of *S. pyrioides* abundance (Shrewsbury & Raupp, 2006), habitat structure has been shown to influence the abundance of *S. pyrioides* (Shrewsbury & Raupp, 2006). *Stephanitis pyrioides* populations are usually less abundant in more complex habitats and have been shown to be extremely abundant, up to 120 times more, in simple habitats when compared to complex habitats, where their populations are more sporadic (Shrewsbury & Raupp, 2006). This difference in pest abundance is due to complex habitats supporting more generalist predators that feed on and reduce *S. pyrioides* survival rates. Therefore changing the complexity of a habitat should influence *S. pyrioides* populations (Nair & Braman, 2012b; Shrewsbury & Raupp, 2006). Most of the studies examining habitat structure on *S. pyrioides* abundance have worked with the more shrub-like azalea plants, while few have examined habitat structure using rhododendrons. Studies with these rhododendrons could be helpful to further understand *S. pyrioides* abundance dynamics (Shrewsbury & Raupp, 2006).

Another way of manipulating the habitat to promote beneficial insects and reduce pests is to add flowering plants to the habitat. This is thought to reduce pest abundance as there is a positive correlation between the number of flowers in full bloom and number of natural enemies present in the area and a negative correlation with *S. pyrioides* populations in a field study by Shrewsbury et al., (2004).

Secondly, biological control can be another alternative way to control *S. pyrioides* abundance and damage. Biological control is a way to mitigate pest damage by using a predator, a parasitoid, or pathogen for population control to a level below an economic

threshold. However, it is often difficult to implement this when herbivores arrive in a new habitat before their natural enemies. Ways to counteract this are the addition of supplemental natural enemies to boost the naturally occurring population (augmentative biological control), encourage population growth of predators already present (conservation biological control), or introduce natural enemies into the habitat (classical biological control).

There are several natural enemies that have been used or may be used to study the control of *S. pyrioides*. Table 1.2 lists the natural enemies most promising to the control of *S. pyrioides* and the information available about these natural enemies. *Stephanitis pyrioides* and most other lace bugs have only a few natural enemies that specifically prey upon them. Only two specialized natural enemies have been reported to attack *S. pyrioides* (Balsdon et al., 1993; Rinehart & Boyd, 2006) a parasitic wasp *Anagrus takeyanus* Gordh (Hymenoptera:Mymaridae) and a predatory mirid, *Stethoconus japonicus* Schumacher (Hemiptera:Miridae) (Balsdon et al., 1996; Nair & Braman, 2012b).

Anagrus takeyanus is an exotic natural enemy present in the eastern United States. It has been studied by Braman et al. (1992), Balsdon et al. (1993), and Balsdon et al. (1996). Braman et al. (1992) and Balsdon et al. (1996) measured its parasitism rates in *Stephanitis* species and development, whereas the study done by Balsdon et al. (1993) examined its biological control potential in conjunction with an insecticide. In their work, it was reported that *A. takeyanus* reduced the population of another *Stephanitis*

species, *S. takeyai*, in Connecticut by approximately 35% by parasitizing overwintering eggs (Braman et al., 1992). Additionally, in two field studies, *A. takeyanus* parasitized an average of 14% *S. pyrioides* eggs (Balsdon et al., 1996) and a range of 3.3-48.8% eggs (Braman et al., 1992) in Georgia. Balsdon et al. (1996), also found that at 24°C, the mean development time is 26.05 ±0.66 days, and that adults live slightly longer than one day with honey-water in laboratory settings. From observations, Baldson et al. (1996) suggested that there are five generations in Georgia and that emergence is either synchronous with, or slightly earlier or later than *S. pyrioides* overwintering emergence. Although this wasp is not species specific, it may be a viable option for release as both *Stephanitis* species are invasive to North America.

The second specialized natural enemy, *S. japonicus*, is a voracious predator of *S. pyrioides* and was first reported in Maryland (Henry et al., 1986). *Stethoconus japonicus* and five other species of *Stethoconus* are exclusive predators of lace bugs (Neal & Haldemann, 1992). The development of *Stethoconus japonicus* at 26.1°C was similar to *S. pyrioides* (Neal & Haldemann, 1992). It also overwinters in the egg stage on the same host plant as its prey and the adults consume about 4 *S. pyrioides* per day (Henry, 1986; Neal et al., 1991; Neal & Haldemann, 1992). Neal & Haldemann (1992) observed that emergence is asynchronous and emerges after the second lace bug generation, indicating that it is prey-dependent. *Stethoconus japonicus* is also capable of preying upon several hosts and could adapt to consuming native *Corythucha* species and *Stephanitis* species (Neal et al., 1991), which may negatively impact the ecosystem. However, it distinctly prefers *S. pyrioides* over other lace bugs, such as the hawthorn lace bug, *Corythucha*

cydoniae Fitch (Neal et al., 1991). In order to introduce this mirid as a biocontrol agent into an area where it has not previously been established, detailed studies would be necessary to test its host specificity in any given region. Furthermore, it was shown that *S. japonicus* was most likely to be present only with high populations (Shrewsbury & Raupp, 2006) of *S. pyrioides*, which perhaps would be too late if plants are used for aesthetic purposes. Although there seems to be enough evidence that the species in the genus *Stethoconus* are all specialized predators of Tingidae (Henry, 1986), *S. japonicus* was not recommended for commercial rearing or release due to the possibility of attacking native species (Sanchez, 1989).

Generalist predators that may prey on *S. pyrioides* include: spiders, minute pirate bugs, plant bugs (Miridae), earwigs, green lacewings, lady beetles, and tree crickets (Rosetta, 2013; Shrewsbury & Raupp, 2006). Shrewsbury and Raupp (2006) compared various generalist predators in a top-down experiment and a bottom-up experiment, testing *S. pyrioides* performance and immigration on azaleas. Top-down experiments measures whether the predator affects prey abundance by keeping their population down as opposed to the population in absence of predators. Bottom-up factors are factors that cause pest population fluctuations due to food and habitat availability (SAGEMAP, n.d.). The study tested predation using the predators listed above and found a statistical significance in top-down factors on *S. pyrioides*. In particular, a spider, *Anyphaena celer* Hentz (Araneae:Anyphaenidae), showed the highest predation pressure when compared to the other predators (Shrewsbury and Raupp 2006). Within the same top-down factor, all of the predators included in the study consumed significantly more nymphs than

adults, except for the snowy tree cricket, *Oecanthus fultoni* Walker (Orthoptera:Gryllidae). No statistical significance was found in bottom-up experiments that measured the proportion of nymph survival, and the development to the adult stage in the absence of natural enemies in both complex and simple habitats. Thus, only the top-down factor, predation, significantly reduced *S. pyrioides* populations.

Because endemic natural enemies of *S. pyrioides* in Oregon are not known and have not been found, a weekly survey by leaf collection, shake sampling and sticky trapping conducted in Chapter 2 surveyed for the potential presence of the egg parasitoid (*A. takeyanus*) and predatory mirid (*S. japonicus*) from reports on the East Coast. Also, natural enemies, predatory arthropods, observed to co-occur with active *S. pyrioides* infestation were recorded in Chapter 2 to help inform future biological control efforts and strategies.

Green lacewing larvae, which are generalist predators, seem to be the most commonly used in augmentative releases for the *S. pyrioides*, specifically, releases using *Chrysoperla carnea* Stephens and *C. rufilabris* Burmeister (Neuroptera:Chrysopidae). In a laboratory study done by Stewart et al. (2002), the green lacewing *C. rufilabris* and a predatory mirid, *Rhinocapsus vanduzeei* Uhler, were observed to determine their kill rate efficiency. *Chrysoperla rufilabris* and *R. vanduzeei* both exhibited a type II functional response, but *C. rufilabris* had a significantly higher kill rate of late 4^{th} and 5^{th} *S. pyrioides* instars (between 0.63 and 8.29 in 24h) than *R. vanduzeei* (between 0.43 and 5.55 in 24h), indicating that augmentative green lacewing release is more suitable than

using *R. vanduzeei* (Stewart et al., 2002). An augmentative release of *C. carnea* by lightly tapping a hexcel unit so the that the larvae dropped onto the plant, lowered the population of *S. pyrioides* by 97% in a commercial nursery (Shrewsbury & Smith-Fiola, 2000). A green lacewing release coupled with the systemic pesticide acephate (Orthene®) significantly reduced the population of *S. pyrioides*, without the chemical adversely affecting green lacewings. Furthermore, *S. pyrioides* was reported to have a higher mortality rate as the number of *C. carnea* larvae increased from 5 to 20 larvae per plant on plants with 40 or 80 lace bugs (Shrewsbury & Smith-Fiola, 2000). However, green lacewing larvae are quite mobile and generalists, and thus green lacewing release to control *S. pyrioides* is seen as a short term solution (Shrewsbury et al., 2004; Shrewsbury & Smith-Fiola, 2000).

A potential way to enhance biological control of *S. pyrioides* is to use herbivore-induced plant volatiles (HIPVs) as they have been reported as strong attractants for arthropods that feed on pest arthropods (Kaplan, 2012). Herbivore-induced plant volatiles are chemicals naturally released by plants as a way to control feeding damage when being fed on by herbivorous insects. The volatiles evaporate in the air and spread, attracting predatory arthropods that feed on the insect causing damage to the plant. HIPVs are potent attractants for predatory arthropods. Several olfactometer trials have shown that HIPVs from damaged plants are better cues for predatory arthropods than chemicals from undamaged plants, or from the prey's frass, or even the prey itself (Kaplan, 2012). However, the efficacy of HIPVs in natural settings is unclear given the complexity of HIPV's effect on predator behavior. Herbivore-induced plant volatiles have been shown

to have an impact in field settings, such as enhancing natural enemy presence in vineyards and hops (James & Price, 2004), increasing parasitism occurrence in cotton (Williams et al., 2008), and increased predation rates on sentinel prey (Kessler & Baldwin, 2001) in various plant species trials (Lee, 2010). Therefore, using a synthetic HIPV applied to a plant may attract beneficial insects and keep them in the area longer (Kaplan, 2012). One common synthetic HIPV used is methyl salicylate (Lee, 2010), which is oil of wintergreen and has a minty scent to it. Coupling both tactics such as an augmentative release of predators and use of HIPV to attract surrounding natural enemies may be a viable mode of biological control. Chapter 4 explores biological control of *S. pyrioides* by manipulative studies with predator releases and deployment of methyl salicylate.

The use of pathogens to control *S. pyrioides* populations may also be an option. Biopesticides have been explored in the study by Nair and Braman (2012a). In their study, they applied Tick Ex which contained *Metarhizium anisopliae* Metsch with or without releases of *C. carnea* larvae, and found that Tick Ex alone significantly reduced adult counts compared to the water control. Although Tick Ex significantly reduced adult counts, it was not as effective as synthetic insecticides also tested in this study. However, more studies examining the effect of *Metarhizium* on *S. pyrioides* are needed to provide clearer information on *Metarhizium* mortality rates.

Lastly, another non-chemical pest control option is to use resistant cultivars or species of rhododendrons and azaleas that may deter heavy *S. pyrioides* feeding (Table 1.3). In one

study, an azalea cultivar, ‘Macrantha’, was found to be the most resistant out of twenty cultivars from a commercial nursery Schultz (1993). ‘Macrantha’ had the lowest average number of eggs present per leaf cutting over a two year sample period, the lowest area of leaf injury and the lowest leaf injury percentage, indicating that this cultivar is promising for determining a mechanism of resistance (Schultz 1993). In another study, all life stages of *S. pyrioides* were reduced up to 72% on the resistant and deciduous, *Rhododendron periclymenoides* Michaux and *R. canescens* Michaux, compared to the susceptible and deciduous azalea cultivars ‘Buttercup’ and ‘My Mary’ (Chappell & Robacker, 2006). Additionally, the azalea species *R. prunifolium* (Small) Millais and *R. canescens* were least suitable to adult survival and most resistant to oviposition when compared to the susceptible azalea control ‘Delaware Valley White’ (Braman & Pendley, 1992).

It was found that resistance of feeding, oviposition, and subsequent population reductions was due to the epicuticular wax of *Rhododendron* spp. previously mentioned. Leaf wax of resistant azalea plants is one of the primary mechanisms of *Rhododendron* spp. susceptibility to *S. pyrioides* (Chappell & Robacker, 2006; Chappell et al., 2004). Applying the wax of resistant varieties to fresh foliage of susceptible varieties resulted in less feeding and oviposition, whereas applying the wax of susceptible varieties did not increase fecal deposition, but did increase oviposition (Chappell & Robacker, 2006; Chappell et al., 2004, 2005). Furthermore, applying resistant leaf wax to resistant varieties resulted in further reductions of oviposition (Chappell & Robacker, 2006). Oviposition resistance and vulnerability were correlated with α- and β-amyrin

constituents of leaf wax, and have been previously reported as possible insect feeding and/or oviposition deterrents (Balsdon et al., 1995). Both are triterpenoid lipids, the dominant lipid component in the leaves of four evergreen azaleas and one deciduous azalea that were tested. Cultivars with lower amounts of these triterpenoids showed a higher susceptibility to *S. pyrioides* damage (Balsdon et al., 1995). This indicates that these two chemicals have some role in resistance and host plant acceptance. A possible way to deter feeding, oviposition, and aesthetic damage is to use leaf wax extract from resistant varieties. Using resistant leaf wax extracts could prove useful if it can be proven effective at a larger scale. Table 1.3 lists the studies that have looked at cultivar resistance and their outcomes. Chapter 3 surveys a wide variety of rhododendrons for potentially resistant species and cultivars that can be recommended to avoid pest damage.

Leaf wax composition is only one of the possible ways to examine for feeding resistance. Leaf trichome density could also play a role in feeding resistance. More specifically, the trichomes located on the underside of the leaves of *Rhododendron* spp., called indumentum, may contribute to feeding resistance. While, to my knowledge, no work has examined indumentum on rhododendrons and *S. pyrioides* feeding, there have been other studies that have looked at trichome density of different host-plants with whiteflies (Hemiptera:Aleyrodidae) in geraniums (Avery et al., 2015), eggplants (Hasanuzzaman et al., 2016), and soybean (do Valle et al., 2012). However, these studies each obtained different results. Avery et al. (2015) found lower host preference and lower oviposition in a geranium with a higher trichome density on the underside of the leaves. Hasanuzzaman et al. (2016) found a positive correlation with trichome density and the

number of adult whiteflies and eggs, and do Valle et al. (2012) found no significant correlation between trichome density and adult attractiveness or oviposition preference. These studies show that trichome density has a different role in different host-plants. Although the effect of trichome density is not yet known in rhododendrons, examining this characteristic could provide useful information to recommend to growers and consumers.

Stephanitis pyrioides has become one of the most important problems affecting rhododendrons and azaleas. While insecticides have effectively suppressed *S. pyrioides* populations, the chemicals can be harmful to beneficial insects and may be harmful to vertebrates. Increasing the landscape complexity could also help reduce *S. pyrioides* populations, as predation pressure can be much higher in complex landscapes than simple landscapes. However, this requires natural enemy populations that feed on *S. pyrioides* to be high. In the absence of natural enemies, complex habitats could be just as suitable for *S. pyrioides*. The two specialist natural enemies *S. japonicus* and *A. takeyanus* have potential but they are not yet known to be in Oregon and both would have to be subject to various target and non-target trials before being introduced. There are also areas of control that have yet to be fully understood, such as pathogens that specifically attack *S. pyrioides*. Spiders are often very abundant predators but are overlooked as they quite often cannibalize each other. Cultivar resistance in rhododendrons is also unknown, as the studies that examined cultivar resistance were done with azaleas. *Stephanitis pyrioides* in Oregon was observed in the adult stages at all times throughout the year as well as feeding on rhododendrons. Knowing its life cycle and voltinism in Oregon are

essential to applying control measures to reduce its population. The thesis presented looks at the presence of natural enemies, the general life cycle of *S. pyrioides* in Oregon, cultivar and species susceptibility, and finally biological control efficacy using a predator and a pathogen.

Literature Cited:

Avery, P. B., Kumar, V., Simmonds, M. S. J., & Faull, J. (2015). Influence of leaf trichome type and density on the host plant selection by the greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera:Aleyrodidae). *Applied Entomology and Zoology*, 50(1), 79-87. doi:10.1007/s13355-014-0308-5

Balsdon, J. A., Braman, K., & Espelie, K. E. (1996). Biology and ecology of *Anagrus takeyanus* Gordh (Hymenoptera: Mymaridae), an egg parasitoid of the azalea lace bug (Heteroptera:Tingidae). *Environmental Entomology, 25*(2), 383-389.

Balsdon, J. A., Braman, S. K., Pendley, A. F., & Espelie, K. E. (1993). Potential for integration of chemical and natural enemy suppression of azalea lace bug (Heteroptera: Tingidae). *Journal of Environmental Horticulture, 11*(4), 153-156.

Balsdon, J. A., Espelie, K. E., & Braman, S. K. (1995). Epicuticular lipids from azalea (*Rhododendron* spp.) and their potential role in host-plant acceptance by azalea lace bug, *Stephanitis pyrioides* (Heteroptera, Tingidae). *Biochemical Systematics and Ecology, 23*(5), 477-&. doi:10.1016/0305-1978(95)00055-y

Braman, Latimer, J. G., Oetting, R. D., McQueen, R. D., Eckberg, T. B., & Prinster, M. (2000). Management strategy, shade, and landscape composition effects on urban landscape plant quality and arthropod abundance. *Journal of Economic Entomology, 93*(5), 1464-1472. doi:Doi 10.1603/0022-0493-93.5.1464

Braman, Pendley, A. F., Sparks, B., & Hudson, W. G. (1992). Thermal requirements for development, population trends, and parasitism of azalea lace bug (Heteroptera, Tingidae). *Journal of Economic Entomology, 85*(3), 870-877.

Braman, S., & Pendley, A. (1992). Evidence for resistance of deciduous azaleas to azalea lace bug. *Journal of environmental horticulture (USA)*.

Braman, S. K., & Beshear, R. J. (1994). Seasonality of predaceous plant bugs (Heteroptera: Miridae) and phytophagous thrips (Thysanoptera: Thripidae) as influenced by host plant phenology of native azaleas (Ericales: Ericaceae). *Environmental Entomology, 23*(3), 712-718.

CABI. (2005). Azalea lace bug, *Stephanitis pyrioides* (Hemiptera: Tingidae). Crop Protection Compendium. CAB International 2005.

Chappell, M., & Robacker, C. (2006). Leaf wax extracts of four deciduous azalea genotypes affect azalea lace bug (*Stephanitis pyrioides* Scott) survival rates and behavior. *Journal of the American Society for Horticultural Science, 131*(2), 225-230.

Chappell, M., Robacker, C., Baden, S., & Byous, A. (2004). The effect of leaf wax extracts from the leaves of eight azalea genotypes (*Rhododendron* spp.) on feeding and oviposition by azalea lace bug *Stephanitis pyrioides* (Scott). *Hortscience, 39*(4), 895-895.

Chappell, M., Robacker, C., Baden, S., & Byous, A. (2005). Border patrol: epicuticular leaf wax mediates resistance of azalea genotypes (*Rhododendron* spp.) to azalea lace bug *Stephanitis pyrioides* (Scott). *Hortscience, 40*(4), 1111-1112.

Cornell University. (1994). Abamectin Retrieved from http://pmep.cce.cornell.edu/profiles/extoxnet/24d-captan/abamectin-ext.html

Cornell University. (1995a). Azadirachtin. Retrieved from http://pmep.cce.cornell.edu/profiles/extoxnet/24d-captan/abamectin-ext.html

Cornell University. (1995b). Cyfluthrin. Retrieved from http://pmep.cce.cornell.edu/profiles/extoxnet/carbaryl-dicrotophos/cyfluthrin-ext.html

do Valle, G. E., Lourenção, A. L., & Pinheiro, J. B. (2012). Adult attractiveness and oviposition preference of *Bemisia tabaci* biotype B in soybean genotypes with different trichome density. *Journal of Pest Science, 85*(4), 431-442. doi:10.1007/s10340-012-0443-0

EPA. (1999). Bendiocarb. Retrieved from http://www.epa.gov/oppsrrd1/REDs/factsheets/0409fact.pdf

EPA. (2001). Acephate Facts. Retrieved from http://www.epa.gov/opp00001/reregistration/REDs/factsheets/acephate_fs.pdf

EPA. (2012). Clothianidin – Registration Status and Related Information. Retrieved from http://pmep.cce.cornell.edu/profiles/extoxnet/carbaryl-dicrotophos/cyfluthrin-ext.html

Hasanuzzaman, A. T. M., Islam, M. N., Zhang, Y., Zhang, C.-Y., & Liu, T.-X. (2016). Leaf morphological characters can be a factor for intra-varietal preference of whitefly *Bemisia tabaci* (Hemiptera:Aleyrodidae) among eggplant varieties. *Plos One, 11*(4), e0153880. doi:10.1371/journal.pone.0153880

Held, D. W., & Parker, S. (2011). Efficacy of soil applied neonicotinoid insecticides against the azalea lace bug, *Stephanitis pyrioides*, in the landscape. *Florida Entomologist, 94*(3), 599-607.

Henry, T. J., Neal, J. W., & Gott, K. M (1986). *Stethoconus japonicus* (Heteroptera: Miridae): a predator of *Stephanitis* lace bugs newly discovered in the United States, promising in the biocontrol of Azalea lace bug (Heteroptera: Tingidae). *Proceedings of the Entomological Society of Washington, 88*.

James, D., & Price, T. (2004). Field-testing of methyl salicylate for recruitment and retention of beneficial insects in grapes and hops. *Journal of Chemical Ecology, 30*(8), 1613-1628. doi:10.1023/B:JOEC.0000042072.18151.6f

Kaplan, I. (2012). Attracting carnivorous arthropods with plant volatiles: The future of biocontrol or playing with fire? *Biological Control, 60*(2), 77-89. doi:DOI 10.1016/j.biocontrol.2011.10.017

Kessler, A., & Baldwin, I. T. (2001). Defensive function of herbivore-induced plant volatile emissions in nature. *Science, 291*(5511), 2141-2144. doi:10.1126/science.291.5511.2141

Klingeman, W. E., Braman, S. K., & Buntin, G. D. (2000). Evaluating grower, landscape manager, and consumer perceptions of azalea lace bug (Heteroptera : Tingidae) feeding injury. *Journal of Economic Entomology, 93*(1), 141-148.

Klingeman, W. E., Buntin, G. D., & Braman, S. K. (2001). Using aesthetic assessments of azalea lace bug (Heteroptera:Tingidae) feeding injury to provide thresholds for pest management decisions. *Journal of Economic Entomology, 94*(5), 1187-1192. doi:Doi 10.1603/0022-0493-94.5.1187

Lee, J. C. (2010). Effect of methyl salicylate-based lures on beneficial and pest arthropods in strawberry. *Environmental Entomology, 39*(2), 653-660. doi:10.1603/EN09279

Nair, S., & Braman, S. K. (2012a). Integration of insecticides with the natural enemy *Chrysoperla carnea* (Stephens) for management of azalea lace bug (Hemiptera:Tingidae). *Journal of Entomological Science, 47*(3), 278-281.

Nair, S., & Braman, S. K. (2012b). A scientific review on the ecology and management of the azalea lace bug *Stephanitis pyrioides* (Scott) (Hemiptera:Tingidae). *Journal of Entomological Science, 47*(3), 247-263.

Neal, J. W., & Douglass, L. W. (1988). Development, oviposition rate, longevity, and voltinism of *Stephanitis pyrioides* (Heteroptera, Tingidae), an adventive pest of azalea, at 3 temperatures. *Environmental Entomology, 17*(5), 827-831.

Neal, J. W., Haldemann, R. H., & Henry, T. J. (1991). Biological-control potential of a japanese plant bug *Stethoconus japonicus* (Heteroptera:Miridae), an adventive predator of the azalea lace bug (Heteroptera:Tingidae). *Annals of the Entomological Society of America, 84*(3), 287-293.

Neal, J. W., & Haldemann, R. L. (1992). Regulation of seasonal egg hatch by plant phenology in *Stethoconus japonicus* (Heteroptera:Miridae), a specialist predator of *Stephanitis pyrioides* (Heteroptera:Tingidae). *Environmental Entomology, 21*(4), 793-798.

Nielsen, G. (1997). Lace Bugs, Entomology Leaflet 153. *Univ. Vermont Ext., Burlington*.

Rinehart, T. A., & Boyd, D. W. (2006). Rapid, high-throughput detection of azalea lace bug (Hemiptera:Tingidae) predation by *Chrysoperla rufilabris* (Neuroptera:Chrysopidae), using fluorescent-polymerase chain reaction primers. *Journal of Economic Entomology, 99*(6), 2136-2141.

Rosetta, R. (2013). Azalea lace bug biology and management in commercial nurseries and landscapes (2013 ed.): Oregon State University.

SAGEMAP, USGS. (n.d.). Top-down versus bottom-up effects. Retrieved from http://sagemap.wr.usgs.gov/ftp/BLM/FtPrtP3V2.pdf

Sanchez, D. (1989). *Stethoconus*, dirty harry of the azaleas. *Agricultural Research, 37*(4), 14-15.

Schultz, P. B. (1993). Host-plant acceptance of azalea lace bug (Heteroptera:Tingidae) for selected azalea cultivars. *Journal of Entomological Science, 28*(2), 230-235.

Shelton, A. (n.d.). *Chrysoperla (=Chrysopa*) *carnea, C. rufilabris* (Neuroptera: Chrysopidae). In table 1.2. Retrieved from http://www.biocontrol.entomology.cornell.edu/predators/Chrysoperla.php

Shrewsbury, P. M., Lashomb, J. H., Patts, J. M., & Casagrande, R. A. (2004). The influence of flowering plants on herbivore and natural enemy abundance in ornamental landscapes. *International Journal of Ecology and Environmental Sciences, 30*(1), 23-33.

Shrewsbury, P. M., & Raupp, M. J. (2006). Do top-down or bottom-up forces determine *Stephanitis pyrioides* abundance in urban landscapes? *Ecological Applications, 16*(1), 262-272. doi:10.1890/04-1347

Shrewsbury, P. M., & Smith-Fiola, D. C. (2000). Evaluation of green lacewings for suppressing azalea lace bug populations in nurseries. *Journal of Environmental Horticulture, 18*(4), 207-211.

Stewart, C. D., Braman, S. K., & Pendley, A. F. (2002). Functional response of the azalea plant bug (Heteroptera:Miridae) and a green lacewing *Chrysoperla rufilabris* (Neuroptera:Chrysopidae), two predators of the azalea lace bug (Heteroptera : Tingidae). *Environmental Entomology, 31*(6), 1184-1190. doi:10.1603/0046-225x-31.6.1184

Torres-Miller, L. (1989). New records of lace bugs from West Virginia (Hemiptera: Tingidae).

Trumbule, R., Denno, R., & Raupp, M. (1995). Management considerations for the azalea lace bug in landscape habitats. *Journal of Arboriculture, 21*, 63-63.

Trumbule, R. B., & Denno, R. F. (1995). Light-intensity, host-plant irrigation, and habitat-related mortality as determinants of the abundance of azalea lace bug (Heteroptera:Tingidae). *Environmental Entomology, 24*(4), 898-908.

Williams, L., III, Rodriguez-Saona, C., Castle, S., & Zhu, S. (2008). EAG-active herbivore-induced plant volatiles modify behavioral responses and host attack by an egg parasitoid. *Journal of Chemical Ecology, 34*(9), 1190-1201. doi:10.1007/s10886-008-9520-5

Table 1.1 Average development time (±SE) of each stage of *S. pyrioides*

	Development ±SE at 24°C (Braman et al. 1992)	Development ±SE at 26°C (Neal and Douglass 1988)
Egg	13.6 ±0.1 days	Not measured
1st instar	2.8±0.1	2.6 ±0.05 days
2nd instar	3.0±0.1	2.6±0.06
3rd instar	2.0±0.1	1.9±0.06
4th instar	2.7±0.1	2.4±0.07
5th instar	4.2±0.1	3.9±0.05
Total nymphal	14.8±0.1	13.4±0.14
Total	28.3±0.1	Not measured

Table 1.2 Natural enemies of *S. pyrioides* and studies that have used assessed them

Natural enemy	**Generalist or specialist**	**Reported locations**	**References**
Anagrus takeyanus Gordh (Hymenoptera:Mymaridae)	Adult stage is a specialist parasitoid on tinged eggs	Japan, Eastern US (CT, MD, GA)	(Nair & Braman, 2012b; Balsdon et al., 1996; Balsdon et al., 1993; Braman et al., 1992; Gordh & Dunbar, 1977)
Stethoconus japonicus Schumacher (Hemiptera:Miridae)	All stages, specialist predator of tingids	Japan, Eastern US (MD, GA)	(Henry et al., 1986; Nair & Braman, 2012b; Neal et al., 1991; Neal & Haldemann, 1992; Sanchez, 1989)
Chrysoperla rufilabris Burmeister (Neuroptera:Chrysopidae)	Generalist, larval stage is predaceous stage	Commercially Available	(Stewart et al., 2002; Shelton, n.d.)
Chrysoperla carnea Stephens (Neuroptera:Chrysopidae)	Generalist, larval stage is predaceous stage	Commercially Available	(Shrewsbury & Smith-Fiola, 2000; Shelton, n.d.)
Rhinocapsus vanduzeei Uhler (Hemiptera:Miridae)	Generalist		(Shrewsbury & Raupp, 2006)
Forficula auricularia Linnaeus (Dermaptera:Forficulidae)	Generalist		
Orius tristicolor White (Hemiptera: Anthocoridae	Generalist		
Anyphaena celer Hentz (Araneae:Anyphaenidae)	Generalist		
Oecanthus fultoni Walker (Orthoptera:Gryllidae)	Generalist		

Table 1.3 Species and cultivars used in various studies and the characteristics examined

Species / cultivar	**Characteristics examined**	**Reference**
'Hino Crimson'	Epicuticular lipid composition	(Baldson et al. 1995)
'Delaware Valley White' (DVW)		
'Higasa'		
'President Clay'		
R. canescens Michaux		
**********	**********	**********
'DVW'	~100% a -nymph survival	(Braman & Pendley, 1992)
R. calendulaceum (Michaux) Torrey	~ 25 b	
R. albamense Rehder	~15% bc	
R. canescens	~10% bc	
R. austrinum (Small) Rehder	~10% bc	
R. prunifolium (Small) Millais	~ <5% c	
**********	**********	**********
'Buttercup'	Susceptible and resistant leaf waxes	(Chappell & Robacker, 2006)
'My Mary'		
R. canescens		
R. periclymenoides (Michaux) Shinners		
**********	**********	**********
'Fourth of July'	Susceptible and resistant leaf waxes	(Chappell et al., 2005)
'My Mary'		
R. austrinum		
R. periclymenoides		
**********	**********	**********
'Blauus Pink'	Host-plant acceptance by oviposition, leaf injury (mm^2) and percent leaf injury. 'Macrantha' had lowest eggs/leaf, as well as lowest leaf injury and percent leaf injury.	(Schultz, 1993)
'Conversation Piece'		
'Coral Bell'		
'Delaware Valley White'		
'Elsie Lee'		
'Gigi'		
'Girards Rose'		
'Hersey Red'		
'Hino Crimson'		
'Hot Shot'		
'Karen'		
'Kathy'		
'Macrantha'		
'Mary Lynn'		
'Mothers Day'		
'Nancy'		

'Poukhanese'		
'Purple Splendor'		
'Sherwood Red'		
'Tradition'		

(Continued)

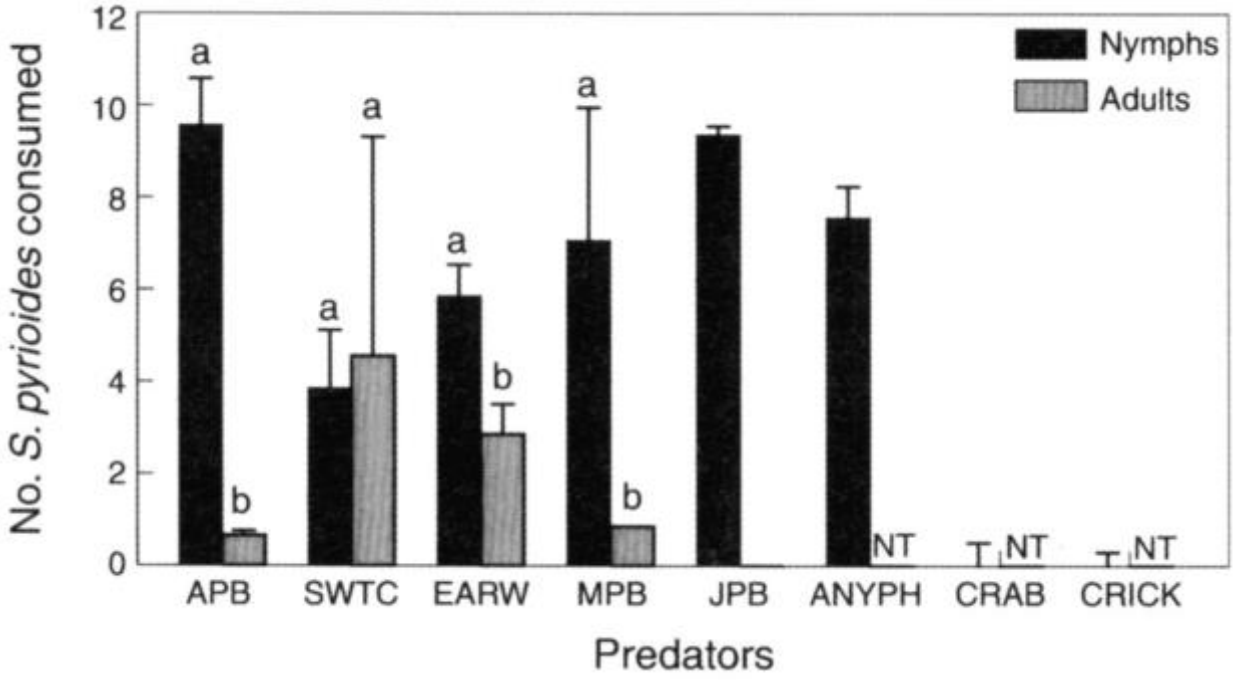

Figure 1.1 Graph provided by the experiments done by Shrewsbury and Raupp (2006) showing that 6 out of 8 predators tested consumed significantly more nymphs than adults, except for the snowy tree cricket. Key to abbreviations: APB, azalea plant bug; SWTC, snowy white tree cricket; EARW, earwig; MPB, minute pirate bug; JPB, Japanese plant bug; ANYPH, *Anyphaena celer*; CRAB, crab spider; CRICK, field cricket.

Chapter 2:

Monitoring and phenological observations of *Stephanitis pyrioides*

Salvador M. Flores, Robin Rosetta, and Jana Lee

Introduction

The azalea lace bug, *Stephanitis pyrioides* Scott, belongs to the family Tingidae (Hemiptera) and as with several other insects in this family, is a pest of plants. *Stephanitis pyrioides* adults and nymphs feed on rhododendron and azalea leaves. Their feeding causes a yellow stippling on the upper side of the leaf, and is the cause of aesthetic damage as well as reduced plant vigor (Buntin et al., 1996). This herbivorous insect has caused a significant amount of damage in a short time to the native and landscape planted rhododendrons and azaleas in the Pacific Northwest and methods of control are needed to mitigate its damage. The most common control measure used is chemical treatment (Klingeman et al., 2001; Nair & Braman, 2012a) and is a relatively cost-effective option, where treating 2,750 azalea plants was estimated to cost $143 in a one-acre nursery scenario or $45 for 10 azalea plants in a landscape scenario (Klingeman et al., 2001). However, there are drawbacks to relying solely on chemical control. Chemical control can be harmful to the environment and other wildlife. Some of the chemicals that have been used to examine *S. pyrioides* control efficacy, such as imidacloprid, have reduced survival of the green lacewing *Chrysoperla carnea* Stephens (Neuroptera:Chrysopidae) (Rogers et al., 2007), as well as the parasitoid *Anagyrus pseudococci* Girault (Hymenoptera:Encyrtidae) (Krischik et al., 2007). They have also been shown to reduce survival of other beneficial insects, such as the lady beetles *Coleomegilla maculata* De Geer, *Harmonia axyridis* Pallas, and *Hippodamia convergens* Guérin-Méneville (Coleoptera:Coccinellidae; Krischik et al., 2015). To mitigate non-target effects, other management options are needed to reduce the use of chemical control.

While it is a relatively new invasive insect pest in the Pacific Northwest, *S. pyrioides* has been established in the eastern United States for almost a hundred years (Nair & Braman, 2012b). It is reported to be mutlivoltine, with up to four generations in Georgia, and the only observed overwintering stage being the egg (Braman et al., 1992). However, the number of generations in the Pacific Northwest is not known, it is also not known if the overwintering stage is the same, due to the differences in weather conditions during each region's winter season. To understand the presence and abundance of *S. pyrioides* throughout the year, I surveyed infested locations to estimate which life stages were present at each month of the year. This information will also help pinpoint the emergence time of the pest and optimal times for control strategies.

Currently, in the eastern U.S., there are two exotic natural enemies, a predatory mirid, *Stethoconus japonicus* Schumacher (Henry, 1986), and a parasitic wasp *Anagrus takeyanus* Gordh (Balsdon et al., 1996). They are not known in the Pacific Northwest. Therefore, sampling for natural enemies present amongst infested plants may enable us to determine which natural enemies are consuming *S. pyrioides*. Once local predators and parasitoids are identified, the natural enemies' community may serve as the means for natural population control. Furthermore, practices to bolster their populations may reduce the use of chemical insecticides.

The objectives of the study were to gather more information on the life stages of *S. pyrioides* from mid-2014 to mid-2016 and to determine what natural predators are present

as well as surveying for the two natural enemies mentioned that are present on the East Coast. I sampled various sites in the Willamette Valley in Oregon to find: 1) what life stages are present throughout a two-year sampling period from leaf collections, and 2) presence or absence of natural enemies (predators and parasitoids) by collecting shake samples and sticky traps and lastly by rearing unhatched *S. pyrioides* eggs for potential egg parasitoids.

Methods

Life stage and egg presence check. To determine what life stages of *S. pyrioides* are present throughout the year, sampling sites were set up throughout the Willamette Valley. Sampled sites from August 2014 to April 2015 were at Hendricks city park in Eugene, a commercial nursery in Dayton, and on the Oregon State University Campus (OSU) in Corvallis. At each site sampled, plants were scanned for infested leaves with a selected number of leaves taken back to the laboratory to count eggs, nymphs, and adult *S. pyrioides*. During the winter months, when *S. pyrioides* adults and nymphs became less abundant, a timed search was implemented to check however many leaves as possible in 20 minutes and collect infested leaves. This was done to increase our survey intensity, therefore this change in protocol varied in the number of leaves collected at each site. Specifics of leaf collection, areas within a site, frequency of sampling, and egg checks can be seen in Table 2.1. For some sites, two infested areas were sampled that were at least 20 m apart.

Starting in May 2015, the number of sampled sites increased and monitoring sites added include the Capitol Mall in Salem, the North Willamette Research and Extension Center in Aurora, Boone's Ferry Park in Wilsonville, Crystal Springs Rhododendron Park in Portland, and Jenkins Estate in Beaverton. Starting in October 2015 and up to present day, sampling sites were pared down and only included the OSU campus, Hendricks City Park, the Capitol Mall, and Jenkins Estate. Sampling specifics for each site are in Table 2.1.

Leaves from sampling sites were collected when they met one or all of the following criteria and are listed by importance in leaf collection: 1) had live *S. pyrioides* adults or nymphs, 2) fecal deposits on underside of leaves, which also indicates feeding and possible egg laying or 3) stippling damage visible on the top of the leaves. Leaves were then taken back to the lab and inspected under the microscope for adults and nymphs. Adults were counted and separated by sex and could be seen at 0.63X magnification or without microscope assistance. Large nymphs, 4^{th}-5^{th} instars, were identified at 0.63-1.00X magnification and small nymphs, 1^{st}-3^{rd} instars, were identified under 1.25-2.0X magnification. The total number of nymphal and adult *S. pyrioides* were recorded at each sampling date per area and site.

In addition to adult and nymphal stage checks, the same leaves were also checked for eggs. Each leaf was washed with warm water and gently cleaned with a sponge to remove fecal deposits and dried of excess water to expose eggs for counting. Leaves were inspected under the microscope and hatched and unhatched eggs were counted

under 1.25-2.0X magnification. All leaves were stored in 10°C to keep the leaves in suitable condition for longer egg viability.

Egg parasitoid checks. For samples from July to October in 2014 and May to October in 2015, egg parasitism was checked. Parasitism was checked from May to October because the egg parasitoid *A. takeyanus* was commonly found in June, August and September in Georgia (Balsdon et al., 1996). All of the leaves with unhatched eggs were placed in a 5 oz. plastic cup with water and then enclosed in a 32 oz. plastic container with a mesh lid. These eggs were then checked 5 weeks later at 2.0-3.2X magnification for any signs of parasitoid emergence by looking for egg exit holes and containers were checked for any emerged adult parasitoids. Eggs were reared for five weeks because development of *A. takeyanus* from egg to adult was about 36 d (Balsdon et al., 1996).

Shake sampling for natural enemies. In conjunction with the leaf sampling, shake samples were taken at the same sampling sites from August 2014 to the present day. This sampling was done with a sweep net, with a branch of an infested rhododendron or azalea plant was shaken into the net and caught any falling arthropods. Each site had ten shakes done and sweep net contents were collected into a large plastic bag. Then the bags were placed in -20°C and all arthropods were counted, while specifically looking for natural enemies. Insects were identified to either order or family. All other arthropods were identified to subclass (i.e., Acari) or order (i.e., Araneae), with the exception of Salticidae and Thomisidae spiders. Only adult lady beetles were keyed to genus or species.

Sticky traps for natural enemies. During the sampling in 2014, sticky traps were set up at three of the sampling sites: Hendricks City Park, OSU, and a commercial nursery. Each site had three weekly collections between August 12 to September 3, 2014. Sticky traps were then taken back to the lab, stored at 10°C, and then arthropods were identified under the microscope as described for the shaking samples.

Data analyses. Because these protocols were performed to observe life stage presence and natural enemies, no statistical tests were done.

Results

Life stage and egg presence check. A total of 8,780 *S. pyrioides* were collected from August 2014 to April 2016 (Table 2.2). Adults were found year round, but were observed in lower numbers in the winter and were the only life stage present, besides eggs, thus far in 2016 with the exception of no adults found in April 2016 (Figure 2.1). More than double the number of females were caught compared to males (Figure 2.3). Sex ratios of adult males and female were checked in 2014 and 2015, where females were consistently the highest proportion of the sampled populations (Figure 2.3). Thus far in 2016, females were almost exclusively caught (Figure 2.3). Nymphs were found in high numbers in spring and summer, with observed smaller collections in the fall and rarely collected in the winter (Figure 2.2). Approximately half of the total *S. pyrioides* nymphs and adults collected were nymphs in 2014 and 2015 (Table 2.2). The proportion of life stages from 2014-2016 were also looked at; there was a spike of large nymphs (4^{th}-5th) in October of 2014 and May 2015 (Figure 2.2). The total number of unhatched eggs was

also slightly above half of the total number of eggs counted in the collected leaves. Eggs were present throughout the year (Figure 2.1) and were, in several months, the most numerous life stage (Figure 2.2).

Egg parasitoid checks. No evidence of the egg parasitoid, *A. takeyanus*, was found. Among the ~520 leaves checked from August through October in 2014 and ~660 leaves from May through October in 2015, there were no observed distinctive exit holes typically made by parasitoids in the egg cap. Also, no dead or live adult parasitoid wasp was found in the leaf rearing containers after 5 weeks.

Shake netting for natural enemies. From August 2014 through December 2015, we found that over half of collected arthropods in sweep nets were adults *S. pyrioides* (Figure 2.4). Approximately 8% of the arthropods collected could be considered potential predators of *S. pyrioides*. The potential predators were either mostly spiders, or the lady beetle *Coccinella septempunctata* Linnaeus. Not all spiders were identified to family, but included Salticidae and Thomisidae. A variety of arthropods were also placed in the "Other" category and included all Miridae, Thysanoptera, non-Coccinellidae Coleoptera, Anthocoridae, other Hemiptera, all Acari, Chrysopidae, Formicidae and a Raphidioptera. Many of these listed in "Other" could have been potential predators of *S. pyrioides*, however each listed accounted for no more than 1% of the total arthropods collected and collectively accounted for only 4% of the total (Figure 2.4)

Sticky traps for natural enemies. From the sticky traps deployed in 2014, we collected and identified a total of 1,583 arthropods. Of these, over half were parasitic Mymaridae wasps (Figure 2.5). *Anagrus takeyanus*, the egg parasitoid of *S. pyrioides*, is a wasp in the Mymaridae, however Mymaridae collected in the sticky traps did not resemble this species that is present in the southern United States and Asia. As with the shaking samples, spiders were not all identified but included Salticidae and Thomisidae. Arthropods placed in the "Other" categories in this sampling method included Cicadellidae, Psocoptera, predatory and non-predatory Thysanoptera, Coleoptera, and all Acari.

Discussion

Checking for life stages and eggs from August 2014 until April 2016 showed that there was at least one stage present in each month. Eggs and adults were present August through December of 2014 and year round in 2015. Egg presence during the winter months is consistent with the study done by (Braman et al., 1992) and the review by Nair & Braman (2012b) stating that the eggs are the overwintering stage. However, to my knowledge, no published reports showed adult presence during the winter months, but in Oregon we consistently found adults in 2014 and 2015 during the winter months, which may be due to the winter in Oregon being more hospitable. James LaBonte, Entomologist at the Oregon Department of Agriculture, also confirmed adult presence during the winter in Oregon (LaBonte & Valente, 2014).

In 2014, there were two substantial increases of large-sized nymphs in August and October, suggesting the possibility of at least two new generations from August to December. In May, July, and October 2015, the large-sized nymphs comprised above 40% of the nymphal and adult population (excluding eggs) possibly suggesting three generations over the course of the year. Whereas large-sized nymphs showed marked increases at the times mentioned, small-sized nymphs only had one large increase in April of 2015, which may have been the first generation of the year. It is possible that population increases of small nymphs did not precede increases in large nymphs throughout the year because of sampling bias or due to the fragility of the small nymphs. Small disturbances in handling could have caused mortality and therefore could have affected sampling counts. In prior studies, four generations were estimated in Georgia by Braman et al. (1992) and Neal and Douglass (1988). Braman et al. (1992) also used the data from Neal and Douglass (1988) to help analyze degree-days (DD) needed for development and found that at the lower threshold temperature of 11.2°C complete development took about 394 DD. Using this lower threshold temperature and an upper temperature threshold of 33°C (Braman et al., 1992), the estimated total number of degree-days in Salem, OR in 2015 was 1403.6 (IPPC Phenology Model, 2016), which suggests that there were about 3.5 generations in 2015. This estimate based on DD is consistent with my observations of three or more generations or more during 2015 based on three population peaks of large nymphs, and is similar to what has been reported in the previously mentioned studies.

Of the adults sampled and sexed, counts of females were consistently higher than males in all months except May of 2015. This increase in males may be due to increased dispersal and need for mating. Females may have also been collected in higher numbers throughout the year due to sampling methods used in the survey. At this point, nothing is known as to whether females spend more time on host plant leaves than males. Females may also be living longer, as suggested by the sex ratio during the winter months. If females are living longer, it would contrast the study done by Neal and Douglass (1988) where they found that paired males lived up to 21 days longer than paired females. While females were caught more often, their longevity in Oregon is not known.

The egg parasitoid *A. takeyanus* was not found in 2014-2015 when the sampled leaves with eggs were reared for potential parasitoid emergence for five weeks. While there were natural predators sampled, none were present at high numbers. In the sticky traps, many parasitoids were caught, but none were confirmed to be *A. takeyanus*. Natural enemy surveys and parasitoid rearing efforts by Oregon Department of Agriculture were also negative for specialist natural enemies, including *A. takeyanus* and *S. japonicus* (LaBonte, pers. comm.). This information gives insight on the temporal presence and absence of *S. pyrioides* throughout the Willamette Valley in Oregon. Further research is needed to accurately determine *S. pyrioides* voltinism in the PNW by rearing eggs to adults in cages with azaleas and rhododendrons to determine how many generations there are per year in this region and how common generation overlap is among these insects. Determining their developmental time in both set temperatures in a laboratory and natural settings will provide better insight on how long it will take to reach sexual maturity and

expected longevity. This information will also help determine when to implement control by estimating when the first generation will emerge.

Literature Cited:

Balsdon, J. A., Braman, K., & Espelie, K. E. (1996). Biology and ecology of *Anagrus takeyanus* Gordh (Hymenoptera:Mymaridae), an egg parasitoid of the azalea lace bug (Heteroptera:Tingidae). *Environmental Entomology*, *25*(2), 383-389.

Braman, Pendley, A. F., Sparks, B., & Hudson, W. G. (1992). Thermal requirements for development, population trends, and parasitism of azalea lace bug (Heteroptera:Tingidae). *Journal of Economic Entomology, 85*(3), 870-877.

Buntin, G. D., Braman, S. K., Gilbertz, D. A., & Phillips, D. V. (1996). Chlorosis, photosynthesis, and transpiration of azalea leaves after azalea lace bug (Heteroptera:Tingidae) feeding injury. *Journal of Economic Entomology*, *89*(4), 990-995.

Henry, T. J., Neal, J. W., & Gott, K. M. (1986). *Stethoconus japonicus* (Heteroptera: Miridae): a predator of *Stephanitis* lace bugs newly discovered in the United States, promising in the biocontrol of azalea lace bug (Heteroptera:Tingidae). *Proceedings of the Entomological Society of Washington, 88*.

IPPC Phenology Model. (2016). Phenology models from uspest.org. Retrieved from http://pnwpest.org/cgi-bin/ddmodel.us

Klingeman, W. E., Buntin, G. D., & Braman, S. K. (2001). Using aesthetic assessments of azalea lace bug (Heteroptera:Tingidae) feeding injury to provide thresholds for pest management decisions. *Journal of Economic Entomology, 94*(5), 1187-1192. doi:Doi 10.1603/0022-0493-94.5.1187

Krischik, V., Landmark, A., & Heimpel, E. (2007). Soil-applied imidacloprid is translocated to nectar and kills nectar-feeding *Anagyrus pseudococci* (Girault) (Hymenoptera:Encyrtidae). *Environmental Entomology, 36*(5), 1238-1245.

Krischik, V., Rogers, M., Gupta, G., & Varshney, A. (2015). Soil-Applied imidacloprid translocates to ornamental flowers and reduces survival of adult *Coleomegilla maculata*, *Harmonia axyridis*, and *Hippodamia convergens* lady beetles, and larval *Danaus plexippus* and *Vanessa cardui* butterflies. *Plos One, 10*(3), e0119133. doi:10.1371/journal.pone.0119133

Labonte, J. R. (2014). [Personal Communication].

LaBonte, James R., & Valente, Thomas E. (2014). Azalea lace bug, *Stephanitis pyrioides* (Scott): a new pest for the Pacific Northwest. Poster session presented at: Entomology 2014, Grand challenges beyond our horizons. 62nd Annual Meeting of the Entomological Society of America; 2014 Nov 16-19; Portland, OR

Nair, S., & Braman, S. K. (2012a). Integration of insecticides with the natural enemy *Chrysoperla carnea* (Stephens) for management of azalea lace bug (Hemiptera:Tingidae). *Journal of Entomological Science, 47*(3), 278-281.

Nair, S., & Braman, S. K. (2012b). A scientific review on the ecology and management of the azalea lace bug *Stephanitis pyrioides* (Scott) (Hemiptera:Tingidae). *Journal of Entomological Science, 47*(3), 247-263.

Neal, J. W., & Douglass, L. W. (1988). Development, oviposition rate, longevity, and voltinism of *Stephanitis pyrioides* (Heteroptera:Tingidae), an adventive pest of azalea, at 3 temperatures. *Environmental Entomology, 17*(5), 827-831.

Rogers, M. A., Krischik, V. A., & Martin, L. A. (2007). Effect of soil application of imidacloprid on survival of adult green lacewing, *Chrysoperla carnea* (Neuroptera:Chrysopidae), used for biological control in greenhouse. *Biological Control, 42*(2), 172-177.

Table 2.1 Sampling protocols for *S. pyrioides*

Date	Sites (areas)	Frequency per month	Leaves collected at each area	Parasitoid egg check
Aug 2014 – April 2015	Hendricks (2) OSU (2) Nursery (1-2)	2 2 2	6-35	Aug 2014-October 2014
May 2015-September 2015	Hendricks (2) OSU (2) Nursery (1-2) Salem (1-2) NWREC (1) Boone's (1) Portland (1-2) Beaverton (1)	1 1 1 1 1 1 1 1	10	April 2015-October 2015
October 2015- Present	Hendricks (2) OSU (2) Salem (1) Beaverton (1)	1 1 1 1	10	October samples only

Table 2.2 Total *S. pyrioides* collected from August-December 2014, All of 2015, and Jan-April 2016

	1	**2**	**3**	**4**	**5**	**Female**	**Male**	**Total Nymphs**	**Total Adults**	**Total *S. pyrioides***	**Unhatched eggs**	**Hatched eggs**
2014	34	61	70	255	412	531	287	832	818	1650	44	106
2015	18	32	130	311	400	694	274	891	968	1859	3218	3708
2016	0	0	0	0	0	25	2	0	27	27	852	1430
Total	52	93	200	566	812	1250	563	1723	1813	3536	4114	5244

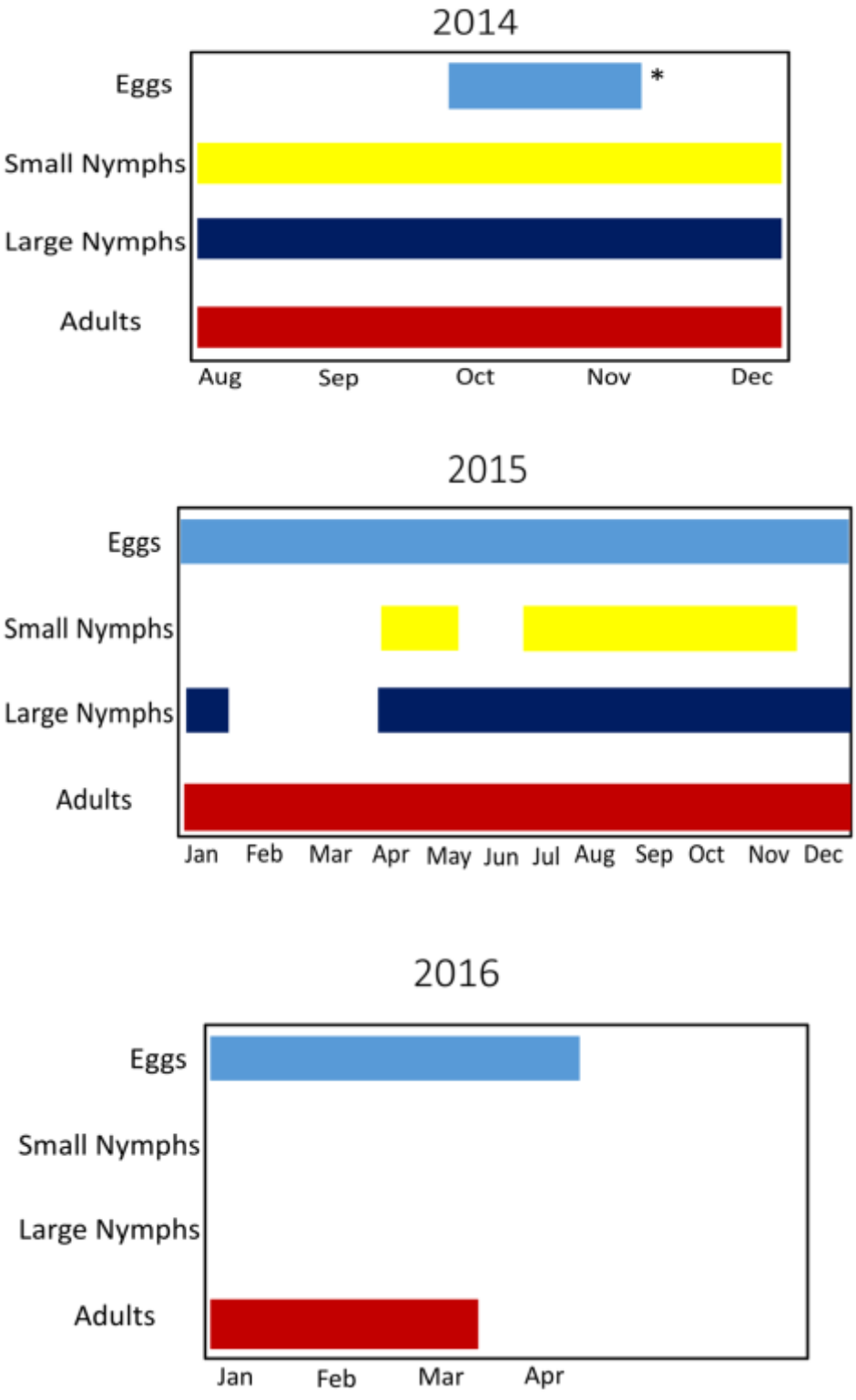

Figure 2.1 Presence of different *S. pyrioides* life stages in 2014-2016. Asterisk in 2014 refers to egg checking only occurring in October and November.

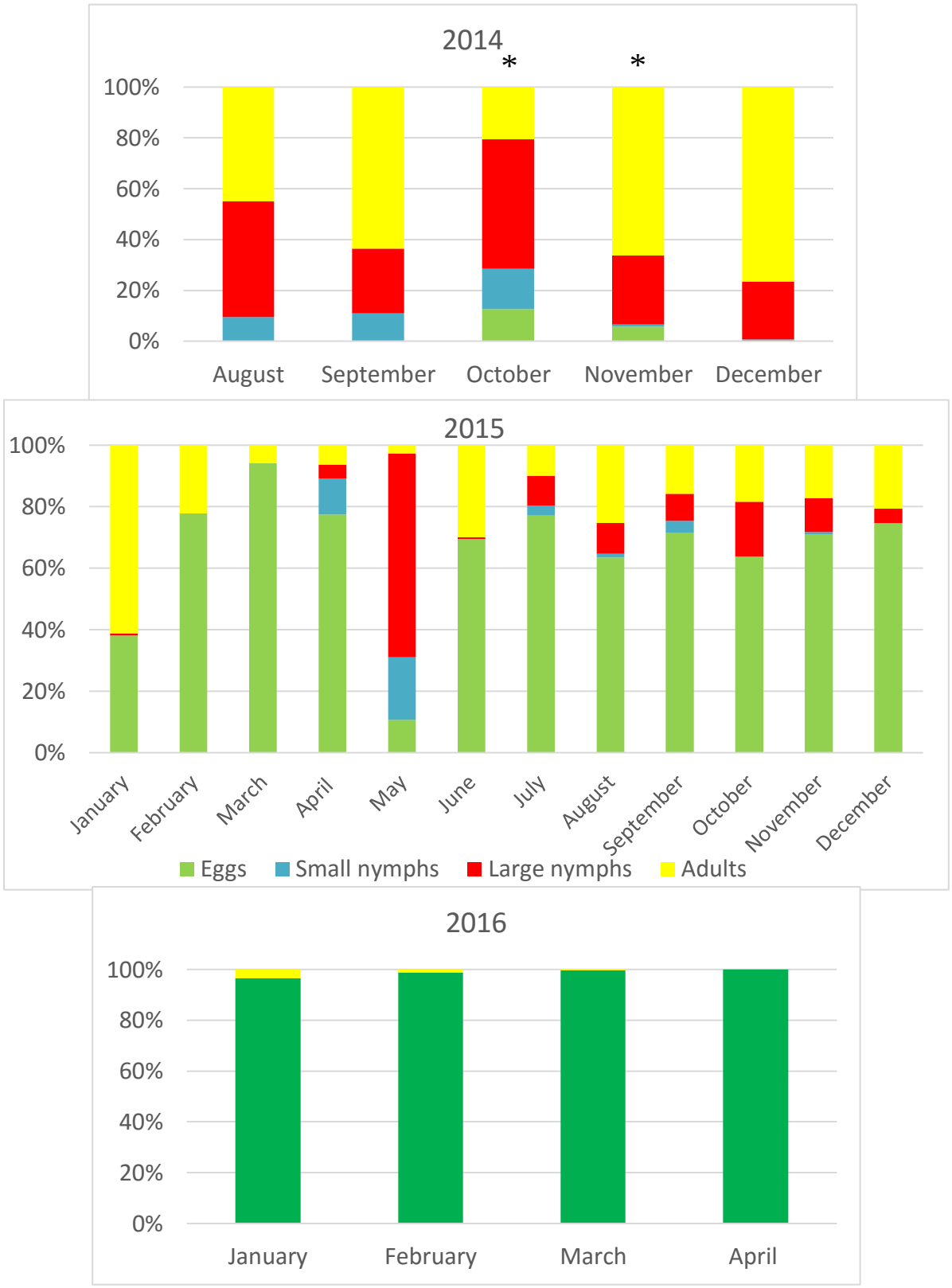

Figure 2.2 Proportion of life stages in the sampled population from 2014-2016. Asterisks in 2014 note the only two months in which eggs were checked.

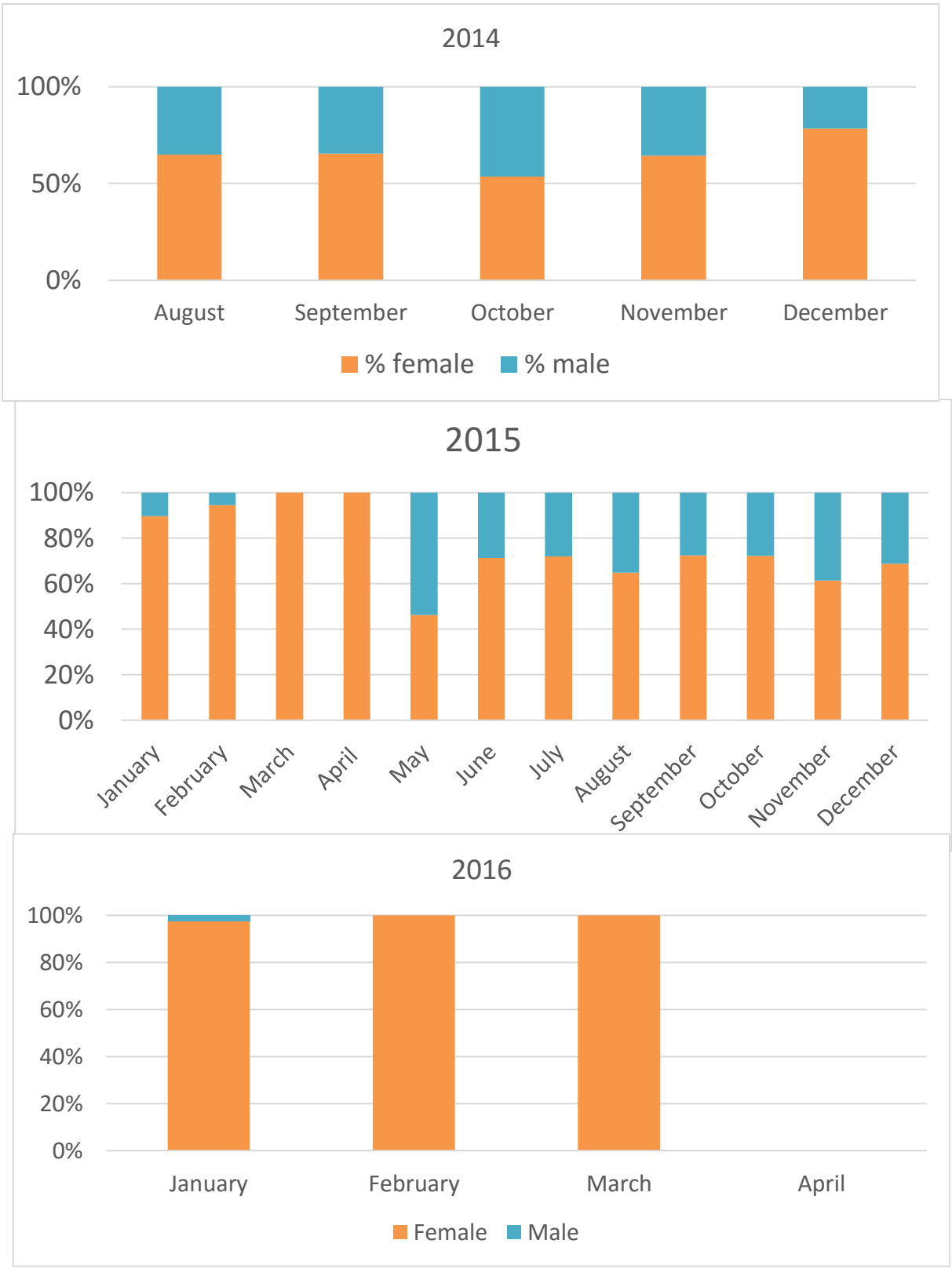

Figure 2.3 Adult *S. pyrioides* sex ratios from 2014-2016, no adults were collected in April 2016.

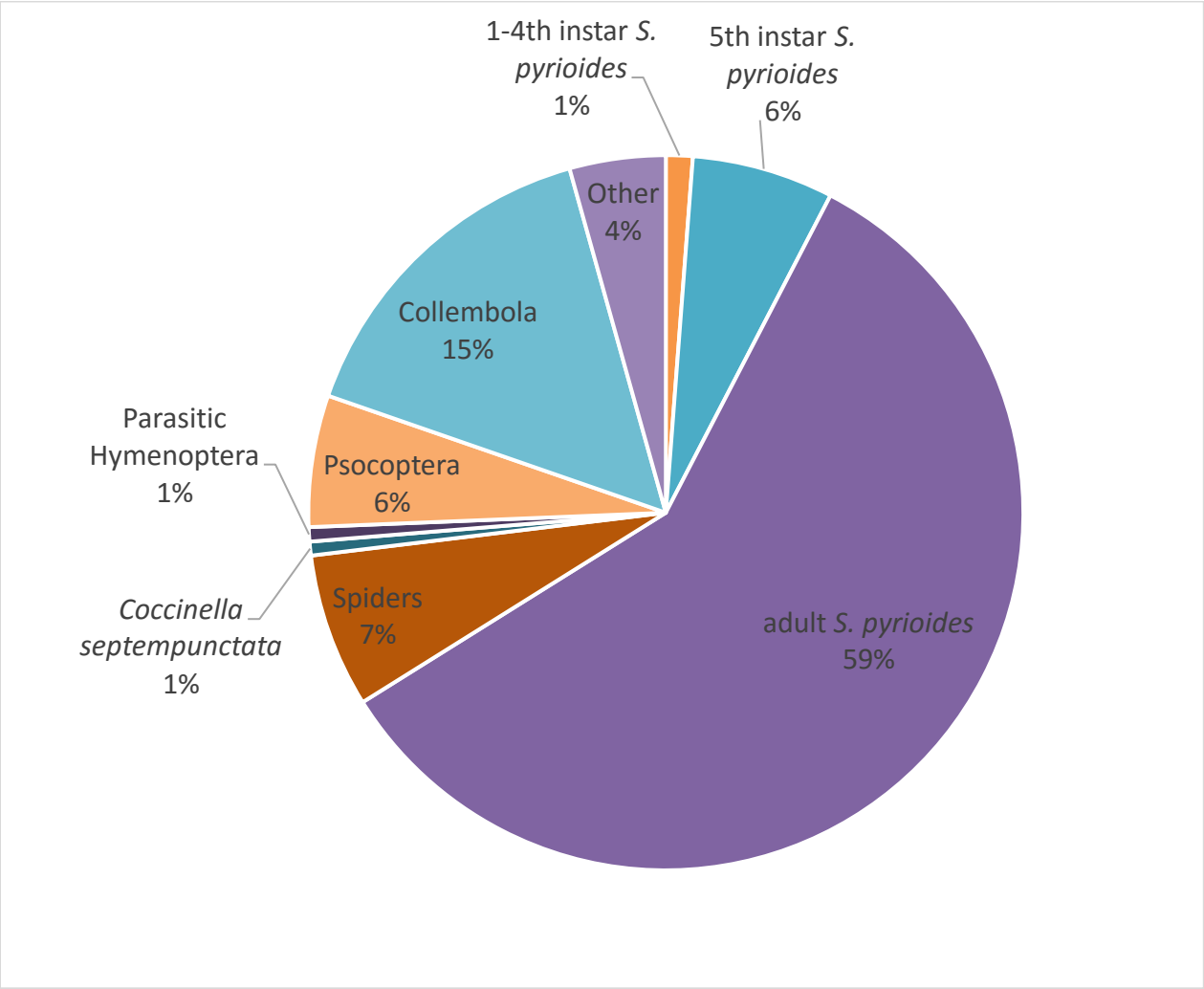

Figure 2.4 Proportion of the types of arthropods collected by shake net sampling.

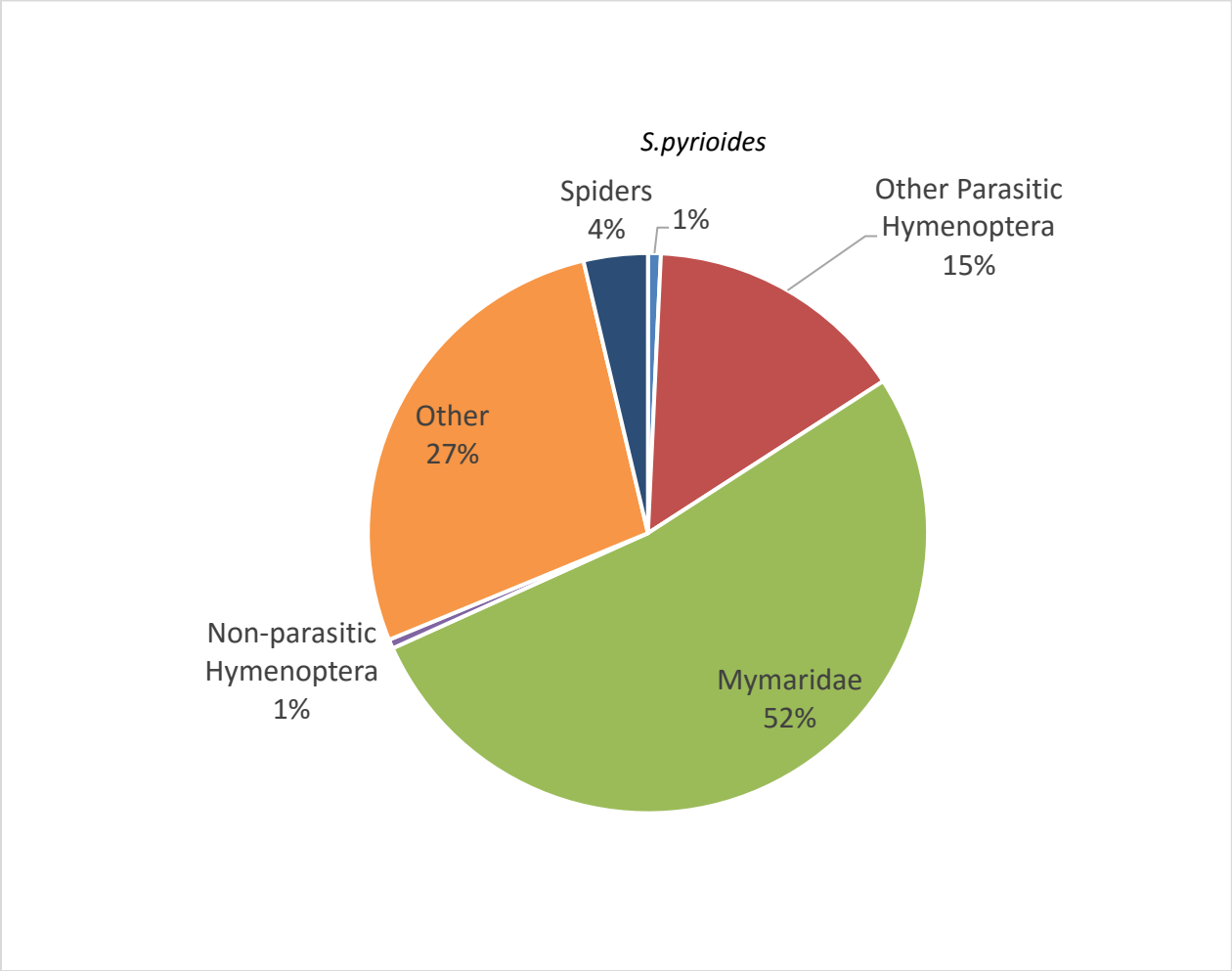

Figure 2.5 Proportion of arthropods collected in sticky traps.

Chapter 3:

Cultivar observations and resistance to *Stephanitis pyrioides* feeding

Salvador M. Flores and Jana Lee

Introduction

Azaleas and rhododendrons are heavily flowering landscape plants. This has made these plants a popular choice in gardens and ornamental settings across Oregon and the rest of the Pacific Northwest. However, a recent invasive pest, *Stephanitis pyrioides* Scott, feeds on plants within the Ericaceae, specializing on *Rhododendron* species, including azaleas, causing aesthetic damage as well as harming the host. This insect can have a potentially large economic impact. In a study by Klingeman et al. (2000), a survey was taken by professionals and consumers using *Rhododendron indica* variety *alba* 'Delaware Valley White' azaleas. The survey showed plant purchase rejection by 50% of the respondents at as little as 1.03% actual injury as well as half of the respondents indicating treatment of established plants to control *S. pyrioides* at 3.3% actual injury. This survey shows that there is a low tolerance to *Rhododendron* injury for purchasing and treatment, the former hindering sales, while the latter increases costs to manage azaleas and rhododendrons.

Feeding by *S. pyrioides* creates a major problem, as the lace bug pierces the stomata and feeds on the chlorophyll (Braman & Pendley, 1992; Buntin et al., 1996). This feeding results in the stippling on the upper side of the leaves (Figure 3.1) and fecal deposition on the lower side of leaves, causing an undesirable appearance on leaves. Feeding has also been shown to reduce net leaf photosynthesis, which can reduce plant photosynthetic capacity, and rate, and diminishing plant vigor (Buntin et al., 1996), with severe damage causing plant death. This reduction can also diminish the number of flowers per plant, thereby reducing their aesthetic quality further. Several studies have looked at azalea cultivars and species resistant to *S. pyrioides* feeding. Balsdon et al. (1995) examined

epicuticular lipid compounds, where they found two triterpenoids were lower in more susceptible plants. Chappell and Robacker (2006) and Chappell et al. (2004) both found that extracting leaf wax from resistant azaleas and applying them to susceptible azaleas reduced feeding, fecal deposition and oviposition. Braman and Pendley (1992) tested several cultivars and showed that deciduous azaleas were more resistant than an evergreen azalea during early season activity, proposing that because deciduous azaleas lose their leaves they are less suitable for oviposition. This is because the loss of leaves during the fall leads to no overwintering eggs, and thus no first generation infestations. The last study, done by Schultz (1993), assessed host plant acceptance by measuring reduced oviposition over two years and found that 'Macrantha' had the lowest oviposition and leaf injury. However, these studies focused on azaleas and *S. pyrioides* can significantly damage other rhododendrons as well. Cultivar studies on what we commonly call rhododendrons, species with large, evergreen and leathery leaves, are lacking. It is important to assess *S. pyrioides* damage in both of these different species or groups in the same genus.

To find resistant rhododendrons and azaleas, observations in locations with many different types of *Rhododendron* species will help with preliminary resistance determination. Finding resistant plants will provide more than one option to deter *S. pyrioides* feeding, as it could not only restrict damage to vulnerable hosts but also control *S. pyrioides* populations. The objective of these studies was to examine potential pest resistance among cultivars, and I used two approaches. The first approach was by observational surveys of natural infestation at two sites over multiple dates, with a large

variety of different cultivars and species. The second approach was to take cultivars and species observed without infestation and test them in the laboratory. This study will give insight and potential options to reduce *S. pyrioides* damage.

Methods

Cultivar Observations. Two unsprayed sites were surveyed for observing natural infestation on various cultivars. The first site surveyed was a commercial nursery within the Willamette Valley in Oregon where rhododendrons were grown in approximately 3 gallon pots under hoop houses and were regularly irrigated. Here multiple plants of a single cultivar were present in one or more hoop houses. At this site, initial cultivar observations were done September 8 and September 18, 2014. Observers recorded the name of the cultivar and then visually scanned the cultivars for signs of damage and looked more closely at damaged plants for stippled leaves, feces, live *Stephanitis pyrioides* adults and nymphs, and nymphal exuviae. Each cultivar was rated a "yes" or a "no" in regards to being infested or not, when one plant was found with infestation and about 30 individuals of each cultivar were checked. After this initial check, all the cultivars rated a "yes" were no longer checked. All cultivars rated "no" were subsequently checked repeatedly to determine if they remained uninfested. Rechecks were done on October 2, 16, 20, and November 12, 2014 as *S. pyrioides* infestations continued to increase. Plans to recheck the "no" cultivars were discontinued in 2015 because the plants were treated with a systemic insecticide.

The second site surveyed was Jenkins Estate, a private rhododendron specimen garden in Beaverton, Oregon where plants were well-established in the ground. At this

site, most cultivars were only represented by 1-3 plants, and plants of the same cultivar were often situated in different areas of the garden. The first observations made for each plant in the garden occurred on June 30, 2015 to allow enough time for non-infested plants to be rechecked for infestation. Observations made at this site were in more detail than that made at the commercial nursery in 2014, due to a more refined protocol. First the name of the cultivar was recorded, then the approximate visual estimate of the plant height and width were recorded. Next, damage was recorded at a scale from 0%, less than 5%, 25%, 50%, or 75% and greater damage among the leaves in the canopy. Lastly, damage intensity on leaves was also ranked as low, medium or high, and intensity was determined by how much stippling was present on the leaves of the cultivar (Figure 3.1). *Rhododendron* plants without an identifying name tag were not included in our observations. A single subsequent check was then done at the Jenkins Estate on September 23, 2015. Similar to the first surveyed site, all observed cultivars given an initial 0% damage rating were rechecked. Observations from both sites then allowed us to generate a list of cultivars that were not yet infested, despite infested cultivars in close proximity. Included in this list were any prominent characteristics that non-infested plants possessed, such as indumentum (dense lower surface hairs), tomentum (upper surface hairs), glossy leaves and hairy leaves.

Cultivar Susceptibility. To determine if rhododendron cultivars and species with dense indumentum deterred S. pyrioides feeding, a laboratory study was conducted. In this study, five different rhododendrons were selected to determine if *S. pyrioides* could feed on them. All five cultivars were either a cultivar, hybrid or a species and cuttings from

these were collected from Jenkins Estate in Beaverton, OR. Four of the five selected were designated as the resistant treatment based on field observations, and were 'Laramie', *Rhododendron makinoi* Tagg ex Nakai, *R. yakushimanum* x *bureavii*, and *R. yakushimanum* x *pachysanthum*. Of these four, 'Laramie', *R. yakushimanum* x *bureavii*, and *R. yakushimanum* x *pachysanthum* have *R. yakushimanum* Nakai in their parentage. All resistant cuttings had dense indumentum and 5 cuttings were collected from each plant. The fifth cultivar, 'Grand Slam', was designated as the control and was susceptible to *S. pyrioides* feeding. After cuttings were brought back to the lab, they were placed in a 5 oz. plastic cups (Solo Cup Co., Lake Forest, IL) filled with water, and the plant stem was fitted through a hole in the lid, with a ball of cotton to keep it upright. Next, each cutting was placed in a 30 x 30 x 30 cm BugDorm arena (BioQuip, Rancho Dominquez, CA). Ten *S. pyrioides* adults were placed in each arena to observe for feeding damage and were handled by the wings with soft forceps, to reduce any bodily damage. These *S. pyrioides* were either collected from natural infestations or from a colony that was constantly repopulated with wild adults. The arenas were then placed in an environmental chamber at temperature of 21°C and 60-70% RH. Arenas were set up on August 25 and September 1, 2015. Checks were done once weekly for two weeks, where each plant cutting was watered as needed and inspected for feeding damage or fecal deposits. After the first week, any observed *S. pyrioides* that had died were replaced, to ensure that their death wasn't due to less fit adults being placed in the arena. Each of the resistant cultivars were replicated 10 times, and the control was replicated eight times, due to the one of the cuttings dying prematurely, for a total of 48 replicates. An ANOVA test was done comparing mortality with cultivar treatment as a fixed effect and each plant

cutting as a random effect. Each plant cutting was treated as a random effect due to each cutting being used twice. Analyses were done in JMP 11.0 (SAS, 2013).

Results

Cultivar observations. After checking cultivars at both the nursery and at the Jenkins estate, a complete list was made of 431 cultivars and species that were observed with or without infestation or damage. Seventy-five of the total were observed without infestation or damage (i.e., rated as "no") after second or multiple checks (Table 3.1). Another list of all cultivars that were infested (i.e., rated as "yes") was also made (Table A.3). Most cultivars were susceptible to attack, only 17% of the total 431 were observed without infestation or damage. About 40% of these non-infested cultivars had either indumentum or tomentum, suggesting that increasingly dense dorsal or ventral leaf trichomes may play a role in feeding deterrence.

Cultivar susceptibility. In each of the observed non-infested cultivars in the lab trials, mortality rate of *S. pyrioides* was over 90% in all four of the resistant cultivars, and no visible feeding damage was observed (Figure 3.2). Mortality rates did not reach 100% on any of the rhododendron cuttings due to some adults not found and subsequently noted as "missing". Compared to the control, 'Grand Slam', mortality rates were significantly higher in resistant cultivars (cultivar: $F_{4.19} = 102.04$, $p < 0.0001$). Feeding damage was also observed in the control plant cuttings. These results suggest that dense indumentum plays a role in deterring *S. pyrioides* feeding on the plant, thereby reducing survivorship.

Discussion

A total of 431 cultivars were checked for infestation or damage at both the Jenkins Estate and at the commercial nursery. Of these 431, about 17%, or 75, cultivars/species were observed without any *S. pyrioides* infestation or damage, despite many of them in close proximity to other infested azaleas or rhododendrons. Furthermore, all of the cultivars/species observed without any infestation or damage were different from the host-plant acceptance study done by Schultz (1993), indicating a greater array of cultivars and species resistant to *S. pyrioides* feeding. This list, along with the susceptible list in Appendix 3 (Table A.3), provides, to my knowledge, one of the first comprehensive lists of rhododendrons observed without infestation or damage, along with susceptible rhododendrons, which could inform growers on which rhododendrons and azaleas may be more resistant to *S. pyrioides*.

Of these 75 cultivars and species, four have stronger evidence of resistance other than field observations from our experiments. The four are 'Laramie', *R. makinoi*, *R. yakushimanum* x *bureavii*, and *R. yakushimanum* x *pachysanthum*. Laboratory trials indicated that two different sets of adults were unable to feed on any of them and subsequently died, and this may be due to the heavy indumentum that all four of these rhododendrons possess. Furthermore, 'Laramie', *R. yakushimanum* x *bureavii*, and *R. yakushimanum* x *pachysanthum* have *R. yakushimanum* as a parent plant, indicating that the dense indumentum is a mode of resistance for these plants. However, the presence of indumentum is not solely indicative of *S. pyrioides* resistance, as several cultivars in Table A.3 had indumentum, mostly light indumentum, and had varying degrees of

infestation intensity. It is possible that the thickness of indumentum or another factor, such as leaf wax composition as shown in the studies of other *Rhododendron* spp. may influence feeding (Chappell and Robacker, 2006; Chappell et al., 2004; Chappell et al., 2005; Balsdon et al., 1995).

Further studies testing the rest of these resistant cultivars in a similar manner as the cultivar laboratory trial would help determine if each of these cultivars or species are truly resistant to feeding or merely less preferred than other cultivars. This would provide a more robust list than the one presented in Table 3.1, as some do not have indumentum but had no observed infestations or damage. Determining which cultivars and species are resistant will provide valuable information to growers and consumers. In order to determine if the indumentum on these rhododendrons is the primary mechanism of defense from feeding, trials where the indumentum is removed could confirm or reject this theory. Another study could be done via an electrical penetration graph (EPG) (Walker & Backus, 2000) which looks at the probing behavior of hemipterans and records voltage fluctuations by measuring ingestion activity inside plant tissues (Bonani et al., 2010). An EPG experiment could then examine *S. pyrioides* feeding attempts on both susceptible and resistant cultivars and see how feeding on each differs. These observed uninfested cultivars and species show promise for feeding and oviposition resistance as they were rechecked, some several times, and could inform growers on future decisions when rearing these plants.

Literature cited:

Balsdon, J. A., Espelie, K. E., & Braman, S. K. (1995). Epicuticular lipids from azalea (*Rhododendron* spp.) and their potential role in host-plant acceptance by azalea

lace bug, *Stephanitis pyrioides* Scott (Heteroptera:Tingidae). *Biochemical Systematics and Ecology, 23*(5), 477-485. doi:10.1016/0305-1978(95)00055-y

Braman, S., & Pendley, A. (1992). Evidence for resistance of deciduous azaleas to azalea lace bug. *Journal of environmental horticulture (USA)*.

Bonani, J., Fereres, A., Garzo, E., Miranda, M., Appezzato-Da-Gloria, B., & Lopes, J. (2010). Characterization of electrical penetration graphs of the Asian citrus psyllid, *Diaphorina citri* Kuwayama, in sweet orange seedlings. *Entomologia Experimentalis et Applicata, 134*(1), 35-49.

Buntin, G. D., Braman, S. K., Gilbertz, D. A., & Phillips, D. V. (1996). Chlorosis, photosynthesis, and transpiration of azalea leaves after azalea lace bug (Heteroptera: Tingidae) feeding injury. *Journal of Economic Entomology, 89*(4), 990-995.

Chappell, M., & Robacker, C. (2006). Leaf wax extracts of four deciduous azalea genotypes affect azalea lace bug (*Stephanitis pyrioides* Scott) survival rates and behavior. *Journal of the American Society for Horticultural Science, 131*(2), 225-230.

Chappell, M., Robacker, C., Baden, S., & Byous, A. (2004). The effect of leaf wax extracts from the leaves of eight azalea genotypes (*Rhododendron* spp.) on feeding and oviposition by azalea lace bug *Stephanitis pyrioides* Scott. *Hortscience, 39*(4), 895-895.

Chappell, M., Robacker, C., Baden, S., & Byous, A. (2005). Border patrol: epicuticular leaf wax mediates resistance of azalea genotypes (*Rhododendron* spp.) to azalea lace bug *Stephanitis pyrioides* Scott. *Hortscience, 40*(4), 1111-1112.

Klingeman, W. E., Braman, S. K., & Buntin, G. D. (2000). Evaluating grower, landscape manager, and consumer perceptions of azalea lace bug (Heteroptera:Tingidae) feeding injury. *Journal of Economic Entomology, 93*(1), 141-148.

SAS. (2013). *JMP® 11.0.0*. Cary, NC: SAS Institute Inc.

Schultz, P. B. (1993). Host-plant acceptance of azalea lace bug (Heteroptera, Tingidae) for selected azalea cultivars. *Journal of Entomological Science, 28*(2), 230-235.

Walker, G. P., & Backus, E. A. (2000). *Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior*. Paper presented at the International Congress of Entomology 1992: Beijing, China).

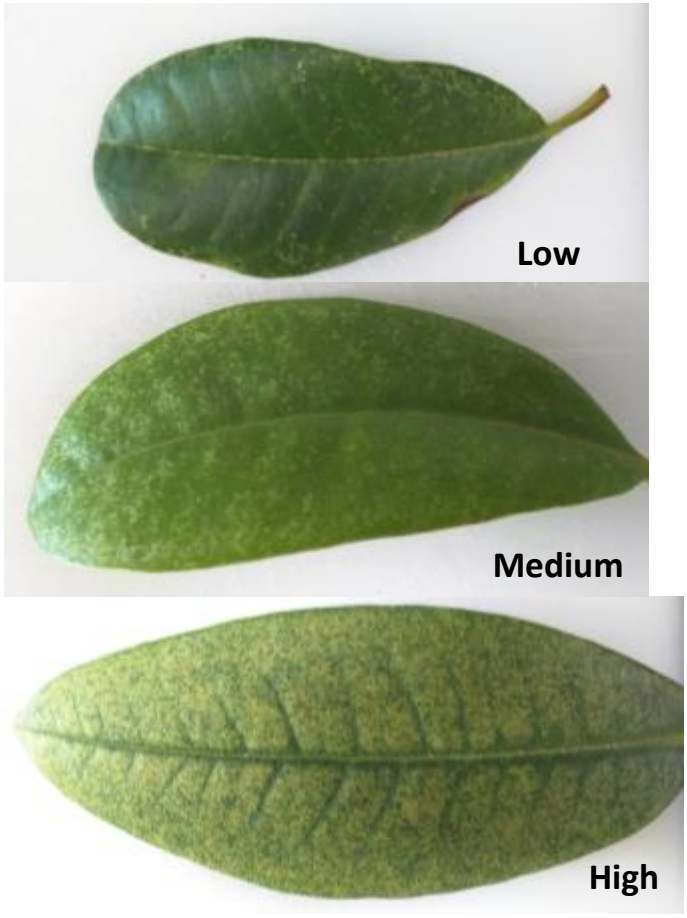

Figure 3.1 Rhododendron damage representing a "low" (top), “medium” (middle), and “high” (bottom) rating for the cultivar observations at Jenkins Estate.

Table 3.1 List of Rhododendrons and azaleas without observed infestation/damage

Name	Noted characteristics	Habit
Arborcum		garden
Atlanticum		garden
Austrinum Moonbeam		garden
Balfourianum	light Indumentum	garden
Beanianum	Indumentum	garden
Bloom-a-thon Lavender		pot
Bloom-a-thon White		pot
Bureavii Lem Form	Indumentum, Glossy	garden
Burovii	Indumentum	garden
Calophytum		garden
Caroline		garden
Cecorum		garden
Chlorops x Campylocarpum x Yak	Indumentum	garden
Clarke's #7363	Indumentum	garden
Crimson Piippin	Indumentum, Tomentum	garden
Degronianum Metlernichii	light Indumentum	garden
Degronianum Yakushimanum	Indumentum	garden
Double Besse		pot
Endre Osto		garden
Etta Burrows	Indumentum, Tomentum	garden
Fred Peste	Indumentum	garden
Impedium	Small leaves, odor	pot
Irnowii	Indumentum	garden
Jonathan Shaw		garden
Laramie	Indumentum	garden
Machmann's Diadem x Unique x Irroratum x Polka dot	Tomentum	garden
Makinoi	Indumentum	garden
Matador	light Indumentum	garden
Mergeratum		garden
Metternichi var. Kyomaruense	Indumentum	garden
Metternichii var. Kyomame	Indumentum	garden

Mikkeli		pot
Morgehrot x Rubicon x Anna's Riplet	Tomentum	garden
Naselle x Bambi x Proteoides	Tomentum	garden
Noyo Dream		garden
Occidentale Stagecoach Cream		garden
Patrick's		garden
Percly Menoides		garden
Perfume/Patrick		garden
Planting Memories	Indumentum	garden
Polar Bear		garden
Polarnacht		pot
Ponticum Variegatum		pot
Pseudochrysanthum Exbury	Tomentum	garden
Purple Passion		pot
Racemosum		garden
Red Eye		garden
Ruby Heart		garden
Saluene	Hairy	garden
Seaview Sunset		garden
September Song x (Bambi x Proteoides x (Yellow saucer x Anna's Riplet)		garden
Serrulatum		garden
Smirnowii	Indumentum	garden
Smirnowii White	Indumentum	garden
Strigillosum	Hairy	garden
Sugar Puff		pot
Thor	Indumentum	garden
Trocadero		pot
Uknown Pink/Dark Eye red	Glossy	garden
Viscosam Rosata		garden
Viscosum		garden
Viscosum Aemulans		garden
Volunteer Seedling/Ruby Heart	Indumentum	garden
Yak Koichiro Wada	Indumentum	garden
Yak Mist Maiden	Indumentum	garden
Yak Pachysanthum	Indumentum	garden
Yak Warpaint		garden
Yak x Arborium	Indumentum	garden
Yak x Bureauvii	Indumentum	garden
Yak x Pretty Place		garden
Yak x Strigilosum	Indumentum	garden

Yak, Yaku angle	Indumentum	garden
Yaku sunrise x (Bambi x proteoides) x Amuas Ripletdwark		garden
Yaku sunrise x Edwin Weber x Bambi x Proteoides #16	Indumentum	garden
Yakushima Dwarf Layer	Indumentum	garden

(Continued)

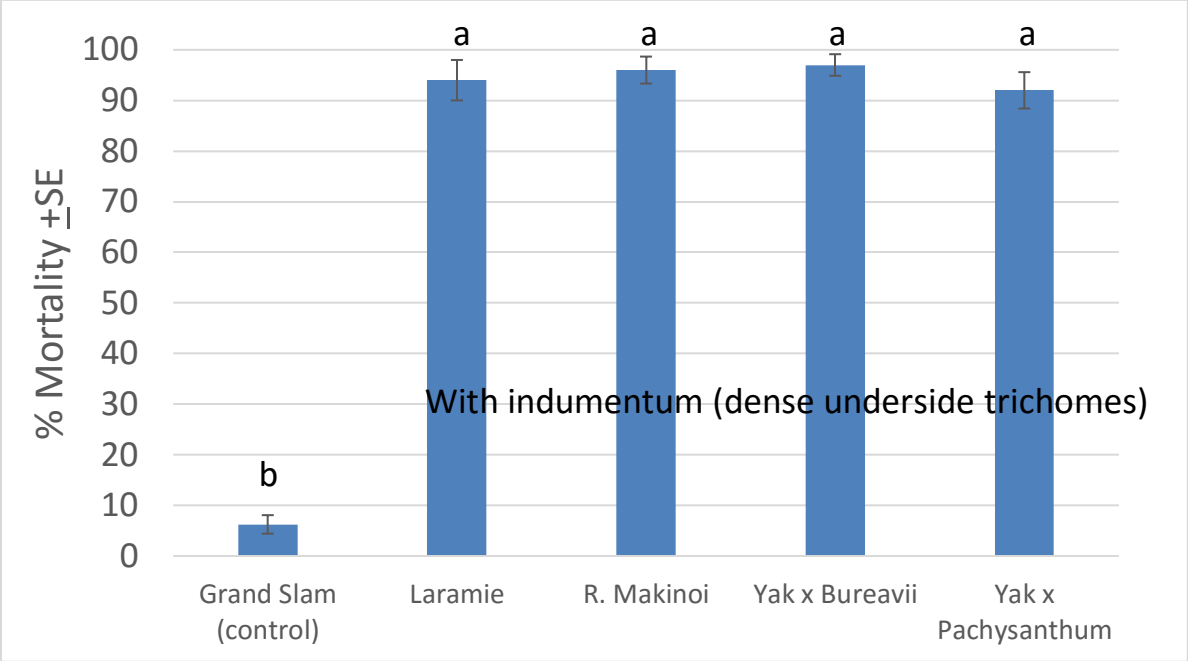

Figure 3.2 Average *S. pyrioides* mortality rate for each cultivar.

Chapter 4

Biological control of *Stephanitis pyrioides*

Salvador M. Flores, Barry Finley, Robin Rosetta, and Jana Lee

Introduction:

Hemiptera is a very large order and these insects are commonly referred to as true bugs. Within this order, there are many species that are agricultural and nuisance pests. One such group of hemipteran pests belong in the family Tingidae, known generally as lace bugs. In this family, the azalea lace bug, *Stephanitis pyrioides* Scott feeds on ericaceous plants. It is native to Japan and has been present on the East coast for almost 100 years (Shrewsbury & Smith-Fiola, 2000). It was recently detected in the Pacific Northwest (PNW), where it was found in Washington in 2008 and Oregon in 2009 (Rosetta, 2013). Rhododendrons and azaleas are important landscape plants, and as such are the most widely planted flowering shrub in the United States (Braman & Beshear, 1994). Since arriving from Japan, it has become a major pest of both rhododendrons and azaleas, becoming the most common pest afflicting these plants (Balsdon et al., 1996; Nair & Braman, 2012b; Rinehart & Boyd, 2006).

Although this pest is quite small (2.8 mm-3.3 mm), it can cause extensive damage when present in large numbers. *Stephanitis pyrioides* feed by inserting their stylets into the stomata on the ventral side of the plant's leaves and removing the chlorophyll contents within the leaves. This causes stippling damage on the upper side, and gives the feeding site a distinct yellow-white or pale discoloration, which leads to overall leaf discoloration. Feeding by *S. pyrioides* can cause chlorosis but can be distinguished from some other forms of chlorosis by the presence of dark brown feces and exuviae on the ventral side of the leaves (Balsdon et al., 1996; Nair & Braman, 2012b; Rinehart & Boyd, 2006). Heavy feeding can reduce photosynthesis and transpiration rates, therefore

negatively affecting plant vigor (Nair & Braman, 2012b) and in severe cases, causing plant death.

Because of the importance of rhododendrons and azaleas in the PNW as landscape and as plants in the natural environment, methods to control *S. pyrioides* populations have been investigate to deter the amount of damage inflicted on rhododendrons and azaleas. Traditionally, chemical treatments have been used, by studying different insecticidal compounds to control *S. pyrioides* populations. Some chemicals that have been used and tested are M-pede, Volck oil, organophosphates, neonicotinoids, bendiocarbs, pyrethroids with varying rates of success (Balsdon et al., 1993; . Braman et al., 2000; Held & Parker, 2011; Nair & Braman, 2012a; Shrewsbury & Smith-Fiola, 2000). However, some of these chemicals can be toxic to humans and other vertebrates as well as honey bees and other beneficial insects. Relying solely on chemical treatment could potentially reduce beneficial insect populations, therefore alternatives to reduce the use of chemicals will mitigate their residual effects on the environment.

As an alternative to chemical treatments, biological control can be a way to control *S. pyrioides* populations that also reduces deleterious effects from pesticide use. Biological control consists of three aspects, using predators, parasitoids, or pathogens as means to control an insect pest. Because this insect is a relatively new pest in the PNW, natural enemies reported to specifically consume *S. pyrioides* in this region are not known, control measures implementing augmentative biological control may help to reduce pest abundance. In past studies, *Chrysoperla carnea* Stephens was the primary chrysopid

predator used in biological control experiments with azaleas. One such experiment by Shrewsbury and Smith-Fiola (2000) tested optimal release rate and larval predation efficacy. In another study, Nair and Braman (2012a) found that integration of *C. carnea* with insecticides offered the best control of *S. pyrioides*. Another method of biological control is the use of pathogens. The study by Nair and Braman (2012a) also tested the effects of using the biopesticide Tick Ex, which contained *Metarhizium anisopliae* (Metchnikoff) Sorokin and found that Tick Ex alone significantly reduced *S. pyrioides* counts but was not as effective as synthetic pesticides.

A main objective of these experiments was to determine if a commercially available predator, *Chrysoperla rufilabris* Burmeister, can control *S. pyrioides* abundance on rhododendrons. While past studies often used *C. carnea*, *C. rufilabris* is the main species available now commercially. We tested the predation of *C. rufilabris* in a laboratory setting on azalea leaves, a nursery setting, on outdoor potted plants, and in an established garden. In the nursery setting, outdoor potted plant, and in the established garden. A secondary objective was to test the effectiveness of a plant volatile, methyl salicylate (MeSA). When synthetic lures are deployed on plants, they can attract generalist predators in the surrounding area which sometimes reduces pest abundance (Kaplan, 2012; Lee, 2010). MeSA has never been tested for *S. pyrioides* control nor for other pests of rhododendrons. Here, MeSA was examined to determine its contribution to pest control either alone or combined with releases of *C. rufilabris*. Enhanced control might be expected if both released predators and incoming predators consumed *S. pyrioides*. While MeSA attracts predators, it is not known whether predators exposed to this volatile

will increase, decrease or have similar feeding rates. Thus, part of this objective was to evaluate the feeding rate of *C. rufilabris* in the presence of MeSA volatiles. The final objective was to test the effects of an insect fungal pathogen spray *M. anisopliae,* now designated as *M. brunneum* Petch (Reddy et al., 2014), to determine if a commercial formulation applied on adult *S. pyrioides* causes mortality.

Methods

Chrysoperla rufilabris* feeding on *Stephanitis pyrioides. To determine the consumption rate of green lacewing larva, *C. rufilabris,* on the azalea lace bug, *S. pyrioides*, nymphs and adults, laboratory trials in small arenas were set up to measure *C. rufilabris* feeding over time. Two shipments of *C. rufilabris* larvae were used and *S. pyrioides* nymphs and adults were obtained from a greenhouse colony, with constant addition of wild *S. pyrioides*. The colony in the greenhouse had wild *S. pyrioides* added to rhododendron and azalea plants in 60 x 60 x 60 cm cages. All *C. rufilabris* used in this and all subsequent *C. rufilabris* experiments were supplied by Evergreen Growers (Clackamas, OR) and were 2nd stage instars. All *C. rufilabris* larvae were held in small individually enclosed cardboard cells, collectively called a hexcel unit, with a prepared provision of *Ephestia* Guenée (Lepidoptera:Pyralidae) eggs to reduce cannibalism and stored at 10°C.

Feeding arenas contained one *C. rufilabris* larva placed in with one of four stages of *S. pyrioides* (Table 4.1). A different number of individuals were used per instar trial depending on the size and availability of the pest stage to measure how much *C. rufilabris* might eat in a day. *Chrysoperla rufilabris* larvae were removed from the

hexcel unit for the trial and not starved, simulating how larvae would be used in common practice. Arenas were lockable 44 mm Petri dishes to keep the predator and prey from escaping and were placed with a dampened filter paper to provide moisture and an unidentified azalea leaf to provide food for *S. pyrioides*. All arenas were placed in an environmental chamber at 21°C and a range of 60-70% RH, with the exception of one trial placed in another environmental chamber, in which the temperature was at 20.6°C, due to the first chamber being cleaned at the time.

After the arenas were placed in the chamber, predation within arenas was checked twice. The first check, at 2 hours after placement, was to determine if any *S. pyrioides* were eaten by *C. rufilabris* larvae. The second check, after 1 day, was to determine how much they might eat and to obtain a daily consumption rate for each stage presented to *C. rufilabris*. A total of 82 replicates over 9 trials were completed with 17-26 replicates for the various stages (Table 4.1). Dates of the trials are listed in Table 4.1. This experiment was only concerned with determining consumption rate at each stage, therefore no statistical tests were performed. The average number of nymphs or adults eaten at both time checks were determined along with their standard errors.

***C. rufilabris* outdoor releases.** To determine whether *C. rufilabris* can control a natural infestation of *S. pyrioides*, trials were set up at the Jenkins Estate in Beaverton, OR. In this experiment, there were three treatment levels: control with no releases, larval releases and egg card releases. Initially, the protocol was to block by choosing groups of three plants of the same cultivars to assign the treatments. However, this protocol resulted in

too few replicates because only a few species or cultivars had multiple plants that were sufficiently infested. Upon closer examination, some of the damaged plants chosen that looked infested did not have any *S. pyrioides* upon inspection of leaves in laboratory checks. Instead, a combination of this protocol was used as well as using plants with high levels of damage. The cultivars used, which were all rhododendrons, in this experiment were six *R. macrophyllum* D. Don ex G. Don plants, three 'Blaney's Blue', three 'Ostbo's Low Yellow', two 'Elizabeth', two 'Anna Rose Whitney', one 'Grand Slam', one 'Everything Nice', one 'Odee Wright', one 'Gills Crimson', one 'Razzle Dazzle', and one unknown cultivar. In many cases, plants were in separate beds. For *R. macrophyllum* plants, all were situated in one large bed, where control plants were separated from larval or egg assigned plants by a buffer zone of 5 m of open space, or by at least two large plants with a space in between of ~ 7 m spacing to reduce predator spill over. In a previous study done by Shrewsbury and Smith-Fiola (2000) with *C. carnea* releases, they found significant differences between their controls and treatments spaced 1.5 m apart, thus the 5-7 m of spacing was considered to be adequate.

After all of the plants to be used in the trials were assigned a treatment, a pre-count at "week 0" for infestation levels was done before applying treatments. To do this, four notably infested leaves from each plant were collected and *S. pyrioides* were counted back in the laboratory under a microscope. After these initial leaves were collected, *C. rufilabris* larvae and eggs were released on their respective plants by attaching hexcel units or egg cards on a branch. Larvae were held in two cut rows of a hexcel unit, and

contained approximately 44 *C. rufilabris* larvae. Two egg cards were placed per plant, for a total of 333 per plant.

After the *C. rufilabris* releases were made, each plant was then sampled weekly for 4 weeks. Ten leaves were collected and all small (1^{st}-3^{rd} instars) and large (4^{th}-5^{th} instars) nymphs and adults of *S. pyrioides* were counted. Because *C. rufilabris* might eat smaller and larger nymphs at different rates, they were distinguished from each other during laboratory checks. During weeks 3 and 4, after *S. pyrioides* were counted, leaves were rinsed with warm water to clean off frass and unhatched eggs were counted under a microscope at 1.25-2.0X magnification. Trials ran from August 7, 2015 to September 11, 2015 and a total of 22 plants were used. The first 16 plants were initiated on August 7, and remaining 6 plants were initiated on August 14, 2015.

The number of small and large nymphs, adults and eggs of *S. pyrioides* were averaged on a per leaf basis. Data from two plants were excluded, as we later found that their initial infestation levels were zero, and subsequent infestation rates were extremely low and statistical tests were not affected by their exclusion. Control plants had a total of 6 replicates, whereas plants with larval and egg releases had 7 replicates per treatment. A separate repeated measures analysis was done per dependent variable (i.e., adults, eggs) with treatment, week, treatment x week interaction as fixed effects, and plant subject as a random effect. Analyses for this experiment and subsequent ones were done in JMP 11.0 (SAS, 2013)

MeSA effect on *C. rufilabris* feeding. To determine if an herbivore-induced plant volatile, methyl salicylate (MeSA), alters *C. rufilabris* feeding rate, feeding rates by *C. rufilabris* were monitored in the presence and absence of MeSA in outdoor stations. Materials used in this study included a synthetic MeSA lure PredaLure (AgBio, Westminster, CO). Prey in this study were pea aphids, *Acyrthosiphon pisum* Harris. *Acyrthosiphon pisum* nymphs were obtained from a greenhouse fava bean colony and were used because they were more readily available than *S. pyrioides* nymphs. Two different shipments of *C. rufilabris* larvae were used for both experimental arenas.

Four stations were set up around the Horticultural Crops Research Unit building, two serving as control stations without MeSA and two with a MeSA 2 g lure. Each station was spaced at least 25 m apart from another with building structures in between to minimize volatile overlap. Each station was also placed in a shaded area, to prevent any heat-induced mortality. In the two stations assigned a MeSA treatment, a MeSA lure was hung approximately 0.5 m aboveground to allow for air distribution. Stations assigned as a control treatment had a white card of similar size to the lure hung aboveground. The location of each station changed each day the trial took place, by finding a previously unused spot or if a spot was repeated with different treatment, there was a minimum of 5 days before the spot was used again. Location of each station was recorded each time.

In the first experiment, to compare consumption rates, small scale arenas were set up at MeSA and control stations. The arena was a 1 oz. plastic cup (Solo Cup Co., Lake Forest, IL), with each cup containing 5 *A. pisum* nymphs that were between 2^{nd}-4^{th} instars

and were placed with a fava bean leaf to feed on. A single 2nd instar *C. rufilabris* larva was placed in each plastic cup with the aphid nymphs. All *Chrysoperla rufilabris* larvae in this and subsequent experiments were not starved beforehand and were maintained with the *Ephestia* egg provisions as mentioned before. The cup was capped with a mesh lid, to allow the volatile to enter the arena. These arenas were placed within 5 cm of the MeSA lure or control paper. Trials took place between May 29 through June 19, 2015. There were 40 replicates per treatment and 1-4 replicates per station. Each cup was checked for *A. pisum* mortality at 1, 2, and 4 hours after *C. rufilabris* larvae were placed in the cup. Temperature and relative humidity were also recorded.

In the second experiment, to compare predation rate and small-scale search efficiency together, a larger arena was also set up at MeSA and control stations to show both predation rate and efficiency. The larger arena was a 32 oz. plastic cup (Solo Cup Co., Lake Forest, IL). Each cup contained 10 *A. pisum* nymphs of 2nd-4th instars on two fava bean leaves to feed on. One 2nd instar *C. rufilabris* larva was also placed inside each cup, and the cup was capped with a mesh lid to allow the volatile to enter. These arenas were placed at the same stations as the small arenas and placed within 15 cm of the MeSA lure. Temperature and relative humidity were also recorded. Unlike the smaller arenas, these were checked only once, after 5 hours. Larger arena trials took place from May 29 through June 19, 2015. The larger arena experiment had 40 replicates total and 1-2 replicates per station. Both stations and arena type set ups are depicted in Figure 4.1.

In both experiments, predation rates included *A. pisum* nymphs visibly eaten as well as nymphs missing. Missing aphids were included in predation rates, as some 5th instar *S. pyrioides* were entirely consumed in predation trials done in the lockable Petri dish trials. Predation rates in the first experiment were analyzed using repeated measures ANOVA with treatment and hour as fixed effects, and the batch (shipment) of *C. rufilabris* and arena subject as random effects. Predation rates in the second experiment were analyzed with an ANOVA test with treatment as a fixed effect and batch as a random effect. To simplify models, the effects of temperature, humidity, and interaction effects were removed from models due to their non-significance and outcomes were similar with or without their inclusion.

***C. rufilabris* and MeSA in a nursery.** To determine if a release of *C. rufilabris* larvae coupled with a plant volatile can reduce infestation by *S. pyrioides*, an experiment was set up at a commercial nursery. The nursery was located in Dayton, OR where *S. pyrioides* were naturally infesting potted rhododendron plants. For the experiment, one treatment was an augmentative release of 10 green lacewings (herein referred to as GLW in treatments), *C. rufilabris*, larvae per plant accompanied with a 2 g MeSA lure hung on the plant, and is referred to as GLW+MeSA. The control treatment were plants containing neither *C. rufilabris* nor MeSA. Twelve *S. pyrioides*-infested rhododendrons from three cultivars were chosen in this study: two 'Lee's Dark Purple' plants, two 'Cunningham's White' plants, and eight 'Anna Rose Whitney' plants, all being rhododendron plants. This provided 6 replicates each treatment. Plants were located in four hoop houses with 1-3 treatment pairs per house. Plants were randomly assigned a

treatment provided that at least one control and one GLW+MeSA plant were present in each hoop house. Treatments within a hoop house were located at least 10 m apart, as space was a limiting factor in this setup. This was to prevent movement of GLW between plants. While this spacing was not ideal in that MeSA volatiles may overlap to control plants, past studies have found higher captures of natural enemies among baited and unbaited traps spaced 10 m apart (James 2003).

Prior to release, each plant was visually inspected using 1.75X Opti-visors (BioQuip, Rancho Dominguez, CA) and all adults and nymphs from infested leaves were identified and counted to ensure that multiple life stages were present and there were sufficient numbers of *S. pyrioides* in each infested plant to use in this experiment. Selected plants had 3 to 25 visible *S. pyrioides* per plant. Treatment and control plants were assigned such that each treatment had a balanced starting number of *S. pyrioides* per leaf, with an average number of 5.2 ± 0.7 in control, and 4.9 ± 0.8 in GLW+MeSA. The total number of adult and nymph *S. pyrioides* on each leaf was recorded, providing a "pre-count" before *C. rufilabris* releases. Infested leaves were tagged for future examination.

C. rufilabris larvae were released August 21, 2014. Ten *C. rufilabris* larvae were added throughout the upper canopy of each treated plant with a wet fine-haired paint brush to lessen the chances of handling mortality, similar to a camel hair brush used in the study by Shrewsbury and Smith-Fiola (2000). Following the protocol from Shrewsbury and Smith-Fiola (2000), six days after release, on August 27, 2014, tagged leaves were re-inspected in the field, providing a visual post-count for adults and nymphs. The tagged

leaves were then collected for a "lab post-count" to measure the accuracy of the in-field visual post-count inspection. Because the counts made visually in-field differed from counts made in the lab on the same leaf, no further assessments were done visually in-field as they would not be highly accurate. Additionally, 24 leaves, two per plant, with evidence of lace bug feeding damage were randomly selected and examined for live lace bugs. The first two leaves with live lace bugs were collected, or if no live lace bugs were found, the last two leaves examined were collected to collect an infested leaf. For these 24 leaves, a maximum of ten leaves per plant were examined before taking the last two. In the laboratory, leaves were viewed under a microscope and the number of small nymphs, large nymphs, and adults were counted. Small nymphs were seen using 1.00-1.5X magnification, whereas large nymphs and adults were seen with the naked eye or at 0.63X magnification. Two, three, four, and six weeks after *C. rufilabris* release, three additional leaves were selected from each of the experimental plants using the same protocol as describe above, and the number of *S. pyrioides* were counted in the field and laboratory.

The impact of releasing *C. rufilabris* coupled with MeSA lures on *S. pyrioides*-infested plants was assessed by comparing the average density of *S. pyrioides* per leaf between control and GLW+MeSA for 6 days to 6 weeks post-*C. rufilabris* release, using the additional leaf counts. A repeated measures tested the number of *S. pyrioides* per leaf with treatment, week, and treatment x week interaction as fixed effects, and each plant subject as a random effect. Because the treatment x week interaction was significant,

each week was separated to compare treatments at 1, 2, 3, 4 and 6-week periods, assuming equal or unequal variances when appropriate.

Outdoor trials with *C. rufilabris* and MeSA combinations. To determine whether the application of methyl salicylate (MeSA) or release of GLW alone or in combination reduces *S. pyrioides* infestation, outdoor field trials with potted a rhododendron, *R. catawbiense* Michaux, were set up. First, potted plants were assigned to one of four treatments: control, MeSA only, GLW only, or GLW+MeSA. Each of the potted plants was infested with *S. pyrioides* approximately 3-4 weeks before beginning the experiment, to create highly visible infestation levels. Each potted plant had a pre-check at "week 0" by removing four to five leaves and counting the number of *S. pyrioides* adults and small and large nymphs per leaf. Small and large nymphs were differentiated to determine if GLW releases reduced one more than the other. Leaves with counted *S. pyrioides* were returned back to their respective plant so that pests were not removed from the plant and would be subject to biological control. *Stephanitis pyrioides* from the pre-checks were visibly counted and barely handled, to reduce any handling damage that may occur.

Trials were conducted at two sites: 1) outside the USDA ARS Horticultural Crops Research Unit (HCRU) in Corvallis, OR and 2) at the experimental USDA ARS North Farm, outside of Corvallis. At each site, four stations were set up at each location, corresponding to each treatment. Trials were repeated twice at HCRU, as the total number of infested plants was a limiting factor. In this experiment, more spacing was available at both sites, so stations were able to be spaced out more. Spacing between

MeSA-containing stations at the HCRU were at least 25 m apart from control or GLW station with buildings in between to prevent volatile drift. The MeSA station at the HCRU was at least 20 m apart from MeSA+GLW station to prevent movement of GLW between pots, and likewise for control and GLW stations. The stations at the North Farm were in an open agricultural field so each station was placed at least 200 m from the next nearest station, to prevent drift as well. Each plant had 15 *C. rufilabris* 2nd instar larvae placed throughout the upper canopy with a wet, fine-haired paint brush. After set up, weekly leaf collections were made from each potted plant, and the number of leaves collected at each potted plant varied depending on the plant's observed vegetation density. Five leaves were collected from foliage-sparse plants and 10 leaves were collected from foliage-dense plants. Each plant was sampled over 4 weeks. Similar to the pre-count, *S. pyrioides* small and large nymphs and total adults per leaf was counted for each potted plant and leaves with the counted *S. pyrioides* were returned to the plant during the first two weeks. For the last two weeks of sampling, leaves were kept to count unhatched eggs before discarding. *Stephanitis pyrioides* counted on these leaves were placed in a Petri dish and shaken back onto each respective plant. Unhatched eggs were counted to see if *C. rufilabris* larvae indirectly reduced egg numbers by reducing adult *S. pyrioides* numbers and therefore negatively affecting *S. pyrioides* reproduction rates. A repeated measures analysis was done with treatment and week, and their interaction as fixed effects, and the individual plant subjects as a random effect.

Effects of *Metarhizium* on *S. pyrioides*. To determine if *Metarhizium* (Metschn.) Sorokīn causes high *S. pyrioides* mortality, trials were performed to assess its efficiency

via direct spray. To do this, control and *Metarhizium* arenas were set up. Each arena was an 85 x 23 mm Petri dish, containing 10 adult *S. pyrioides*, five males and five females. *Metarhizium* dishes were sprayed, with Met52 EC (Novozymes Biologicals, Inc., Salem, VA), which contains an 11% solution of *Metarhizium anisopliae* strain F52 spores. *Metarhizium* dishes were sprayed with 2 mL of the Met52 EC solution. The Met52 EC solution was mixed the same day of spraying at a rate of 7.81 mL per liter of water according to this formula: (1fl.oz Met52 EC/gallon water X 29.57mL/fl. oz. X 1gal./3.785L). Control dishes were sprayed with 2 mL of sterile distilled water. Both control and *Metarhizium* sprays were sprayed at a pressure of 103.421 kPa or 15 PSI. Sprays were done with a Potter spray tower (Burkard Scientific, Uxbridge, UK), located at the Agricultural Life Sciences building of Oregon State University. Prior to spraying, *S. pyrioides* were chilled at or slightly below 0°C for 5-10 minutes to immobilize them during the spray and handling by grasping the wings with soft forceps, to avoid any bodily harm to the insect. Before the spraying treatments commenced, a potato dextrose agar (PDA) plate was sprayed with water as a negative control to an indicate whether fugal contaminants were present in the spray apparatus. Next, control plates were sprayed with sterilized distilled water, followed by *Metarhizium* plates. For a positive control, two PDA plates were sprayed with *Metarhizium* as an indicator that the solution had viable spores. All Petri dishes with *S. pyrioides* had a filter paper placed inside them prior to spraying to absorb excess spray residue and prevent drowning. Afterwards, the Potter tower was cleaned with acetone sprays to prevent residual *Metarhizium* build up for subsequent trials or usage.

Following spraying, *S. pyrioides* from each replicate were transferred into a new arena for monitoring. The arena consisted of a 32 oz. clear plastic container with a mesh lid, with an unidentified azalea cutting in a 5 oz. plastic cup filled with water (both Solo Cup Co., Lake Forest, IL). Then each arena was placed in an incubator at 26°C with 16:8 L:D and 40-50% RH. Each arena was checked for *S. pyrioides* mortality three times a week for 2 weeks for a total of seven checks. If mortality was observed, dead *S. pyrioides* from *Metarhizium* sprays were subsequently placed in small 56 mm Petri dishes with wet filter papers to retain moisture. These were then placed in a separate incubator at 26°C to promote fungal growth with a 0:24 L:D photoperiod and 40-50% RH. Petri dishes were checked after approximately two weeks for sporulation. Fifteen replicates were done for each treatment over three spray dates: August 5, August 12, and August 21, 2015. The total number of *S. pyrioides* sporulated and not sporulated as well as percentage sporulated and not sporulated were compared graphically. A repeated measures test compared the percent mortality with treatment, day, and treatment x day interaction as fixed effects, and the spray trial date and arena subject as random effects. Percent was arc-sin transformed.

Results

***C. rufilabris* feeding on *S. pyrioides*.** After the first 2 hours, *C. rufilabris* larvae consumed, on average, 2.72 3rd instars, 1.12 4th instars, 0.95 5th instars, and 0.07 adult *S. pyrioides* based on the 6, 7, 5, and 3-5 individuals presented to *C. rufilabris*, respectively (Figure 4.2). *Chrysoperla rufilabris* consumed numerically more 3rd instars than all other life stages. After one day, *C. rufilabris*, on average, consumed 5.06 3rd instars, 4.71 4th

instars, 3.30 5th instars, and 0.58 adult *S. pyrioides*. Adults were seldom consumed: over both time periods, wherein only half of the replicates (12) did *C. rufilabris* consume adult *S. pyrioides*.

C. rufilabris outdoor releases. The total number of *S. pyrioides* per leaf was numerically lower in both the larval and egg card releases than the control plants, but they were not significantly different by treatments nor treatment x week interactions (Table 4.2, Figure 4.3). The numbers of nymphs and adults also did not vary by treatment (Table 4.2). Given that the starting density of *S. pyrioides* per plant was numerically higher among control plants at week 0, *post*-hoc analyses were done with starting density as a covariate in repeated measures analyses. These results were also similar (not reported). This shows that our release rates of *C. rufilabris* eggs or larvae had no statistically significant impact on *S. pyrioides* populations in established rhododendrons. There was also no significant difference in the number of eggs per leaf in either larval or egg release treatments when compared to the control plants (Table 4.2, Figure 4.4). Thus, the experimental releases of *C. rufilabris* had no detectable effect on *S. pyrioides* reproduction.

MeSA effect on *C. rufilabris* feeding. While the percent of *A. pisum* consumed by *C. rufilabris* was numerically higher in MeSA than control treatment, there was no significant difference between treatments (treatment: $F_{1,77.43} = 1.60$, $p = 0.21$; hour: $F_{2,145.9} = 34.99$, $p < 0.0001$; Figure 4.5). While predation rates in the 32 oz. cups of MeSA treatments were also numerically higher than in control, there was no significant difference by treatment ($F_{1,72} = 1.32$, $p = 0.25$; Figure 4.6). Thus, *Chrysoperla rufilabris*

predation rates and search efficacy together were not affected by the presence of MeSA in their area.

***C. rufilabris* and MeSA in a nursery.** Coupling MeSA and *C. rufilabris* larvae together showed an overall significant difference between the treatments (Figure 4.7; treatment $F_{2,10} = 7.048$, $p = 0.024$). Furthermore, our results also showed a significant difference in the number of *S. pyrioides* counted per week and a significant treatment x week interaction (week $F_{4,40} = 3.79$, $p = 0.010$; treatment*week $F_{4,40} = 3.58$, $p = 0.013$). To further examine treatment x week interactions, GLW+MeSA and control plants were compared each week. During the first two weeks, exposure to GLW+MeSA significantly reduced *S. pyrioides* counts (Week 1: t-ratio = -3.84, $p = 0.003$; Week 2: t-ratio = -2.78, $p = 0.03$). GLW+MeSA plants showed reductions of *S. pyrioides* by 78.6%, and 85.9% during week 1 and 2, respectively. During weeks 3, 4, and 6 there was no significant difference in the number of *S. pyrioides* counted per leaf between treatments ($p = 0.15$, 0.94, and 0.61, respectively).

Outdoor trials with *C. rufilabris* and MeSA combinations. The average number of *S. pyrioides* collected per leaf in all of the treatments (GLW, GLW+MeSA, and MeSA) did not differ significantly from the control (Table 4.3, Figure 4.8). A release of 15 *C. rufilabris* per plant did not reduce *S. pyrioides* counts at a higher rate when exposed to MeSA, nor did MeSA alone reduce average *S. pyrioides* collected per leaf. There was also no significant difference when separated by life stage (egg, nymphs, adults; Table 4.3), which also suggests that *C. rufilabris* and MeSA did not significantly reduce

numbers of any one life stage collected per leaf. While there wasn't a significant reduction, all treatments were numerically lower than the control (Figure 4.8; Figure 4.9).

Effects of *Metarhizium* on *S. pyrioides*. Spraying *S. pyrioides* adults with *M. anisopliae* (Met52 EC) resulted in numerically higher deaths, and killed marginally more adults than spraying with just water, but results were not statistically significant (treatment: $F_{1,17}$ = 3.21, p = 0.091; Figure 4.10). Spraying *S. pyrioides* with *Metarhizium* resulted in a mortality rate of ~83%, while water sprays resulted in ~75% mortality rate. High mortality rates with water sprays may be the result of the fragility of the insect, as 103.421 kPa (15 PSI) may have caused traumatic damage. In *Metarhizium* sprays, a total of 77 died out of the total of 92 adults used in the trials. Of these, 60 out of the 77 sporulated, corresponding to ~78% of adult *S. pyrioides* showing visible sporulation, suggesting that *Metarhizium* can have a high rate of infection in adult *S. pyrioides*.

Discussion

Laboratory studies showed that *C. rufilabris* larvae predate on more *S. pyrioides* nymphs than adults. This may be due to the nymphs generally moving more slowly than adults and the flightlessness of nymphs. In observing *C. rufilabris* attacking an adult *S. pyrioides*, I noticed the adult dropped from the leaf of a cutting, allowing it to escape predation. However, it is not conclusive that this is how adults evade predation, as it was observed only once. *Chrysoperla rufilabris* also had difficulty grasping adults due to the wings the main body. This may be why nymphs, especially the smaller nymphs, are predated upon at a higher rate. Smaller nymphs (1^{st}-3^{rd}) are much less mobile than the

larger nymphs (4^{th}-5^{th}) which may be why 3^{rd} instars were, on average, predated on in higher numbers than 4^{th} or 5^{th} instars. It is also possible that due to the smaller size of 3^{rd} instars, *C. rufilabris* had to consume more to meet nutritional needs.

In 2014, we got promising results at a commercial nursery when using *C. rufilabris* larvae combined with MeSA during the first two weeks after release. Results showed that *S. pyrioides* numbers per leaf were significantly reduced in MeSA+GLW versus the control plants for weeks 1 and 2, with a reduction of up to 85.9% of *S. pyrioides* after the second week. This reduction over the first two weeks was likely due to the developmental rate of *C. rufilabris* larvae, as the larval stage lasts about 11 to 16 days when fed live prey at 25°C and 20°C, respectively (Nino & Cave, 2015). After larval development and pupation, adults may leave the area, explaining why long-term control was not observed. Because of this most beneficial insect suppliers recommend repeated releases every 1-3 weeks (Vinje, 2012). However, this experiment did not separate whether *C. rufilabris* alone was causing *S. pyrioides* reduction or if MeSA had a complementary effect in lowering pest counts as well as attracting other predators. The laboratory study with MeSA and *C. rufilabris* predation on *A. pisum* aphids showed that predation was similar in the presence or absence of MeSA. To follow up on the 2014 experiment, in 2015 a more in-depth study was done to examine whether *C. rufilabris* alone was reducing counts of *S. pyrioides* or if MeSA also had an impact. However, none of the combinations (GLW, GLW+MeSA, and MeSA) significantly reduced pest counts per leaf versus the control plants. There was no evidence that MeSA attracted or retained *C. rufilabris* on rhododendrons.

In 2015, a larger area was used at Jenkins Estate in Beaverton, Oregon to test the efficacy of larval and egg releases of *C. rufilabris*. While we did find that resulting *S. pyrioides* per leaf was numerically lower with both egg and larval releases, neither were significantly different from the control after four weeks. Releases of eggs and larvae at a rate of 333 eggs (~196 hatched) and 44 larvae per plant was not sufficient for *S. pyrioides* pest counts on a large scale. In comparison, the study by Shrewsbury & Smith-Fiola (2000) tested 5 to 20 *C. carnea* larvae with 40 or 80 *S. pyrioides* nymphs per azalea plant and found that having a higher predator-to-prey ratio significantly influenced *S. pyrioides* mortality. In our studies, *S. pyrioides* populations were visually obvious in the private garden and the deliberately infested potted plants in 2015 than in the commercial nursery in 2014. While we do not know the predator:prey ratios in each of the trials, we suspect that the release of predators in 2015 was not sufficient for the level of *S. pyrioides* infestation, and thereby explain the lack of treatment effect.

Using the entomopathogenic fungi, *M. anisopliae*, we obtained a mortality rate of ~83%. However, water sprays gave slightly lower but similar mortality rates, therefore mortality by water versus *Metarhizium* was not significantly different. Although mortality rates were similar, we showed that *M. anisopliae* had a relatively high infection rate of ~78% of the adults that died in *Metarhizium* sprays, showing that adults are susceptible to infection. While this study shows that *S. pyrioides* is susceptible to *M. anisopliae*, it did not cause mortality at high enough rates to justify the cost of purchasing the pathogen, as

water sprays had a similar effect. Further trials with water sprays and unsprayed controls are needed to verify this.

While there was some pest reduction found following the release of *C. rufilabris*, further studies are needed. Future predator release trials may need to be carefully timed to coincide with the nymphal stage of the pest, when they are more vulnerable to *C. rufilabris* predation. Studies examining the optimal release of *C. rufilabris* eggs and larvae for sufficient control, below economic damage thresholds, would provide valuable information for *S. pyrioides* management. Studies examining the efficacy of other lacewings, such as the brown lacewing, *Sympherobius barberi* Banks, may be promising. Since these lacewings are predaceous at both the adult and larval stages, they could be more effective than *Chrysoperla* spp. as the adults may feed on *S. pyrioides* adults controlling all stages of *S. pyrioides*. Given that water sprays of *S. pyrioides* in the *Metarhizium* trials yielded high mortality, further studies are warranted to examine water pressure, such as using a high pressure backpack sprayer as a lace bug control technique. However, this would require sprays on the underside of the leaves, which may not be practical. Initial water spraying on the underside of the leaves followed with *C. rufilabris* releases could complement each other and decrease pest abundance. Water sprays may initially clean off the plants, and *C. rufilabris* releases may keep pest pressure low. This might be a convenient method for both homeowners and growers.

Literature cited:

Balsdon, J. A., Braman, K., & Espelie, K. E. (1996). Biology and ecology of *Anagrus takeyanus* Gordh (Hymenoptera:Mymaridae), an egg parasitoid of the azalea lace bug (Heteroptera:Tingidae). *Environmental Entomology, 25*(2), 383-389.

Balsdon, J. A., Braman, S. K., Pendley, A. F., & Espelie, K. E. (1993). Potential for integration of chemical and natural enemy suppression of azalea lace bug (Heteroptera:Tingidae). *Journal of Environmental Horticulture, 11*(4), 153-156.

Braman, Latimer, J. G., Oetting, R. D., McQueen, R. D., Eckberg, T. B., & Prinster, M. (2000). Management strategy, shade, and landscape composition effects on urban landscape plant quality and arthropod abundance. *Journal of Economic Entomology, 93*(5), 1464-1472. doi:Doi 10.1603/0022-0493-93.5.1464

Braman, Pendley, A. F., Sparks, B., & Hudson, W. G. (1992). Thermal requirements for development, population trends, and parasitism of azalea lace bug (Heteroptera:Tingidae). *Journal of Economic Entomology, 85*(3), 870-877.

Held, D. W., & Parker, S. (2011). Efficacy of soil applied neonicotinoid insecticides against the azalea lace bug, *Stephanitis pyrioides*, in the landscape. *Florida Entomologist, 94*(3), 599-607.

James, D.G. (2003) Synthetic herbivore-induced plant volatiles as field attractants for beneficial insects. Environmental Entomology 32:977-982.

Kaplan, I. (2012). Attracting carnivorous arthropods with plant volatiles: The future of biocontrol or playing with fire? *Biological Control, 60*(2), 77-89. doi:DOI 10.1016/j.biocontrol.2011.10.017

Lee, J. C. (2010). Effect of Methyl Salicylate-Based Lures on Beneficial and Pest Arthropods in Strawberry. *Environmental Entomology, 39*(2), 653-660. doi:10.1603/EN09279

Nair, S., & Braman, S. K. (2012a). Integration of insecticides with the natural enemy *Chrysoperla carnea* Stephens for management of azalea lace bug (Hemiptera:Tingidae). *Journal of Entomological Science, 47*(3), 278-281.

Nair, S., & Braman, S. K. (2012b). A scientific review on the ecology and management of the azalea lace bug *Stephanitis pyrioides* (Scott) (Hemiptera:Tingidae). *Journal of Entomological Science, 47*(3), 247-263.

Nino, A. A., & Cave, R. D. (2015). Suitability of Microtheca ochroloma (Coleoptera: Chrysomelidae) for the Development of the Predator Chrysoperla rufilabris (Neuroptera: Chrysopidae). *Environmental Entomology, 44*(4), 1220-1229. doi:10.1093/ee/nvv084

Reddy, G. V., Zhao, Z., & Humber, R. A. (2014). Laboratory and field efficacy of entomopathogenic fungi for the management of the sweetpotato weevil, *Cylas formicarius* (Coleoptera:Brentidae). *Journal of Invertebrate Pathology, 122*, 10-15.

Rinehart, T. A., & Boyd, D. W. (2006). Rapid, high-throughput detection of azalea lace bug (Hemiptera:Tingidae) predation by *Chrysoperla rufilabris* (Neuroptera:Chrysopidae), using fluorescent-polymerase chain reaction primers. *Journal of Economic Entomology, 99*(6), 2136-2141.

Rosetta, R. (2013). Azalea lace bug biology and management in commercial nurseries and landscapes (2013 ed.): Oregon State University.

SAS. (2013). *JMP® 11.0.0*. Cary, NC: SAS Institute Inc.

Shrewsbury, P. M., & Smith-Fiola, D. C. (2000). Evaluation of green lacewings for suppressing azalea lace bug populations in nurseries. *Journal of Environmental Horticulture, 18*(4), 207-211.

Vinje, E. (2012, December 08). The Good Bugs: Beneficial Garden Insects | Planet Natural. Retrieved July 15, 2016, from http://www.planetnatural.com/good-bugs/

Table 4.1 Experimental set up of the arenas and life stage used for *C. rufilabris* consumption

S. pyrioides Stage	Number of Individuals per arena	Replicate Arenas	Dates
3rd	6	18	July 10-28, 2015
4th	7	17	July 13-27, 2015
5th	5	21	July 10-27, 2015
Adult	3-5	26	June 19-July 27, 2015

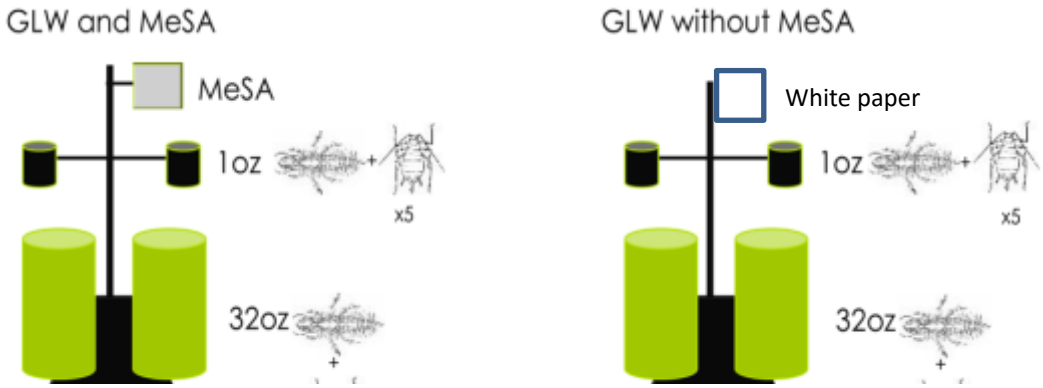

Figure 4.1 Each station's configuration with MeSA treatment and without (control) and the position of each arena relative to the MeSA lure.

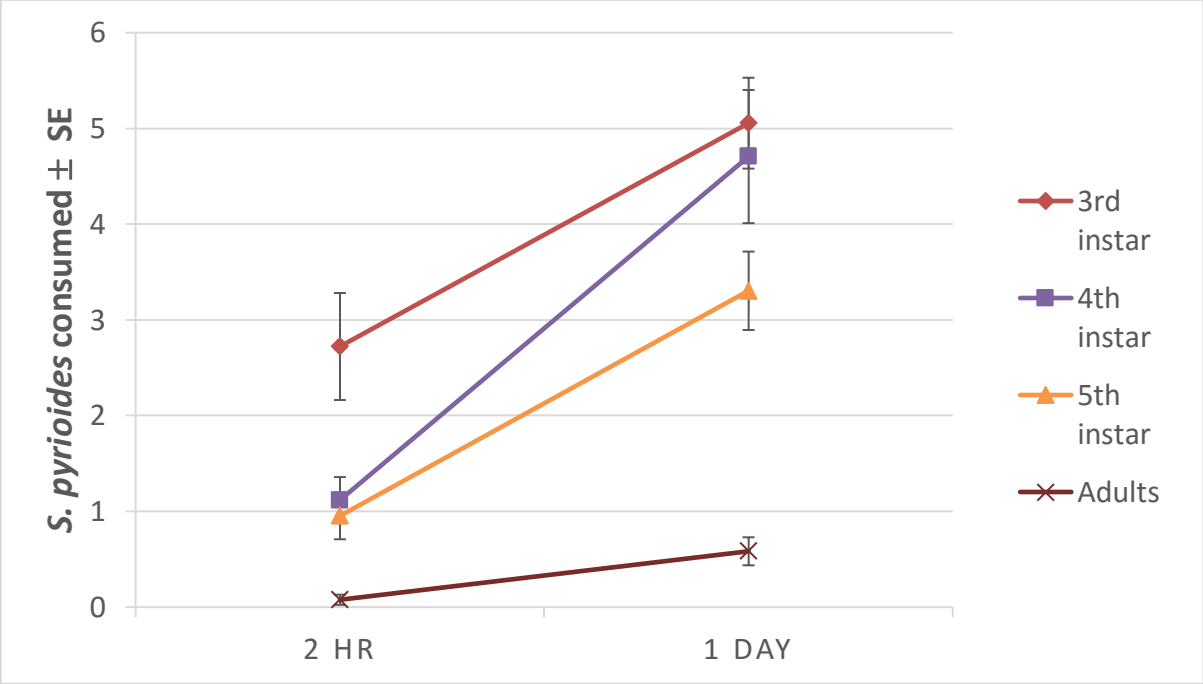

Figure 4.2 Average *C. rufilabris* consumption rates of *S. pyrioides* life stages.

Table 4.2 Statistical outcomes with repeated measures, significance is at p <0.05

Dependent variable	Effect	ndf, ddf	F	p-value
Total *S. pyrioides*	Treatment	2, 17	1.86	0.186
(nymphs + adults)	Week	3, 51	1.58	0.204
	Treatment*week	6, 51	1.85	0.108
S. pyrioides nymphs	Treatment	2, 17	2.39	0.122
	Week	3, 51	0.494	0.688
	Treatment*week	6, 51	1.26	0.291

S. pyrioides adults	Treatment	2, 17	0.979	0.396
	Week	3, 51	2.43	0.076
	Treatment*week	6, 51	1.67	0.147
S. pyrioides eggs	Treatment	2, 11.14	1.80	0.210
	Week	3, 51	1.58	0.204
	Treatment*week	6, 51	1.85	0.108

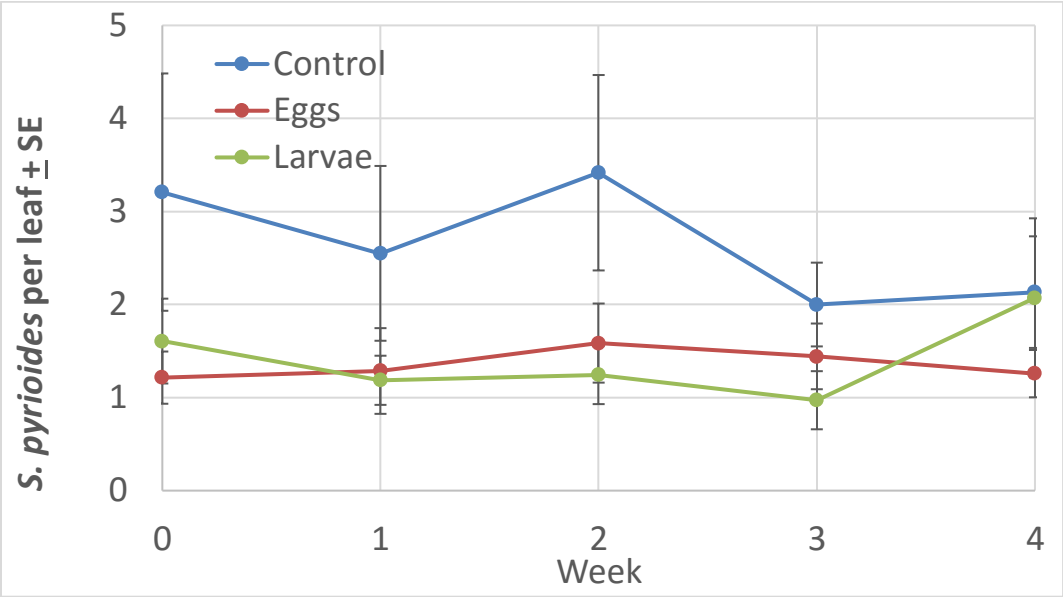

Figure 4.3 The average number of *S. pyrioides* (nymphs + adults) per leaf in the three treatments.

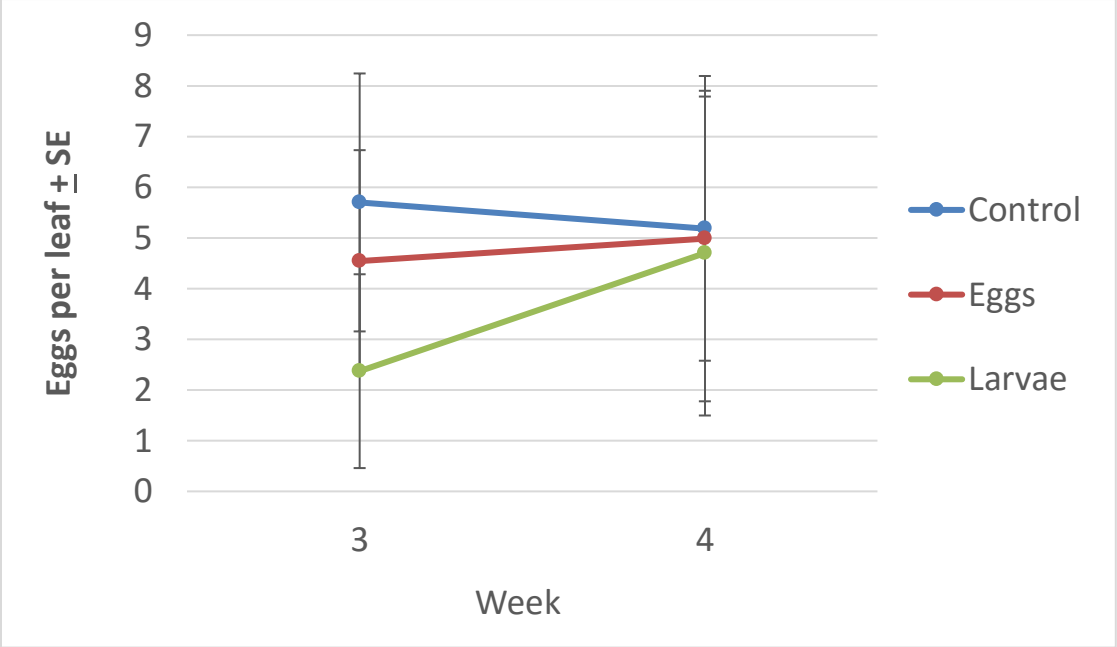

Figure 4.4 The average number of *S. pyrioides* eggs per leaf over the last two weeks for all three treatments.

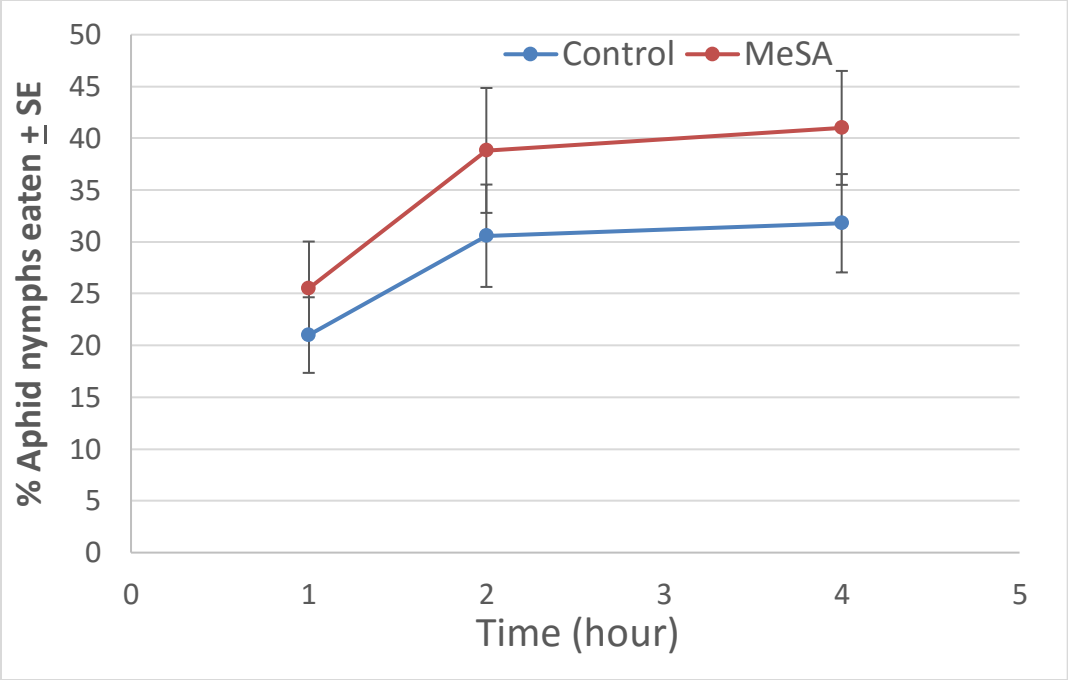

Figure 4.5 Percentage of *A. pisum* nymphs eaten by *C. rufilabris* at each hour in the 1 oz. plastic cups.

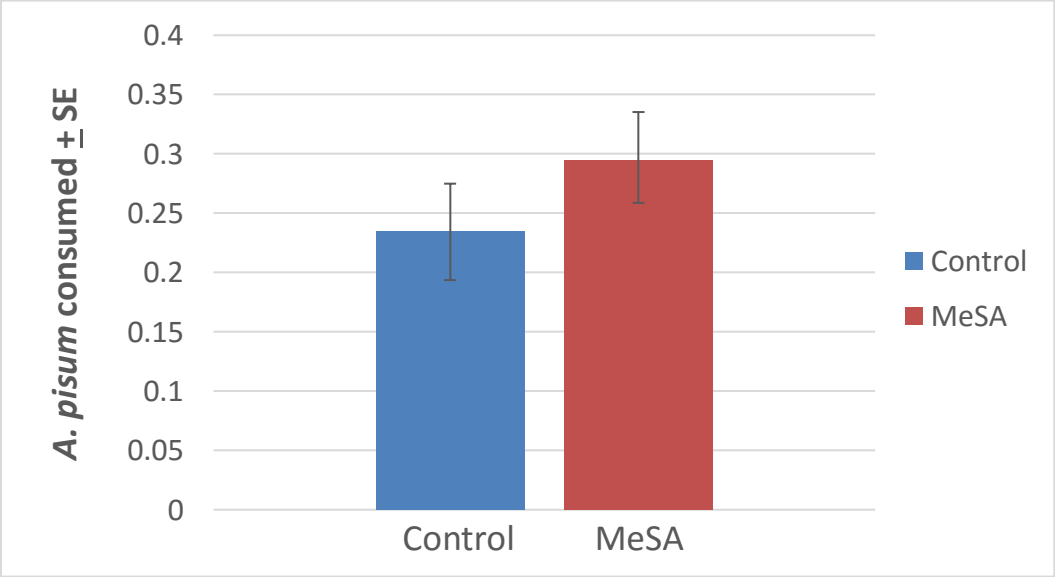

Figure 4.6 Percent of *A. pisum* nymphs eaten by *C. rufilabris* in 32 oz. cups after 5 hours.

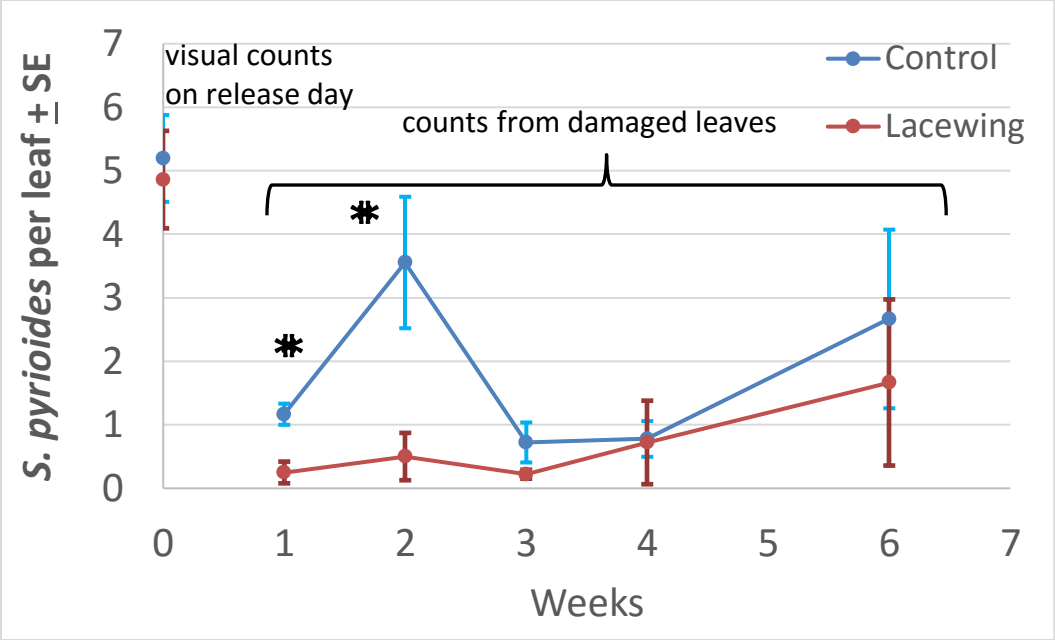

Figure 4.7 Average *S. pyrioides* counted per leaf, asterisks show a significant difference at each week ($p < 0.05$).

Table 4.3 Statistical results of the average number of *S. pyrioides* counted per leaf

Dependent variable	Effect	ndf, ddf	F	p-value
Total *S. pyrioides*	Treatment	3, 20	0.662	0.585
(nymphs + adults)	Week	3, 60	9.54	<0.0001
	Treatment*week	9, 60	1.28	0.266
S. pyrioides nymphs	Treatment	3, 19	0.781	0.519
	Week	3, 60	4.92	0.004
	Treatment*week	9, 60	1.27	0.272
S. pyrioides adults	Treatment	3, 60	0.448	0.721
	Week	3, 60	8.12	0.0001
	Treatment*week	9, 60	1.22	0.299
S. pyrioides eggs	Treatment	3, 19	0.871	0.473
	Week	1, 20	0.094	0.762
	Treatment*week	3, 20	0.321	0.81

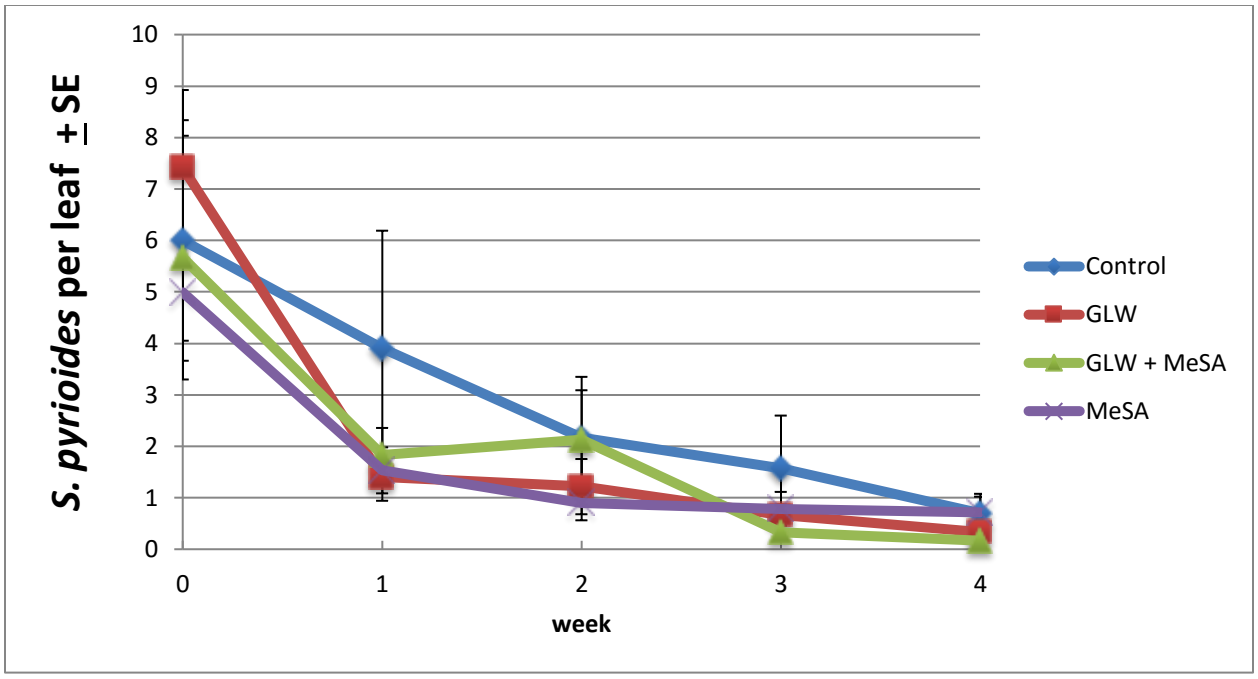

Figure 4.8 Average counts of *S. pyrioides* per leaf for four weeks' after release.

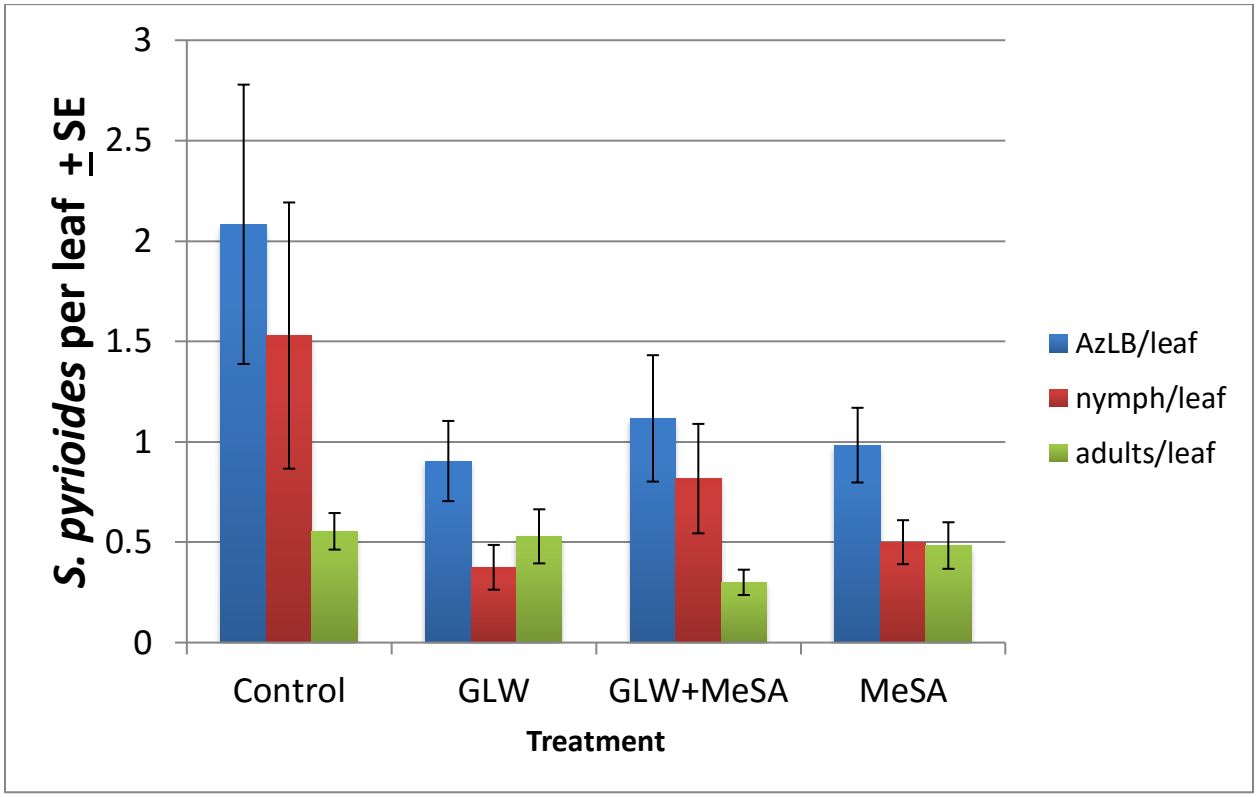

Figure 4.9 Average *S. pyrioides* per leaf after 4 weeks post-release.

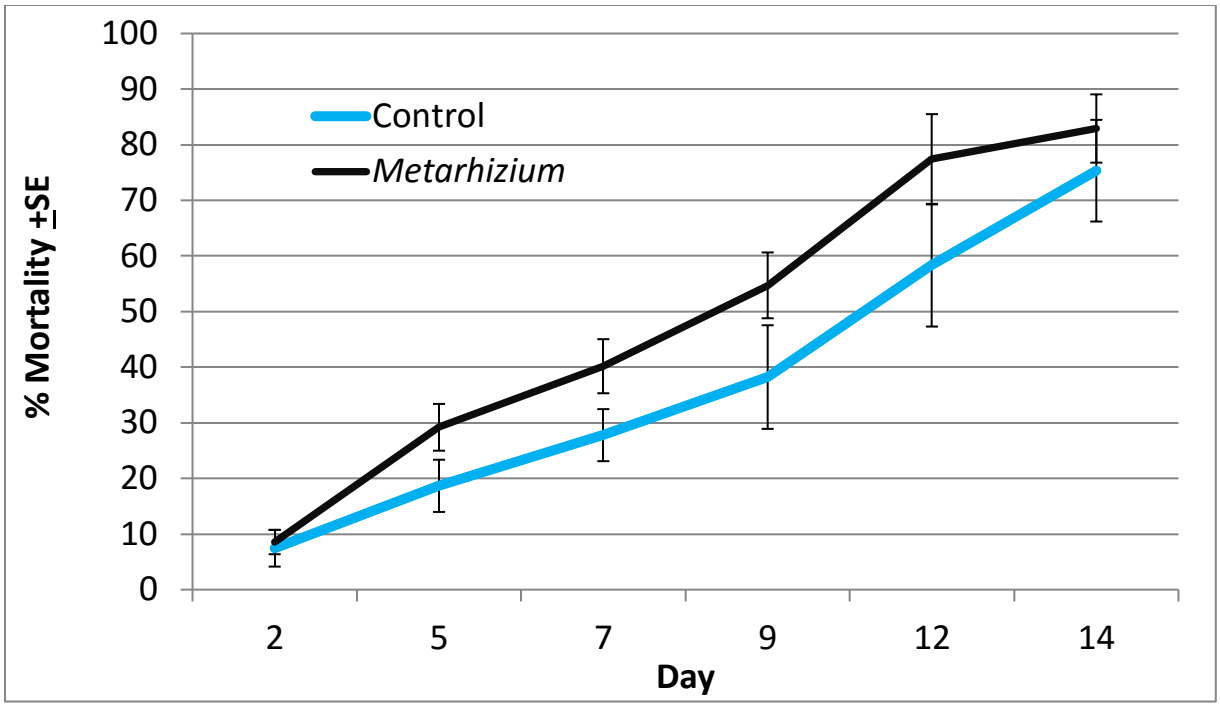

Figure 4.10 Mortality rate of *S. pyrioides* in both spraying regimes.

Chapter 5:

Conclusion

Salvador M. Flores

The Pacific Northwest (PNW) is home to several native rhododendrons and azaleas, and other *Rhododendron* spp. brought in from elsewhere, as well as other ericaceous plants. The recent introduction of the azalea lace bug, *Stephanitis pyrioides* Scott, has become a major concern since its detection in Oregon in 2009 (Rosetta, 2013) due to the damage it causes by feeding. Azaleas and rhododendrons are very floriferous plants, and are therefore sought after in landscapes and other public and private green spaces. However, *Rhododendrons* are, generally, pest-prone and damage by *S. pyrioides* is aesthetically displeasing to growers and consumers. This damage causes a refusal to purchase any noticeably damaged rhododendrons (Balsdon et al., 1996; Braman & Beshear, 1994; Klingeman et al., 2000; Klingeman et al., 2001; Shrewsbury & Raupp, 2000). This is due to the visual damage caused by the insect, as the upper side of the leaves have a stippled appearance that superficially resembles iron chlorosis. This stippled appearance is caused by feeding. *S. pyrioides* by which they feed by inserting their stylets on the underside of the plant's leaves, entering through the stomata and removing the chlorophyll contents within the leaves (Nair & Braman, 2012b). Aesthetic damage is also apparent on the underside of the leaves, there are many fecal deposits as well as nymphal and adult *S. pyrioides.* Besides the aesthetic damage that *S. pyrioides* causes, it also causes permanent damage to the plant. It has been shown to negatively affect net leaf photosynthesis, which can reduce plant photosynthetic capacity, as well as negatively affect plant vigor (Buntin et al., 1996; Nair & Braman, 2012b), with severe damage causing plant death. This reduction in both photosynthesis and vigor may reduce the number of flowers produced per plant, thereby further reducing their aesthetic quality. However, damage is not limited to *Rhododendron* species. LaBonte and Valente (2014)

reported several new host records including salal, *Gaultheria shallon* Pursh, which has also been noted in our field observations and is a native to western North America.

Because this insect is a recently invasive pest, the voltinism and generation time is not known for the PNW. Monitoring this insect over two years has provided us with important information to predict when the first generation will emerge in the PNW (April-May) and we also found that they persist, notably the adults, throughout the year and into the winter. This monitoring has also been important in cataloging natural enemies in the same area, which have been shown to be in low numbers in our sampling. This in part may explain the high level of damage seen on some rhododendrons, along with other factors such as the weather, species and cultivars present, and host abundance. Using the degree-day developmental data from previous studies (Braman et al. 1992; Neal and Douglass 1988) we estimated 3.5 generations per year in the PNW, a similar number to three generations reported from the East Coast. This data will prove most useful when implementing control strategies to target a certain life stage(s) to effectively reduce lace bug populations and mitigate the damage to host plants.

Several studies have looked at cultivar resistance. The studies done by Chappell et al. (2004), Chappell et al. (2005) and Chappell and Robacker (2006) all examined azalea epicuticular wax and found that wax was a main mechanism of resistance after applying wax from resistant types to susceptible types and finding increased resistance to feeding and oviposition. Balsdon et al. (1995) looked at epicuticular composition and found two triterpenoids that were present in lower levels in more susceptible azaleas. Braman and Pendley (1992) examined deciduous azaleas and found that deciduous were preferred less

over evergreen azaleas in early season emergence of *S. pyrioides*. However, deciduous azaleas have been observed to be heavily attacked here, noted from personal observations and as reported by LaBonte (pers. comm.). Schultz (1993) looked at host-plant acceptance and percent damage and compiled a list of 20 azaleas noting each cultivar's leaf damage and mean number of eggs per cutting. He found that 'Macrantha' had the lowest leaf damage and number of eggs. Our study observed both azaleas and rhododendrons in natural and nursery type settings and compiled two lists of every rhododendron and azalea that was identifiable (uninfested, Table 3.1; infested, Table A.3). We also confirmed that four different rhododendrons with heavy indumentum were resistant to *S. pyrioides* feeding in laboratory testing along with field observations. Of these four, three had parentages from *R. yakushimanum* Nakai, giving evidence that indumentum is a mode of resistance. These two lists, as well as our laboratory study will provide valuable information and recommendations for azaleas and rhododendrons to grow with the least vulnerability to *S. pyrioides* feeding.

Because this insect is a recently invasive pest and lacks indigenous natural enemies in the PNW and the two exotic natural enemies (*Stethoconus japonicus* Schumacher, and *Anagrus takeyanus* Gordh), it has caused an extensive amount of damage in a short time, raising concern for its control. Traditionally, chemical treatment is the first option for most pests. But many of these chemicals used in studies with *S. pyrioides* (Balsdon et al., 1993; . Braman et al., 2000; Held & Parker, 2011; Nair & Braman, 2012a; Shrewsbury & Smith-Fiola, 2000), are toxic to other animals, including beneficial insects. They can also be harmful to humans, and because this is a landscape/garden plant, humans could

potentially be constantly exposed to these chemicals if used to treat *S. pyrioides* infestations. So, alternatives must be explored. This work explored these alternatives, with a commercially available predator, *Chrysoperla rufilabris* Burmeister, a green lacewing, which was also used by Stewart et al. (2002), as well as using a novel approach with methyl salicylate (MeSA). While we found promising results in 2014, these were not reflected in 2015 trials, although lace bug counts were numerically lower in *C. rufilabris* releases than their respective controls, no results showed statistical significance. Therefore, more studies must be done to examine the effect of *C. rufilabris* and MeSA as control measures. Most of the past studies also focused on the more shrub-like azaleas, whereas ours focused on what is commonly referred to as rhododendrons, which have larger and sparser distribution of leaves. There are no known pathogens that specifically target *S. pyrioides*. We tested a commercially available product, Met52 EC, but found that percent mortality was similar to spraying lace bugs with water. However, examining other commercially available entomopathogenic fungi, such as *Beauveria bassiana* (Bals.) Vuill., may be a promising method of control.

Stephanitis pyrioides is an economically important insect in rhododendrons and azaleas, because it can be costly to treat these plants. Klingeman et al. (2000) reported that professionals and consumers had a low tolerance threshold for purchasing *S. pyrioides*-damaged azaleas, where damage levels at as little as 1.03% actual injury prompted half of the respondents to reject the purchase of an azalea. This survey also showed that at 3.3% actual damage, half of the responents would initiate treatment to control lace bugs. These "quick to pass up, quick to treat" thresholds may exacerbate the economic impact *S.*

pyrioides can have. While chemical treatment is the current method of control, this work demonstrates the great need for more alternatives to control *S. pyrioides*. The use of biological control can reduce the chemical footprint in public green spaces as well as a way to reduce control costs, watershed contamination and immediate and residual chemical effects to beneficial non-target insects.

Bibliography

Avery, P. B., Kumar, V., Simmonds, M. S. J., & Faull, J. (2015). Influence of leaf trichome type and density on the host plant selection by the greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera:Aleyrodidae). *Applied Entomology and Zoology*, 50(1), 79-87. doi:10.1007/s13355-014-0308-5

Balsdon, J. A., Braman, K., & Espelie, K. E. (1996). Biology and ecology of *Anagrus takeyanus* Gordh (Hymenoptera:Mymaridae), an egg parasitoid of the azalea lace bug (Heteroptera:Tingidae). *Environmental Entomology, 25*(2), 383-389.

Balsdon, J. A., Braman, S. K., Pendley, A. F., & Espelie, K. E. (1993). Potential for integration of chemical and natural enemy suppression of azalea lace bug (Heteroptera:Tingidae). *Journal of Environmental Horticulture, 11*(4), 153-156.

Balsdon, J. A., Espelie, K. E., & Braman, S. K. (1995). Epicuticular lipids from azalea (*Rhododendron* spp.) and their potential role in host-plant acceptance by azalea lace bug, *Stephanitis pyrioides* (Heteroptera:Tingidae). *Biochemical Systematics and Ecology, 23*(5), 477-&. doi:10.1016/0305-1978(95)00055-y

Bonani, J., Fereres, A., Garzo, E., Miranda, M., Appezzato-Da-Gloria, B., & Lopes, J. (2010). Characterization of electrical penetration graphs of the Asian citrus psyllid, *Diaphorina citri*, in sweet orange seedlings. *Entomologia Experimentalis Et Applicata, 134*(1), 35-49.

Braman, Latimer, J. G., Oetting, R. D., McQueen, R. D., Eckberg, T. B., & Prinster, M. (2000). Management strategy, shade, and landscape composition effects on urban landscape plant quality and arthropod abundance. *Journal of Economic Entomology, 93*(5), 1464-1472. doi:Doi 10.1603/0022-0493-93.5.1464

Braman, Pendley, A. F., Sparks, B., & Hudson, W. G. (1992). Thermal requirements for development, population trends, and parasitism of azalea lace bug (Heteroptera:Tingidae). *Journal of Economic Entomology, 85*(3), 870-877.

Braman, S., & Pendley, A. (1992). Evidence for resistance of deciduous azaleas to azalea lace bug. *Journal of environmental horticulture (USA)*.

Braman, S. K., & Beshear, R. J. (1994). Seasonality of predaceous plant bugs (Heteroptera:Miridae) and phytophagous thrips (Thysanoptera:Thripidae) as influenced by host plant phenology of native azaleas (Ericales:Ericaceae). *Environmental Entomology, 23*(3), 712-718.

Bonani, J., Fereres, A., Garzo, E., Miranda, M., Appezzato-Da-Gloria, B., & Lopes, J. (2010). Characterization of electrical penetration graphs of the Asian citrus

psyllid, *Diaphorina citri* Kuwayama, in sweet orange seedlings. *Entomologia Experimentalis et Applicata, 134*(1), 35-49.

Buntin, G. D., Braman, S. K., Gilbertz, D. A., & Phillips, D. V. (1996). Chlorosis, photosynthesis, and transpiration of azalea leaves after azalea lace bug (Heteroptera:Tingidae) feeding injury. *Journal of Economic Entomology, 89*(4), 990-995.

CABI. (2005). Azalea lace bug, *Stephanitis pyrioides* (Hemiptera: Tingidae). Crop Protection Compendium. CAB International 2005.

Chappell, M., & Robacker, C. (2006). Leaf wax extracts of four deciduous azalea genotypes affect azalea lace bug (*Stephanitis pyrioides* Scott) survival rates and behavior. *Journal of the American Society for Horticultural Science, 131*(2), 225-230.

Chappell, M., Robacker, C., Baden, S., & Byous, A. (2004). The effect of leaf wax extracts from the leaves of eight azalea genotypes (*Rhododendron* spp.) on feeding and oviposition by azalea lace bug *Stephanitis pyrioides* (Scott). *Hortscience, 39*(4), 895-895.

Chappell, M., Robacker, C., Baden, S., & Byous, A. (2005). Border patrol: Epicuticular leaf wax mediates resistance of azalea genotypes (*Rhododendron* spp.) to azalea lace bug *Stephanitis pyrioides* (Scott). *Hortscience, 40*(4), 1111-1112.

Cornell University. (1994). Abamectin. Retrieved from http://pmep.cce.cornell.edu/profiles/extoxnet/24d-captan/abamectin-ext.html

Cornell University. (1995a). Azadirachtin. Retrieved from http://pmep.cce.cornell.edu/profiles/extoxnet/24d-captan/abamectin-ext.html

Cornell University. (1995b). Cyfluthrin. Retrieved from http://pmep.cce.cornell.edu/profiles/extoxnet/carbaryl-dicrotophos/cyfluthrin-ext.html

do Valle, G. E., Lourenção, A. L., & Pinheiro, J. B. (2012). Adult attractiveness and oviposition preference of *Bemisia tabaci* biotype B in soybean genotypes with different trichome density. *Journal of Pest Science, 85*(4), 431-442. doi:10.1007/s10340-012-0443-0

EPA. (1999). Bendiocarb. Retrieved from http://www.epa.gov/oppsrrd1/REDs/factsheets/0409fact.pdf

EPA. (2001). Acephate Facts. Retrieved from http://www.epa.gov/opp00001/reregistration/REDs/factsheets/acephate_fs.pdf

EPA. (2012). Clothianidin – registration status and related information. Retrieved from http://pmep.cce.cornell.edu/profiles/extoxnet/carbaryl-dicrotophos/cyfluthrin-ext.html

Hasanuzzaman, A. T. M., Islam, M. N., Zhang, Y., Zhang, C.-Y., & Liu, T.-X. (2016). Leaf morphological characters can be a factor for intra-varietal preference of whitefly *Bemisia tabaci* (Hemiptera:Aleyrodidae) among eggplant varieties. *Plos One, 11*(4), e0153880. doi:10.1371/journal.pone.0153880

Held, D. W., & Parker, S. (2011). Efficacy of soil applied neonicotinoid insecticides against the azalea lace bug, *Stephanitis pyrioides*, in the landscape. *Florida Entomologist, 94*(3), 599-607.

Henry, T. J. N. J. W. G. K. M. (1986). *Stethoconus japonicu*s (Heteroptera:Miridae): a predator of *Stephanitis* lace bugs newly discovered in the United States, promising in the biocontrol of azalea lace bug (Heteroptera:Tingidae). *Proceedings of the Entomological Society of Washington, 88*.

IPPC Phenology Model. (2016). Phenology models from uspest.org. Retrieved from http://pnwpest.org/cgi-bin/ddmodel.us

James, D., & Price, T. (2004). Field-testing of methyl salicylate for recruitment and retention of beneficial insects in grapes and hops. *Journal of Chemical Ecology, 30*(8), 1613-1628. doi:10.1023/B:JOEC.0000042072.18151.6f

James, D.G. (2003) Synthetic herbivore-induced plant volatiles as field attractants for beneficial insects. Environmental Entomology 32:977-982.

Kaplan, I. (2012). Attracting carnivorous arthropods with plant volatiles: The future of biocontrol or playing with fire? *Biological Control, 60*(2), 77-89. doi:DOI 10.1016/j.biocontrol.2011.10.017

Kessler, A., & Baldwin, I. T. (2001). Defensive function of herbivore-induced plant volatile emissions in nature. *Science, 291*(5511), 2141-2144. doi:10.1126/science.291.5511.2141

Klingeman, W. E., Braman, S. K., & Buntin, G. D. (2000). Evaluating grower, landscape manager, and consumer perceptions of azalea lace bug (Heteroptera : Tingidae) feeding injury. *Journal of Economic Entomology, 93*(1), 141-148.

Klingeman, W. E., Buntin, G. D., & Braman, S. K. (2001). Using aesthetic assessments of azalea lace bug (Heteroptera:Tingidae) feeding injury to provide thresholds for pest management decisions. *Journal of Economic Entomology, 94*(5), 1187-1192. doi:Doi 10.1603/0022-0493-94.5.1187

Krischik, V., Landmark, A., & Heimpel, E. (2007). Soil-applied imidacloprid is translocated to nectar and kills nectar-feeding *Anagyrus pseudococci* (Girault) (Hymenoptera:Encyrtidae). *Environmental Entomology, 36*(5), 1238-1245.

Krischik, V., Rogers, M., Gupta, G., & Varshney, A. (2015). Soil-Applied imidacloprid translocates to ornamental flowers and reduces survival of adult *Coleomegilla maculata*, *Harmonia axyridis*, and *Hippodamia convergens* lady beetles, and larval *Danaus plexippus* and *Vanessa cardui* butterflies. *Plos One, 10*(3), e0119133. doi:10.1371/journal.pone.0119133

Labonte, J. R. (2014). [Personal Communication].

LaBonte, James R., & Valente, Thomas E. (2014). Azalea lace bug, *Stephanitis pyrioides* (Scott): a new pest for the Pacific Northwest. Poster session presented at: Entomology 2014, Grand challenges beyond our horizons. 62nd Annual Meeting of the Entomological Society of America; 2014 Nov 16-19; Portland, OR

Lee, J. C. (2010). Effect of methyl salicylate-based lures on beneficial and pest arthropods in strawberry. *Environmental Entomology, 39*(2), 653-660. doi:10.1603/EN09279

Nair, S., & Braman, S. K. (2012a). Integration of insecticides with the natural enemy *Chrysoperla carnea* (Stephens) for management of azalea lace bug (Hemiptera:Tingidae). *Journal of Entomological Science, 47*(3), 278-281.

Nair, S., & Braman, S. K. (2012b). A scientific review on the ecology and management of the azalea lace bug *Stephanitis pyrioides* (Scott) (Hemiptera:Tingidae). *Journal of Entomological Science, 47*(3), 247-263.

Neal, J. W., & Douglass, L. W. (1988). Development, oviposition rate, longevity, and voltinism of *Stephanitis pyrioides* (Heteroptera:Tingidae), an adventive pest of azalea, at 3 temperatures. *Environmental Entomology, 17*(5), 827-831.

Neal, J. W., Haldemann, R. H., & Henry, T. J. (1991). Biological-control potential of a japanese plant bug *Stethoconus japonicus* (Heteroptera:Miridae), an adventive predator of the azalea lace bug (Heteroptera:Tingidae). *Annals of the Entomological Society of America, 84*(3), 287-293.

Neal, J. W., & Haldemann, R. L. (1992). Regulation of seasonal egg hatch by plant phenology in *Stethoconus japonicus* (Heteroptera:Miridae), a specialist predator of *Stephanitis pyrioides* (Heteroptera:Tingidae). *Environmental Entomology, 21*(4), 793-798.

Nielsen, G. (1997). Lace bugs, entomology leaflet 153. *Univ. Vermont Ext., Burlington*.

Nino, A. A., & Cave, R. D. (2015). Suitability of *Microtheca ochroloma* (Coleoptera:Chrysomelidae) for the Development of the Predator *Chrysoperla rufilabris* (Neuroptera:Chrysopidae). *Environmental Entomology, 44*(4), 1220-1229. doi:10.1093/ee/nvv084

Reddy, G. V., Zhao, Z., & Humber, R. A. (2014). Laboratory and field efficacy of entomopathogenic fungi for the management of the sweetpotato weevil, *Cylas formicarius* (Coleoptera:Brentidae). *Journal of Invertebrate Pathology, 122,* 10-15.

Rinehart, T. A., & Boyd, D. W. (2006). Rapid, high-throughput detection of azalea lace bug (Hemiptera:Tingidae) predation by *Chrysoperla rufilabris* (Neuroptera:Chrysopidae), using fluorescent-polymerase chain reaction primers. *Journal of Economic Entomology, 99*(6), 2136-2141.

Rogers, M. A., Krischik, V. A., & Martin, L. A. (2007). Effect of soil application of imidacloprid on survival of adult green lacewing, *Chrysoperla carnea* (Neuroptera:Chrysopidae), used for biological control in greenhouse. *Biological Control, 42*(2), 172-177.

Rosetta, R. (2013). Azalea lace bug biology and management in commercial nurseries and landscapes (2013 ed.): Oregon State University.

SAGEMAP, U. (n.d.). Top-down versus bottom-up effects. Retrieved from http://sagemap.wr.usgs.gov/ftp/BLM/FtPrtP3V2.pdf

Sanchez, D. (1989). *Stethoconus*, dirty harry of the azaleas. *Agricultural Research, 37*(4), 14-15.

SAS. (2013). *JMP® 11.0.0*. Cary, NC: SAS Institute Inc.

Schultz, P. B. (1993). Host-plant acceptance of azalea lace bug (Heteroptera:Tingidae) for selected azalea cultivars. *Journal of Entomological Science, 28*(2), 230-235.

Shelton, A. (n.d.). *Chrysoperla* (=*Chrysopa*) *carnea, C. rufilabris* (Neuroptera:Chrysopidae). Retrieved from http://www.biocontrol.entomology.cornell.edu/predators/Chrysoperla.php

Shrewsbury, P. M., Lashomb, J. H., Patts, J. M., & Casagrande, R. A. (2004). The influence of flowering plants on herbivore and natural enemy abundance in ornamental landscapes. *International Journal of Ecology and Environmental Sciences, 30*(1), 23-33.

Shrewsbury, P. M., & Raupp, M. J. (2006). Do top-down or bottom-up forces determine *Stephanitis pyrioides* abundance in urban landscapes? *Ecological Applications, 16*(1), 262-272. doi:10.1890/04-1347

Shrewsbury, P. M., & Raupp, M. J. (2000). Evaluation of components of vegetational texture for predicting azalea lace bug, *Stephanitis pyrioides* (Heteroptera:Tingidae), abundance in managed landscapes. *Environmental Entomology, 29*(5), 919-926. doi:Doi 10.1603/0046-225x-29.5.919

Shrewsbury, P. M., & Smith-Fiola, D. C. (2000). Evaluation of green lacewings for suppressing azalea lace bug populations in nurseries. *Journal of Environmental Horticulture, 18*(4), 207-211.

Stewart, C. D., Braman, S. K., & Pendley, A. F. (2002). Functional response of the azalea plant bug (Heteroptera:Miridae) and a green lacewing *Chrysoperla rufilabris* (Neuroptera:Chrysopidae), two predators of the azalea lace bug (Heteroptera:Tingidae). *Environmental Entomology, 31*(6), 1184-1190. doi:10.1603/0046-225x-31.6.1184

Torres-Miller, L. (1989). New records of lace bugs from West Virginia (Hemiptera: Tingidae).

Trumbule, R., Denno, R., & Raupp, M. (1995). Management considerations for the azalea lace bug in landscape habitats. *Journal of Arboriculture, 21*, 63-63.

Trumbule, R. B., & Denno, R. F. (1995). Light-Intensity, host-plant irrigation, and habitat-related mortality as determinants of the abundance of azalea lace bug (Heteroptera:, Tingidae). *Environmental Entomology, 24*(4), 898-908.

Vinje, E. (2012, December 08). The Good Bugs: Beneficial Garden Insects | Planet Natural. Retrieved July 15, 2016, from http://www.planetnatural.com/good-bugs/

Walker, G. P., & Backus, E. A. (2000). Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Paper presented at the International Congress of Entomology 1992: Beijing, China).

Williams, L., III, Rodriguez-Saona, C., Castle, S., & Zhu, S. (2008). EAG-active herbivore-induced plant volatiles modify behavioral responses and host attack by an egg parasitoid. *Journal of Chemical Ecology, 34*(9), 1190-1201. doi:10.1007/s10886-008-9520-5

Appendices

Appendix 1 Clip cage trials

In 2014, clip cage trials were conducted to monitor egg laying and rearing. Nine clip cages were set up, six in a greenhouse and three outside in a screen cage. Each clip cage arena had a one male and one female *S. pyrioides*. Clip cages were set up July 31, 2014 and ceased August 28, 2014 and were clipped to the underside of rhododendron leaves. Trials were ceased due to the lack of feeding and eggs being laid. Feeding wasn't observed until August 4, 2014. Furthermore, eggs were laid in only three of the nine clip cages, with two eggs the most being laid by one pair. It is possible that the small arena may have affected their behavior. Due to the low success rate of acquiring infestation with clip cages, I did not continue to use clip cages to start infestations.

Appendix 2 Caged voltinism

Attempts were made to rear a cohort of eggs to adults and calculate their time of development in total and between each stage. This was done by keeping the leaves checked for egg presence in Chapter 2, and circling each egg or egg cluster and waiting for hatching inside the laboratory at room temperature. After 1st instars were hatched, they would then be transferred with a wet fine-haired camel brush to the outdoor cage with rhododendrons to track their development. However, handling and transporting the 1st instars caused high mortality due to their fragility and could not be continued due to the timing of generation time in the wild becoming asynchronous with the caged voltinism.

Appendix 3 Cultivar observations

In addition to the cultivars observed without infestation listed in Table 3.1, Table A.3 lists the cultivars that were observed with infestation and together provides a complete list of all the cultivars observed. Only cultivars with notable characteristics have their infestation intensity listed in Table A.3; all others are infested but their infestation density is not reported in this table.

Table A.3 Table of infested cultivars from observations

Name	Notable characteristics	Infestation density	Habit
(Yakushimanum x Norman Gill) x (Yellow Saucer x Anna S Riplet Dwarf			garden
1000 Butterflies			garden
Aberconway 1			garden
Aberconway x David x Crest			garden
Aberreonway II			garden
Albamense			garden
Alice Spring	Indumentum	Low	garden
Allison Johnstone			garden
Anah Kruscke			garden
Anna Rose Whitney			pot, garden
April Rose			garden
Arborescens			garden
Arborescens Layer	Glossy	Low	garden
Arborescens Sweet Azalea			garden
Arboreum Connamomeum	Indumentum	Low	garden
Arboreum Hybrid			garden
Augustinii			garden
Augustinii Bergeii Bump			garden
Augustinii Blue			garden
Augustinii Bump B/W Cross			garden
Augustinii Charmanthum Blue Cloud			garden
Augustinii Chasman Willad grey			garden
Augustinii Chasmanthum			garden
Augustinii Copera	Indumentum	High	garden

Augustinii Crater Lake			garden
Augustinii Dark			garden
Augustinii Exbury			garden
Augustinii Lacamas Blue			garden
Augustinii Senora Meidon			garden
Augustinii St. Trudy			garden
Augustinii Vilmoranium Blue			garden
Augustinii White			garden
Auriculatum	Light Indumentum, Sticky	Med	garden
Austrinum			garden
Avalanche			garden
Azalea Fuschia			pot
Azalea GA Karen			pot
Azalea Hino Crimson			pot
Azalea Luteum			garden
Azalea Renee Michelle			pot
Azalea Rose			pot
Azalea GA Rosebud			pot
Azalea White Rosebud			pot
Barto			garden
Barto/Macrophyllum White			garden
Basilicum			garden
Belle Heller			garden
Belleaus Toy	Light Indumentum	Low	garden
Bessie Balcom			garden
Best Purple			garden
Biscuit Colored Daydream			garden
Black Eye			garden
Black Magic			garden
Blaney's Blue			garden
Bloom-a-thon Pink			pot
Blue Baron			garden
Blue Cloud			garden
Blue Diamond			garden
Blue Jay			garden
Blue Peter			garden
Bob Bovee			garden
Bob's Blues			garden
Borde Hill			garden
Bow Bells			garden

Bronze Wing			garden
Brown Eyes			garden
Buzzer Beater			pot
Cacasicum x Fred Rose			garden
Candi			garden
Captain Jack	Light Indumentum	High	garden
Cardiobasis			garden
Carmen			garden
Cary Ann			garden
Cataw Grandiforum			pot
Catawbiense Forma Insularis	Leathery	Low	garden
Cawtawbiense Baursalt			garden
Chaetomallum			garden
Charmont			garden
Chasman William Gay			garden
Chasmanthum			garden
Cherry Cheescake			pot
China			garden
Chlorops			garden
Chlorops x Yak x Skipper			garden
Christmas Cheer			garden
Ciliatum			garden
Cinnabarinum var. Pearly Glow			garden
Cinnabarinum Xanthocodon			garden
Cinnabarum Xanthocodon Purpurellum			garden
Cipinense			garden
Circus			garden
Clark's Mardis Gras	Indumentum, Tomentum	Low	garden
Clipense			garden
Corona x Yak			garden
Creole Bell			garden
Crest			garden
Crimson Pippin's Sister x (Ernest Inman x Edwin O. Weber)			garden
Cuneatum			garden
Cunningham's White			pot, garden
Dan's Early Purple			garden
Daphnoides			pot, garden
Dauricum Alba			garden

David x Crest			garden
Davidsonianum			garden
Diaprepes			garden
Dido x Williamsianum			garden
Dora Amateis			garden
Dr. Stocker x self			garden
Dreamland			pot
Dredoxa var. Fargisii			garden
Edith Bosley			pot
Electra Mel Reeves			garden
Electra's Son			garden
Eliott's Red Whalloper			garden
Elisabeth Hobbie			garden
Elizabeth R. Shum Picnic	Light Indumentum	High	garden
Elvira			pot
English Roseum			pot
Evening Glow			garden
Everything nice			garden
Exbemia			garden
Exury Azalea	Indumentum, Tomentum	Low	garden
Falvidur x Lady Roseberry	Glossy	High	garden
Fastuosum Flore Pleno			garden
Firestorm			pot
Fortunei Pink Chiffon			garden
Fred Hamilton			garden
Friday			garden
Furnivall's Daughter			garden
Gall's Crimson			garden
Garten-Direktor Gloker			garden
George Delight			garden
Ginny Gee			garden
Gold Mour			garden
Golden Gate			garden
Goldstrike			garden
Gomer Waterer			pot, garden
Grace Seabrook			garden
Grand Slam			garden
Green Eye			garden
Greenland			pot
Guy Nearing	Tomentum	Med	garden

Haaga			pot
Hachmanns Diadem x Yellow Saucer x Anna's Riplet			garden
Hachmanns Polaris x Yellow saucer x Anna's Riplet Dwarf			garden
Hackmans Polaris			garden
Halfdan			garden
Halfdan Lem			garden
Hanceanum Nanum			garden
Hardi JZ ER's Beauty Azalea			garden
Heart's Delight			garden
Hellikki			pot
Helsinki University			pot
Henry's Red			pot
Hensley Anum			garden
Holy Moses			garden
Hookeri			garden
Hoopla			pot
Hoppy			garden
Horizon Lakeside			garden
Hotei			pot, garden
Huskymania			pot
Hyperthrum	Light Indumentum, Glossy	Low	garden
Ignatius Sergeant			pot
Ilam Violet			garden
Irroratum			garden
Isabel Pierce			garden
Jan Dekins			garden
Jean Marie de Montague			pot, garden
Jock			garden
Johnsteanum	Hairy	High	garden
Jumbo			garden
Karen Triplett			garden
Karin			garden
Keiskei Yaku Fairy			garden
Kevin	Indumentum	Low	garden
Kilmanjaro			garden
Kiusianum var Shp			garden

Kiusilaum Album			garden
Kristin			garden
Lacanas Blue			garden
Lady Clementine Hotford			garden
Last Chance	Light Indumentum	High	garden
Ledum			garden
Ledum Glanoulosum			garden
Lee's Dark Purple			pot
Lemon Tart			garden
Lems 121			garden
Lem's Cameo			garden
Lems Oregon Sunset			garden
Len's Monarch			garden
Leo			garden
Leonna			garden
Leutiem			garden
Lissabon			garden
Little Beth			garden
Lodauric x R. Hemsley Anum			garden
Loderi King George			garden
Loder's White			garden
Looking Glass			pot
Lord Roberts			pot
Lucky Strike			garden
Lucy Lou	Hairy	Med	garden
Lutescens FCC			garden
Lutescens FCC Layer			garden
Luteum			garden
Macabeanum			garden
Macrophyllum			garden
Macrophyllum L. Lum			garden
Madan Fr. J. Chauvin			garden
Malamute			garden
March Madness			pot
Mark EET A's Prince			garden
Markeeta's Prize			garden
Marley Hedges			garden
Maryke			garden
Maximum Fetlerhoffs Ivory Bump	Light Indumentum	Low	garden
Maximum White/Bump			garden
McGuire Hybrid	Glossy		garden

Medusa	Light Indumentum	Low	garden
Mid Summer			garden
Midnight Mystigame			garden
Miss Portland			garden
Morii			garden
Moser's Maroon			garden
Mount Everest			garden
Mrs. 4 De La Mere x Purple Splendor			garden
Mrs. Betty Robertson			garden
Mrs. Charles Pearson			garden
Mrs. EC Sterling			garden
Mrs. G W Leak			garden
Mrs. P.D. Williams x Lacteum			garden
Mrs. W.C. Slocock			garden
Mucronulatum			garden
Nakaharai			garden
Naomi Astarte			garden
Naselle x (Lem's cameo x Recurvoides)	Light Indumentum	Med	garden
Nelda Peach			garden
Nightwatch			garden
Normandy			pot
Nova Zembla			pot
Oblonfolium			garden
Occidentale			garden
Occidentale Olivever			garden
Occidentales S M 29			garden
Ocean Lake			garden
Odee Wright			garden
Olive			garden
OR Tricanthum			garden
Orbiculare			garden
Oreotrephes			garden
Oreotrephes exbury			garden
Ostbo's Low Yellow			garden
Paprika Spiced			garden
Peppermint Stick			garden
Perfume			garden
Peter Faulk	Light Indumentum	Low	garden
Phyllis Kern			garden
Phyllis Kor			garden
Pink Pearl			garden

PJM			pot, garden
Point Defiance			garden
Popeye No. 2			garden
Praevernum			garden
President Roosevelt			pot, garden
Primula Sibddii "Pink Spray"			garden
Princess Elizabeth x Elizabeth			garden
Puget Sound			garden
Purple Elegans			garden
Purple Gem			pot
Queen of Waaldheim			garden
Rac Roc Rose	Small Leaves	Low	pot
Rainbow			garden
Ramapo			pot
Razzle Dazzle			garden
Red Walloper			garden
Ririei			garden
Rockett			pot
Roseum elegans			pot
RSF #77-787			garden
Rubicon	Light Indumentum	High	garden
Ruby Bowman			garden
Rukizon			garden
Russatum			garden
Sapphire			garden
Sarita Loder			garden
Sausalito			garden
Scarlet Wonder Dwarf			pot
Schlippenbachii			garden
Scintillation			garden
Searsiae			garden
Seedling JE 30 x Avalanche			garden
September Song			pot
Serotinum x Skipper			garden
Seta			garden
Shamrock			garden
Skipper			garden
Slam Dunk			pot
Smithii Clarks	Indumentum	Low	garden
Smokey			garden

Sneezy			pot
Solent Queen			garden
Soulei			garden
Soulei hybrid			garden
Spatter			garden
Spatter Paint			garden
Special White	Leathery	Med	garden
Spring Glory			garden
St. Tudy			garden
Star Sapphire			garden
Star Trek			garden
Starfish			garden
Sugar Pink			garden
Sunset Bay			garden
Sunspray			garden
Sutchuenense	Indumentum	Low	garden
Tahitian Dawn			garden
Taurus			pot, garden
Tempest Sister			garden
Thomsonii			garden
Tortoise Shell Orange			garden
Town Court			garden
Trail Blazer			garden
Tricanthum	Indumentum	High	garden
Trilby			pot
Trube Waterer			garden
Unique			garden
Unnamed Rothschild Yellow			garden
Uvarifolium			garden
Vanness Sensation			garden
Vaseyi Pink Shell Azalea			garden
Vernicosum			garden
Vibrant Violet			garden
Vicosum Davis Layer			garden
Virginia Richards			pot, garden
Voleit			garden
Voleit Cornubia			garden
Vulcan			pot, garden
Vulcan's Bells			garden

Wallachii			garden
Wardir			garden
Warlock			garden
William Falconer Layer			garden
Wind River			garden
Windbean			garden
Winsome	Light Indumentum	Low	garden
Wizard			garden
Wonjar's Purple			pot
Yakushimanum Hybrid	Indumentum	Low	garden
Yakushimanum x Norman Gill			garden
Yaku Dreamland			pot
Yaku Percy Wiesman			pot
Yaku Princess			pot
Yellow Petticoat			pot
Yellow Rolls Royce			garden

(Continued)