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Title: Alternative Controls for *Stephanitis pyrioides* in the Pacific Northwest

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Jana C. Lee

The azalea lace bug (*Stephanitis pyrioides* Scott) is a global pest of rhododendrons and azaleas (*Rhododendron* spp.). It is originally from Asia, has been established in the eastern U.S. for the past century, and was recently detected in the Pacific Northwest in 2008-2009. *Stephanitis pyrioides* feeds on the underside of leaves, removing the chlorophyll from the mesophyll layer causing leaf chlorosis. It leaves unattractive residues on the underside of leaves as a result of frass deposition and molting. *Rhododendron* is an economically important genus for the nursery and landscape industries in the state of Oregon. Presently *S. pyrioides* is controlled using systemic insecticides, but the possible environmental impacts and possible development of resistance compel efforts to find alternative controls. Previous research has offered regional solutions which cannot be used at a large scale in Oregon. The research presented in this thesis aimed to add to the body of knowledge about alternative ways to control this pest. We set out to determine 1) if *S. pyrioides* infestations can be controlled using plant volatiles, a blend of herbivore-induced plant volatiles (HIPV) and floral volatiles, to attract naturally-occurring green

lacewing, a known voracious lace bug predator, and 2) if *Rhododendron* spp. resistance to *S. pyrioides* can be enhanced with supplemental silicon.

In the first study, two out of three different blends of plant volatiles attracted green lacewing compared to a control in farm landscapes, none in urban landscapes. The blends comprised of methyl salicylate + acetic acid +2-pheylethanol, and acetophenone + acetic acid + 2-phenylethanol. One out of four experiments that used the volatile blends to attract lacewing effectively controlled *S. pyrioides* using the blend with acetophenone (above). Other natural enemies were also monitored for attraction: the blends with methyl salicylate or acetophenone (above) recruited some predators and the floral blend with phenylacetaldehyde + methyl salicylate + acetic acid recruited some parasitoids, but associated reductions of *S. pyrioides* were not large enough to draw any correlations.

In past studies, increasing host plant resistance by supplementing plants with elemental silicon has enhanced the defense systems of monocots and some dicots. The silicon may increase cell wall strength making it more difficult for herbivores to feed on or, may affect plant chemistry and palatability. Rhododendrons supplemented with silicon had reduced numbers of *S. pyrioides* eggs and frass spots in choice experiments with whole plants and detached leaves albeit post-supplementation tests showed no increase in silicon content. If rhododendrons are unable to absorb and accumulate supplemental silicon, a potential topical effect of foliar applications is conceivable but the mechanism for similar results obtained with soil applications is unclear. Tri-trophic interactions in varying landscapes are complex and examples of using plant volatiles to successfully manipulate natural enemies for effective biological control are still scant. This study is another example of that complexity. A more practical tool for the control of *S. pyrioides* in Oregon may be the use of silicon to enhance host resistance. Further research in

this area is needed to bridge the gap between small scale experimental success and large scale, practical alternative controls.

Alternative Controls for *Stephanitis pyrioides* in the Pacific Northwest

by

Katerina Velasco-Graham

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APPROVED:

Major Professor, representing Horticulture

Head of the Department of 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.

Katerina Velasco-Graham, Author

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CONTRIBUTION OF AUTHORS

Katerina Velasco-Graham designed experiments, supervised data collection and processing and analyzed data. Man-Yeon Choi provided guidance on testing volatiles and gas chromatography, and Jana Lee provided guidance on experimental design, sorting sticky trap samples, and draft revisions.

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DEDICATION

To all the young women who may struggle with family/career decisions.

No one decision is the right one, just the one that is right for you

…but never give up figuring it out.

Onward!

CHAPTER 1

General Introduction

Katerina Velasco-Graham

The azalea lace bug *Stephanitis pyrioides* Scott, 1874 (Tingidae: Hemiptera) is native to Japan. In the past century, it has spread to the rest of the world due to the movement of ornamental plants, especially azaleas (Nair and Braman 2012). It was first reported in the U.S. in New Jersey in 1916, then detected in Pennsylvania and Washington D.C. and since then, it has spread to many other U.S. states and other European, Asian, North African and South American countries. The increased trend of introductions into Europe was possibly due to the importation of ornamental plants from North America (Nair and Braman 2012). Within the Hemipteran order, Tingidae is considered as the most damaging to ornamental trees and shrubs (Nair and Braman 2012). The genus includes over 60 species and many are pests of fruit and ornamental trees and shrubs in tropical and temperate regions of the world. Three species in the genus *Stephanitis* Stal exist in North America, the azalea lace bug (*S. pyrioides* Scott), the andromeda lace bug (*S. takeyai* Drake and Maa) and the rhododendron lace bug (*S. rhododendroni)* Horvath; a fourth species from this genus is believed to be extinct (Nair and Braman 2012). The avocado lace bug (*Pseudacysta perseae* Heidemann) is another economically important lace bug which belongs to a separate genus. Scott first described the azalea lace bug in 1874 and named it *Tingis globulifera*. Uhler and Mitzukuri listed it again in 1896 using the same name. It was renamed by Horvath in 1905 as *Stephanitis azalea* and in 1908 by Oshanin to *Stephanitis pyrioides.* The common name was approved by the American Association of Economic Entomologists in 1942 and by the Entomological Society of America in 1965 (Nair and Braman 2012). Hybrids from *S. pyrioides* females and *S. takeyai* males have been reported (Nair and Braman 2012). All three species in the genus *Stephanitis* attack woody ornamentals in the Ericaceae family but especially azaleas and rhododendrons. Range of damage extends from insignificant to plant death, with *S. pyrioides* considered as the most injurious in terms of spread and economic damage (Nair and Braman 2012).

Biology of *Stephanitis pyrioides*

Stephanitis pyrioides range from 2.8 - 3.3 mm in length for males and 2.9 - 3.3 mm for females. Adults are weak fliers and flight usually takes place when they have run out of food or when the host plant has been disturbed. Adults show cold hardiness and do not diapause. Experimental temperature comparisons show a tolerance for lower temperatures whereas high temperatures are

not conducive to *S. pyrioides* longevity. Single males lived up to 246 d and females 253 d at 20.6° C (Neal and Douglass 1988). Adults have been reported present as late as December in Maryland and late February in North Carolina (Neal and Douglass 1988) and throughout the winter months in Oregon (Lee et al. 2019), possibly due to the mild Oregon winter. Courtship and mating behavior are not well described (Nair and Braman 2012). Oviposition of a single egg takes between 2 – 3 min (Nair and Braman 2012); eggs are white, oval or flask-shaped, 0.36 - 0.43 mm long and 0.16 -0.23 mm wide with a bent neck (Nair and Braman 2012). The soft membranous eggs are most often laid into the midrib and secondary, lateral veins of the underside of a leaf although oviposition on the leaf margin has also been observed (Nair and Braman 2012); occasionally they are laid on the upper surface of a leaf (Neal and Douglas 1988, Shrewsbury and Smith-Fiola 2000). After oviposition, the female covers the egg with a dark brown liquid which adheres to the leaf as it hardens forming a protective coating. For most zones, the egg stage is the overwintering stage. In outdoor studies, first instars appeared as early as March 23rd in Georgia and the first week of April in Maryland (Neal and Douglass 1988), and in April in Oregon (Lee et al. 2019). Significantly higher oviposition has been observed in June and July, winter oviposition has also been recorded (Nair and Braman 2012). In environmental chambers, egg development median times were 22.3, 13.0 and 11.3 d. at 20.6°C (69.08°F), 26.1°C (78.98 F), and 31.7°C (89.06 F) respectively (Neal and Douglas 1988). Nymphs, colorless upon hatching, turn black and spiny as they develop. Five instars range in length from 0.1 - 1.8 mm. Wing pads appear after the fourth molt in both males and females; wings are transparent with brown patches and a lace-like pattern which are held flat over the dorsum. Early instars congregate on the underside of leaves, later instars disperse as they seek food, with a preference for the leaf underside throughout their lifetime. Complete development from egg to adult takes approximately one month. In an environmental chamber experiment, development of five instars took a mean of 22.9 d at 20.6°C and 10.5 d at 31.7°C (Neal and Douglass 1988). Tri-voltinism was reported in southern New Jersey; fifth instars in late May and adults as late as the beginning of December in Maryland, point to at least 4 generations per year (Neal and Douglass 1988). Plant stress may also affect voltinism (Nair and Braman 2012). Braman et al. (1992) determined threshold temperatures and degree day accumulations for egg, nymphal and complete development.

Plant damage

The genus *Rhododendron* has over 1,000 species of woody ornamentals including azaleas which make up 2 of the 8 subgenera of the genus. Some of the most widely cultivated ornamental landscape plants, azaleas are native to North America, Europe and Asia (Nair and Braman 2012). In a survey of pest occurrence in landscapes, Shrewsbury and Smith-Fiola (2000) revealed that *Rhododendron* was one of the most pest prone genera of landscape plants, with greater than 50% of plants attacked by pests. Although the genus *Stephanitis* is considered monophagous (eating only one kind of food), there are reports of *S. pyrioides* attacking plants of families other than Ericaceae (Nair and Braman 2012). *Stephanitis pyrioides* nymphs and adults avoid young tender leaves and instead feed on the underside of older mature leaves of ericaceous plants. Females cause significantly more injury per day than males or nymphs. However, the amount of injury caused over a lifetime is similar for both genders (Nair and Braman 2012) because reportedly males live longer than females (Neal and Douglass 1988). *Stephanitis pyrioides* insert their stylets through stomatal openings and remove plant chlorophyll from the palisade mesophyll of the leaf (Shrewsbury and Smith-Fiola 2000, Nair and Braman 2012). Chlorophyll removal leads to chlorosis which results in a stippled or bleached appearance of the upper surface of the leaves. Feeding also causes increased stomatal resistance which reduces rates of photosynthesis and transpiration. Severe damage causes the leaf to dry with subsequent abscission. Dark brown frass and cast skins (exuviae) results in aesthetic damage to the leaf.

Stephanitis pyrioides survive better, are more fecund, and prefer to feed and oviposit on shade grown plants. Further, *S. pyrioides* fitness has been positively associated with morphology, plant chemistry and leaf-water relations, which are markers of plant vigor (Trumbule and Denno 1995). Studies of the distribution of *S. pyrioides* in landscape habitats suggest two causal interacting factors in explaining patterns: sun exposure and the abundance of natural enemies related to that exposure. Despite occurring more abundantly on azaleas growing in the open sun, it is not believed that *S. pyrioides* prefer to feed on light-stressed azaleas relative to shade-grown plants (Neal and Douglas 1988). Instead, Trumbule et al. (1995) attributes lower *S. pyrioides* infestations on shaded azaleas to *natural enemies* which are more abundant in shaded habitats, effectively suppressing *S. pyrioides* in these microclimates and consequently relegating them to azaleas growing in sunny locations. Thus, *Rhododendron* spp. in exposed areas experiencing

stress from intense solar radiation and insufficient water due to their adaptation to the forest understory's low light conditions, combined with the loss of photosynthetically active tissue if heavy infestations are present, will result in leaf desiccation, premature leaf-drop, and plant death.

Control methods

If left unchecked, a severe infestation of *S. pyrioides* can cause plant death, but even a light infestation means economic loss for growers. According to a survey by Klingeman et al. (2000), 2% surface damage on 11% of leaves causes consumers to reject plants and could be used as the aesthetic injury level (though azalea bushes do show a tolerance to *S. pyrioides* feeding above this aesthetic threshold). For this reason, a range of control methods have been investigated over the years including host resistance, cultural, botanical, chemical and biological control.

Host resistance. Some deciduous azalea species are less suitable for *S. pyrioides* feeding, oviposition and nymphal development than an evergreen variety but in general, *Rhododendron* spp. and cultivars exhibit a continuous range of resistance from susceptible to highly resistant (Nair and Braman 2012). Possible mechanisms of resistance proposed and investigated include stomatal characteristics and leaf moisture content, epicuticular waxes and, leaf pubescence (Nair and Braman 2012). Studies evaluating leaf water content and stomata size have not correlated with *S. pyrioides* feeding preference. Those dealing with epicuticular waxes seem to indicate that the difference in the cuticle, specifically, the leaf wax which is composed of surface lipids n-alkenes and triterpenoids play a role in *S. pyrioides* resistance. One study showed that if a cultivar had very large proportions of α-amyrin, β-amyrin and n-nonacosane it belonged to susceptible deciduous genotypes, conversely, if it was high in ursolic acid, n-hentriacontane and n-nonacosane levels, it belonged to resistant genotypes. Furthermore, if leaf wax extracts from resistant genotypes were applied to susceptible cultivars, it resulted in resistance to feeding and oviposition in the susceptible group and vice versa (Nair and Braman 2012). Studies evaluating leaf pubescence (Nair and Braman 2012) reported that only *Rhododendron canescens*, a cultivar with high indumentum (fine leaf hairs) density, showed high resistance to *S. pyrioides* feeding. Knomnenus and Lee (2018) found similar results suggesting that indumentum provided a barrier to feeding. They placed *S. pyrioides* into arenas with indumentum covered leaves and with leaves without indumentum. *Stephanitis pyrioides* with the indumentum leaves died after a week, those

placed on de-fuzzed of the same cultivar, or glabrous leaves of another cultivar survived longer. Lace bugs chose the indumentum-free leaves 100% of the time in a choice test in the same study. Furthermore, compared to "fuzzy" leaved cultivars, significantly higher feeding and oviposition took place on de-fuzzed and glabrous leaves.

Cultural control. Habitat manipulation and preventing water deficit has also been successful in controlling infestations. Examples of habitat manipulation include type of groundcover and container position of above-ground containers (Nair and Braman 2012). Shrewsbury and Raupp (2000) noted that adding non-host vegetation and other flowering plants lowered *S. pyrioides* survival because of higher numbers of natural enemies and increased abundance of alternative prey. Nitrate: ammonium ratios applications may also affect *S. pyrioides* feeding. In *Rhododendron austrinum*, damage reduction occurred with ammonium but increased with an increase in nitrate concentrations. Neither *S. pyrioides* performance nor fitness appeared to be influenced by nitrogen fertilization yet they did show a preference for plants with high nitrogen and water level applications. Plant growth regulator use appeared to slow the development of *S. pyrioides* due to reduced nutrient availability (Nair and Braman 2012). Water spraying is another non-toxic and easy cultural control method for homeowners and landscape managers to immediately dislodge *S. pyrioides*. A field efficacy study showed significant reduction of adults per leaf in randomly sampled leaves. At the end of the trial, water sprayed plants had a lower percentage of damaged leaves compared to the control group. A consistently lower number of eggs per cm^2 on treated plants was also observed, probably due to the removal of adults resulting in oviposition reduction (Lee et al. 2018).

Botanical control. Although they are not as effective as chemical pesticides, homeowners and landscapers prefer botanical and natural compounds due to safety concerns. Several essential oils have shown promise for the management of *S. pyrioides* including Pelargonium, Cinnamomum, Hedychium, *Tagetes*, *Juniperus saltuaria*, *J. squamata var. fargesii*, and *Eupatorium capillifolium* (Nair and Braman 2012). Insecticidal soaps may have several mechanisms of action including structure and permeability disruption of cell membranes leading to leakage of cell contents and insect death. Horticultural oils suffocate the operculum of the egg causing egg mortality and prevent proper gas exchange in nymphs and adults causing

respiration disruption and death (Nair and Braman 2012). Neem oil works in a similar fashion but also acts as a repellent (Isman 2006).

Chemical control. Insecticides used to control *S. pyrioides* in the distant past have included whale oil soap and white oil in combination with powdered derris root; thimet, dimethoate and phosphamidon (Nair and Braman 2012). Balsdon et al. (1993) compared efficacy of pre-neonicotinoid era pesticides including acephate, dimethoate, bendiocarb, cyfluthrin, abamectin, azadirachtin, insecticidal soap and horticultural oil. Acephate was the most cost-effective and persistent in suppression of *S. pyrioides* with the added benefit of not affecting the development of the egg parasitoid *A. takeyanus* (Balsdon et al. 1993). Systemic neonicotinoid insecticides such as imidacloprid, thiamethoxam, dinotefuran and clothianidin have been successful in the control of infestations since their appearance on the market. In the case of imidacloprid, an early April application of a granular form provides year-long pest control (Joseph 2019). However, due to their potential effect on pollinators and natural enemies, the popularity of treating *Rhododendron* spp. with neonicotinoids, whether for sale or established in a landscape, has declined in recent years. During the same time, pyrethroid use has increased, but not without its own negative effects on beneficials which may result in secondary pest outbreaks (Joseph 2019). Insect growth regulators or IGRs, which affect the molting process in immature insect stages, have been investigated recently. The use of novaluron, buprofezin, and azadirachtin resulted in the reduced development of young instars when *S. pyrioides* eggs were exposed to IGRs. The use of an adjuvant and novaluron made the IGR effective even when it was sprayed adaxially (on the upper part of the leaf), suggesting translaminar activity (Joseph 2019).

Biological control. The use of natural enemies to suppress pest populations below damaging levels is known as biological control. *Stephanitis pyrioides* has several known generalist and specialist natural enemies (Table 1). In Oregon, generalists are present, but specialist predators and parasitoids were not detected in a two-year survey (Lee et al. 2019). *Stephanitis pyrioides* is susceptible to *Metarhizium* and sporulate after a direct spray but it was deemed an ineffective option considering that many adults dies after water sprays as well (Skillman et al. 2018). Nymphs and adults secrete fluid droplets from specialized abdominal setae and antennae. These secretions which possess bactericidal, fungicidal and nematicidal

properties, may also play a role against predation, parasitization and/or as an alarm pheromone system (Nair and Braman 2012).

Green lacewing for biological control

Chrysoperla spp. (Neuroptera: Chrysopidae) are important natural enemies of several pests of economic importance due to their polyphagous feeding habits (Amarasekare and Shearer 2013). They are present on every continent except for the Antarctic. In North America, *Chrysoperla plorabunda* is the dominant species from Texas to the west coast, *Chrysoperla rufilabris* from Texas to the east coast, *Ceraeochrysa cubana* joins *C. rufilabris* in Florida and *Chrysopa oculata* and *nigricornis* join *C. plorabunda* in the northern US and Canada (Duelli 2001). The genus *Chrysoperla* are generalist predators in their larval stage but feed on nectar, pollen and honeydew (palyno-glycophagous) as adults whereas species of the genus *Chrysopa* are characterized by predatory adults. The *Chrysoperla* genus was divided into four groups by Brooks in 1994: the *carnea*- and *pudica*- groups include natural enemies of field crop pests and the *comans*- and *nyerina*- are mainly arboreal (Duelli 2001). The *carnea* group contains 20 or more "cryptic sibling" species. Details of the systematics of this group is beyond the scope of this study nonetheless, it is worth pointing out that *C. plorabunda* is part of the *carnea*-group and is adapted to live in fields and gardens occurring in a wide range of habitats. *Chrysoperla rufilabris* is part of the *pudica* group and is better adapted for living in trees; since they are dominant and better adapted to the southern U.S. compared to *C. plorabunda* (Nair and Braman 2012) they may be more useful in humid greenhouses, irrigated crops, or humid parts of the country (Kvedaras et al. 2010).

Chrysoperla adults are light green with long slender antennae, golden eyes and long delicately veined wings that are ½ to ¾ inch (1.27 – 1.9 cm) long; *C. plorabunda* displays a reddish- or yellowish-brown coloration at diapause (Duelli 2001). Adults are active at night feeding on pollen, nectar and honeydew. Mated and fed females lay eggs in singly or in groups. Fecundity is strongly influenced by the nutritional acquisition experienced by the larval stages and by environmental conditions (Szentkiralyi 2001). Oviposition takes place nocturnally with an apparent preference for the tops of hairs or bristles in various species (Szentkiralyi 2001).

White eggs are laid on top of ¼ inch (0.635 cm) slender pedicels. Embryonic development varies for different species, the *carnea* group reportedly taking between 13 to 2.5 days with 59° F to 95° F (15°C - 35°C) respectively (Canard and Volkovich 2001).

Chrysoperla spp. undergo complete metamorphosis. After hatching, the new larva is motionless and defenseless for a few hours. They have spindle-shaped bodies with prominent sickle-shaped jaws (mandibles) which make them resemble tiny alligators. Their jaws contain tubes uses to inject venom, paralyze prey and suck out body fluids. If it gets disturbed, the larval jaw grooves won't align correctly hindering its ability to eat efficiently (Canard and Volkovich 2001). Larvae grow to ½ inch (1.27 cm) long; three instars or larval stages take two to three weeks to develop before spinning into a spherical white silken cocoon. The adult emerges in about 5 days. Winter is spent in the cocoon or adult stage, depending on species. Duelli (2001) reports three types of flight behavior for the dispersal strategy of *Chrysoperla*: migration flights to and from overwintering sites, preovipository migration flights and continued nomadism during their reproductive period (Duelli 2001).

Migration to and from overwintering sites may be specific to the *carnea*- group species of Europe. These migration flights may occur at any time during and after diapause; only temperatures below 10° C, not short daylength, seems to limit flight activity. In California, *C. plorabunda* adults disperse widely after emerging from their cocoons during preovipository migration flights in spring and summer. Because they have immature gonads, they can't mate nor oviposit. After two nights of flights, females will copulate and start to oviposit after another two to four days. During these preoviposition flights, females do not get distracted by either vegetative stimuli nor potential mating partners whereas pregnant females do react to food stimuli and land. This age-dependent reaction was shown both in the field and in laboratory experiments using artificial food sprays (Duelli 2001). It is believed this obligatory preovipository migration flight is an adaptive dispersal strategy that displaces *Chrysoperla* spp. downwind, mimicking the downwind dispersal flights of aphids, their major prey. Depending on the wind speed, the dispersal capacity may be over dozens of kilometers. Unlike most insects which become sedentary after they begin their reproductive period, *Chrysoperla* exhibit nomadism throughout their reproductive period. In California and in Switzerland, reproductively active females remain in agricultural fields for an average of only two days (Duelli 2001). If they

are flying lower than 16.4 feet (5 m) above ground, they are probably in an “appetitive” flight, meaning they will be strongly attracted to the scent of honeydew and nectar. This is the case whether the flight is back from their overwintering site or post-migratory (Duelli 2001). *Carnea* group species are reported to have reproductive diapause between August and May in mild climates. Adults with reproductive diapause have a longer longevity than those that do not (Canard and Volkovich 2001).

Augmentative biocontrol. In a nursery setting where natural enemies may be absent or not abundant enough to suppress pest populations, augmentative control is a viable management option (Shrewsbury and Smith-Fiola 2000). Both *C. plorabunda* and *C. rufilabris* are suitable for augmentative release, but *C. rufilabris* is the species currently sold by commercial insectaries. The larvae, sometimes called “aphid lions”, are known to be predaceous on many soft-bodied insects and mites, including insect eggs, thrips, mealybugs, immature whiteflies and small caterpillars. They can consume over 200 aphids or other prey per week. *Chrysoperla* spp. are sold as adults in deli containers and as larvae in bottles with vermiculite or “hexcel units” where each larva is confined in its own cell to prevent cannibalism. Adults fly off once the container is opened thus may be appropriate for greenhouse environments. Both bottles and hexcel units can be tapped to release *Chrysoperla* larvae in a controlled manner making them practical release methods. Suppressing *S. pyrioides* populations below damaging levels with commercially available *Chrysoperla* seems feasible given *Chrysoperla* are found associated with *S. pyrioides* in landscape habitats. Indeed, lab studies have demonstrated that *Chrysoperla* larvae feed on *S. pyrioides* (Shrewsbury and Smith-Fiola 2000). Furthermore, in a no-choice lab study, *Chrysoperla* larvae hardly fed on adult *S. pyrioides* when placed together in a Petri dish (Lee et al. 2018).

Though in the same genus, rhododendrons and azaleas have a different architecture. Whereas studies in the eastern U.S. have mostly focused on azaleas, in the west the studies have focused on rhododendrons. In greenhouse studies, releasing *Chrysoperla* larvae onto azaleas using a camel hair brush significantly increased *S. pyrioides* mortality; greater mortality was achieved with the release of higher number of predators no matter the size of the prey population (Shrewsbury and Smith-Fiola 2000). In the same study, the authors reported *Chrysoperla* larvae disappeared from the treatment area after only two days, possibly due to dispersion or to

cannibalism. An additional experiment was conducted to determine whether *Chrysoperla* could control *S. pyrioides* in a production nursery. Foliage from azaleas within each treatment group were just touching and a plant-free zone of 4.9 feet (1.5 m) was created between each group. Hexcel units were lightly tapped over the azaleas to knock *Chrysoperla* onto the plants. This reduced *S. pyrioides* by 97% with a release average ratio of 1:2.6 (predator:prey), showing that the tapping method was practical and appropriate in nurseries where hot spots of *S. pyrioides* have been identified among azalea plants (Shrewsbury and Smith-Fiola 2000). Since rhododendrons have widely spaced leaves compared to the tight leaf arrangement of azaleas there was concern that tapped *Chrysoperla* larvae tapped may fall through to the ground depending on how densely the leaves were arranged. This in fact, is not the case. Lee et al. (2018) determined 99% of larvae tapped landed onto larger pots, 88% onto smaller pots of large-leaved rhododendrons, and 98% onto small pots of small-leaved rhododendrons. Additionally, shaking eggs from a bottle, resulted in 38% and 79% of eggs landing on dry and wet leaves, respectively (Lee et al. 2018). Egg cards may be more suitable where pest infestations are not concentrated. In a separate experiment, *Chrysoperla* release was more effective when most *S. pyrioides* were in the nymph stage than when they were in the adult stage possibly due to the higher mobility of adults and that nymphs were more susceptible to predation. When released on potted rhododendrons, *Chrysoperla* larvae controlled *S. pyrioides* nymphs + adults (70-86% reduction) in the short-term, but differences were marginal or non-significant 3-6 weeks later (Lee et al. 2018).

In an efficacy comparison between *Chrysoperla* versus the organophosphate acephate, the average control of *S. pyrioides* was 84% and 100%, respectively (Shrewsbury and Smith-Fiola 2000). However, the tendency of *Chrysoperla* larvae to disappear once *S. pyrioides* populations had declined meant *Chrysoperla* would be unable to give season-long control. Thus, multiple releases or integration with other management tactics would be necessary which, due to the higher costs and additional management, may not be permissive. The authors conclude that trying to justify the use of *Chrysoperla* as a biocontrol method given the long-term suppression (~ 3 months) provided by systemic chemical controls could be a challenge (Balsdon et al, 1993, Shrewsbury and Smith-Fiola 2000).

Attracting naturally occurring Chrysopidae. Volatile organic compounds (VOCs) are chemicals produced and emitted by plants in response to abiotic and biotic stresses (Lucchi et al. 2017). VOCs are considered semiochemicals which mediate interactions between individuals belonging to different species and benefit both the emitter and the receiver (Rodriguez-Saona et al. 2012). They act as mediators in intra- and interspecific plant interactions as well as between plants and other organisms especially arthropods belonging to different trophic levels. When the biotic stressor is an herbivore, the compounds are referred to as herbivore-induced plant volatiles or HIPVs. HIPVs include green-leaf volatiles such as C6 aldehydes, alcohols and acetates, terpenes and aromatic compounds. Arthropod predators use these HIPVs to locate their prey (Salamanca et al. 2017).

HIPVs influence the structure and dynamics of ecosystems by inducing indirect host plant resistance, repelling phytophagous insects, concentrating natural enemies into a specific area (Lucchi et al. 2017) and sometimes regulating herbivore reproduction (Mallinger et al. 2011). Variable and contradictory results exist in the literature regarding the specificity of HIPVs to target species. HIPVs can increase the diversity and density of beneficial arthropod species which result in a decrease in pest numbers and crop damage but, in some cases, using HIPVs results in more insect pest damage and only a slight attraction on parasitoids. Interactions among organisms can be influenced by several factors so the role of VOCs can change depending on the environmental context, developmental stage of the target species and the number of compounds perceived by the organism (Lucchi et al. 2017).

Various studies have used HIPVs in innovative strategies. The “push-pull” approach uses the HIPV to both repel the pest and attract the parasitoid. For example, when *Melinis multiflora* was intercropped with maize in Kenya, the HIPVs of *M. multiflora* resulted in increased parasitism of the stem-borer larva as well as repelling the adult (Rodriguez-Saona et al. 2012). Conversely, HIPVs can cause undesirable effects such as decreasing natural enemies in the surrounding areas due to the concentration in treated areas or HIPVs may stimulate plants to produce other VOCs influencing the dynamics of the ecosystem by attracting other insects (either non-target natural enemies or additional herbivores) (Jones et al. 2011).

Different genera of Chrysopidae are highly attracted to semiochemicals that they produce themselves (Toth et al. 2009). For example, the elliptical epidermal glands found only on the

male abdominal sternites of *Chrysopa oculata* Say, secretes a powerful aggregation pheromone. No specific pheromones have been found yet for *Chrysoperla* (Toth et al. 2009), however it is known various scents do attract *Chrysoperla*. Salamanca et al. (2017) summarized *Chrysoperla*'s attraction to methyl salicylate (MeSA). MeSA is a phenolic compound which has been identified in the herbivore-induced volatile blends from at least 13 crop plant species (James and Price 2004) and has been shown to increase natural enemies in a crop field including Coleoptera, predaceous Heteroptera, Diptera, parasitic Hymenoptera, and Neuroptera (Lucchi et al. 2017). MeSA may also repel pests from crop plants, possibly by affecting their behavior (Mallinger et al. 2011). MeSA has been documented to itself induce the salicylic acid defense pathway causing the release of MeSA in healthy plants thus extending the spatial range of this volatile's effects in an agroecosystem (Mallinger et al. 2011).

Although MeSA has been shown to attract several species of Chrysopidae, such as *Chrysopa nigricornis*, *Chrysopa oculata* and *Chrysoperla rufilabris*, but not to *C. plorabunda* under field conditions (Salamanca et al. 2017), some studies suggest combinations of HIPVs result in a higher attractiveness to Chrysopidae relative to single compounds alone. With the goal of achieving optimal trapping of *Chrysoperla* spp., Jones et al. (2016) tested several compounds singly and in combinations. MeSA and acetic acid (AA) were hardly effective as single compound lures but did seem to act synergistically if combined with other plant volatiles as described below; Jones in reviewing a meta-analysis of MeSA studies by Rodriguez-Saona et al. (2011) sorts out the possible reasons for the differences between studies. Acetic acid is thought to indicate fermentation of various food sources and may explain attraction of various types of insects (Toth et al. 2009). Overall the acetophenone (AP) + acetic acid (AA) + 2-phenylethanol (PE) combination was the most attractive lure. Other findings included the relative interchangeable nature of phenylacetaldehyde (PAA), PE and AP due to a shared common mode of action. The PE + AP combination resulted in some benefits over those of PE + PAA, AA acted additively with AP, and PAA acted synergistically with PE (Jones et al. 2016). An MS + AA + PE blend seems to be specific to *Chrysoperla* and not attractive to other Chrysopidae species, nor other natural enemies such as Syrphidae, Braconidae or Ichneumonidae (Lucchi et al. 2017). In Lucchi's study, Malaise traps set up near the HIPV blend caught an abundance of Braconidae and Ichneumonidae, while the sticky traps near the HIPV blend caught very few. In contrast, a low number of *Chrysoperla* were caught in the Malaise trap while a high number

were caught in baited sticky traps (although the authors remarked that other studies have found Malaise traps may be inadequate for trapping Chrysopidae). A “ternary” blend of PAA + MS + AA by Toth et al. (2009) has been shown to be very effective attracting *Chrysoperla carnea* (Koczor et al. 2015). Finally, simultaneously attracting *Chrysoperla* and *Chrysopa* species using a lure combination that is attractive to both has been studied and may be beneficial for biocontrol efforts since it simultaneously attracts species that predate as larvae (*Chrysoperla* spp.) and as adults (*Chrysopa* spp.) (Koczor et al. 2019).

The atmospheric concentration of attractants is an important factor in the success of multicomponent lures in the sustainable control of pests. The type of deployment device, initial amount loaded into the device, release rate, temperature, spatial range of the volatile, and foraging range of *Chrysoperla* affect recommended density of lure per hectare (James and Price 2004). Ensuring the presence of a volatile compound in the “right” concentration is far from being fine-tuned. High loads of MeSA have been reported to repel *Chrysoperla* and other predators (Pålsson et al. 2019). MeSA sachets used by James and Price released 30 mg/d in lab conditions but possibly up to 60 mg/day under summer field conditions; Predalure, a commercial MeSA lure, deploys 35 mg/d at 30° C whereas those designed by Jones et al. (2016) released 78.6 mg/d.

Studies suggest a short range of attraction of *Chrysoperla* to MeSA lures. Mallinger et al. (2011) saw a decrease in attraction above 1.5 m, Lee (2010) reported declines at approximately 10 m or less from the point source for a Predalure dispenser, and Salamanca et al. (2017) documented 80% of captured *C. rufilabris* were very close to the release site with captures declining significantly at distances greater than 5 m with a maximum range of 20-30 m. If synthetic volatiles are capable of triggering plant to plant signaling, then this range would be greatly expanded (James and Price 2004, Mallinger et al. 2011). Finally, devices may not only deliver the initial loaded compounds but also breakdown products or impurities derived from synthesis; how the latter affects predator attraction is not clear (Pålsson et al. 2019).

It is known that vertical intrahabitat distribution varies by *Chrysoperla* species (Szentkiralyi 2001). Studies report a range of heights for lure placement based on observations of the most frequent flight heights. Examples include 1.5 - 2.0 m in the crown of trees (Toth et al. 2009), 1.5 - 3m high in a tree canopy (Jones et al. 2011), 1.8 - 2.0 m above ground in an orchard

(Koczor et al. 2015), 1.6 m above ground for an attraction longevity test in an orchard and at vegetation height when measuring biological control on barley plants (Pålsson et al. 2019).

Many studies have clearly shown Chrysopidae can be manipulated with HIPVs, some have even documented the ability to influence oviposition and overwintering. Eggs were found laid at a close distance to the lures (Koczor et al. 2015, 2017) and the number of *Chrysoperla* spp. was clearly different between baited and unbaited overwintering chambers (Koczor et al. 2015). Despite this knowledge, it is still not clear if HIPVs can be used successfully to improve biological control. In the case of *C. carnea* for example, it is known that newly emerged adults respond to a vegetative stimulus only at the end of a "migratory flight". That is, they may fly up to 40 km away from their point of emergence with a favorable wind before showing sedentary behavior, descending to foliage level and mating. In other words, for an HIPV lure to be effective it would have to attract several adult generations of *Chrysoperla* during an infestation because every new generation may potentially migrate (Lucchi et al. 2017). Another reason why the manipulation of HIPVs may not be directly tied to successful biological control may be the role played by phytophagous populations. Based on previous reports, Lee (2010) and Gadino et al. (2012) speculate that if prey availability is low or absent when lures are present, predators may learn to avoid HIPV-baited areas.

Silicon supplementation to enhance resistance

Silicon, the second most abundant element on Earth, comes in various forms. Pure silicon is a mineral but is rarely found in nature. Silica, also known as silicon dioxide, is the oxide form of silicon and the most common form is quartz. Quartz is also part of a larger family known as the silicates. Silicone is any group of compounds which use a Si/O/Si chemical chain as a backbone and they frequently have properties of oils, greases or rubber.

Roots absorb the bioavailable form of silicon in soil solution, silicic acid. Plants can also take up ionic silicon when combined with common plant essential nutrients (Klittich, 2016). Silicon is translocated from the root upwards through the xylem vessels. Large amounts of silicon get deposited in cell walls of xylem vessels possibly to prevent compression of the vessels when transpiration rates are high (Ma and Yamaji 2006). At the leaf level, as the

epidermal cells form and expand, the dissolved but concentrated silica enters the cells and deposits as polymerized hydrated amorphous silica or pytholiths. Pytholiths are immobile once they get deposited, thus more silicon accumulates in older tissues (Ma and Yamaji 2006). In rice, a 2.5 micrometer layer forms beneath the cuticle layer of the rice blade. Various studies have revealed that the pattern and location of pytholiths may be more important than the content. Resistant cultivars tend to have a dense pattern, closer silica chains or more complete and even deposits (Alhousari & Greger 2018).

Although all terrestrial plants contain some silicon in their tissues, concentrations vary greatly. In general, there are three categories. Members of the Cyperaceae, Poaceae, and Balsaminaceae are high accumulators, accumulating >4% silicon on a dry weight basis. Moderate accumulators, 2-4%, include members of the Cucurbitacea, Urticacea, and Commelinaceae families. Low accumulators include most dicots ((Ma and Yamaji 2006, Alhousari and Greger 2018). Within the same family, ranges also differ for different species genera, for example within the Poeaceae barley ranges from 1.2 to 3.8 mg of Si /g, sugarcane 6.4- 10.2 mg Si/g, and rice 39mg Si/g. Within species ranges can differ also, japonica rice cultivars tend to accumulate more than indica. If supplemented, some plant groups will uptake silicon more readily than others even in dicots. For example, silicon supplemented zinnias had a higher silicon leaf content than the control (2.2% vs 0.49%) (Klittich 2016).

The differences in absorption, thus accumulation, appears to be due to the difference in expression of transporter genes (Liang et al. 2015, Deshmukh and Belanger 2016). The presence of an influx transporter protein (nodulin 26-like intrinsic proteins or NIPs) seems indispensable for a plant to absorb silicon, whereas the presence of both an influx and efflux transporter seem key for a plant to be able to accumulate silicon (Deshmukh and Belanger 2016). In rice two types of transporters exist at the root plasma membrane. Rice Lsi1 is the influx transporter and Lsi2 is the efflux transporter. Lsi1 belongs to the subfamily of aquaporins and moves silicon from soil solution into the root cells. Aquaporins are cell membrane proteins that form pores which conduct water into cells more rapidly than by diffusion alone; they help plant cells quickly recover turgor when water becomes available. Lsi2 is driven by a proton gradient, thus it can transport silicic acid against the concentration gradient out of the cells into the stele of the root.

Lsi1 and Lsi2 have been identified in monocots (rice, barley, maize, wheat) and dicots (pumpkin and horsetail) (Liang et al. 2015).

As far back as 1917 silicon's effect was first noted by Odonera on *Magnathorpe grisea*, a fungal pathogen (Klittich 2016), it took a few more years for scientists to begin documenting the resistance to insects. In the last 20 years, more and more studies have been published on the benefits of silicon – most of it on monocots, but studies on dicots are surfacing in the literature. A plethora of studies, in some cases contradictory, have evaluated the effect of silicon supplementation on arthropod herbivory involving Lepidoptera, Hemiptera, Diptera, Thysanoptera, Coleoptera and the mites (Table 2).

Mechanism of action of silicon mediated resistance

The hypothesized mechanisms to silicon mediated resistance to arthropod herbivory fall into two main categories: physical and chemical, each may have direct and indirect effects. The direct effects include abrasiveness, reduced digestibility, and interference with oviposition. The indirect effects are the consequences of delayed insect penetration into the plant including longer exposure to natural enemies, to adverse environmental conditions and to chemical controls. Both direct and indirect effects lead to the delay of development and decrease in population intensity by slowing the population growth.

Physical. The first hypothesized direct mechanism proposes silicon increases plant strength. Silicified leaves are abrasive, and evidence suggests they cause irreversible wear of mouthparts and deters chewing insects. However, if a larva must chew through the cuticle, it will result in mandible wear, but if it feeds on soft tissues between the epidermal layers, then no damage to incisor teeth are apparent (Alhousari and Greger 2018). Another hypothesis is that silicon damages the structure of the midgut causing reduced digestibility. Dos Santos et al. (2015) discovered leaf miners (*Tuta absoluta*) experienced detachment of epithelial cells from the basement layer surrounding the midgut when they treated tomato plants with various silicon products. Detachment of epithelial cells possibly affects food digestion and conversion to nutrients by digestive enzymes, slowing down insect growth. It could also reduce insecticide detoxification. Dos Santos et al. also observed the presence of cell protrusions analogous to

apoptotic bodies being released in the midgut lumen. This suggest a cytotoxic effect of silicon like that of Cry1 from *Bacillus thuringiensis.* Some studies have looked at the effect of silicon on plant hairs or trichomes. Chrysanthemums treated with additional silicon showed a significant difference in the length of trichomes and a numerical difference in the number of trichomes between treated and untreated groups. Increase in length and number of trichomes could possibly impede movement, affect oviposition preference and feeding rate (Klittich 2016). The impact of silicon on leaf miner population was a four-fold decrease in second generation adults accompanied by a decrease in stippling and a 54 % reduction of mines in chrysanthemums. Klittich concluded that the overall impact of silicon could be a perceived decrease in suitability of the host plant. Indirectly, these mechanical defenses may lead to delayed development and slow population growth of various herbivores. Han et al. (2015) looked at several parameters of development in the rice leaf folder (*Cnaphalocrocis medinalis*) in a silicon-mediated resistance study with a susceptible rice variety. Larval survival and pupation rate were significantly reduced apparently due to a significant decrease in the efficiencies of both ingested and digested foods of third instars.

Chemical. The second proposed major mode of action is a chemical one. Silicon in solution could influence plant biochemistry as a ligand of an organic metabolite affecting up and down gene regulation when plants are undergoing some sort of stress (Epstein 2009). Direct and indirect chemical hypotheses are discussed in the literature. In a direct path, silicon may elicit changes in the defensive chemicals of plants which are involved in the synthesis of lignin and suberin, in the production of chemicals with antibiotic properties, in the catalysis of compounds that lead to a reduction in the nutritional quality of food and decreased protein digestibility or in the production of phenolic compounds with deterrent and toxic properties (Reynolds et al. 2009). This mechanism was highlighted in a study by Goussain et al. (2005) in which wheat aphids (*Schizaphis graminum*) in silicon treated groups withdrew their stylets more often, hence reducing probing time. Pereira et al. (2010) used an electrical penetration graph (EPG) to further determine the probing behavior of the wheat aphid. There was a difference between treatment and control for total non-probing duration and the total duration of sap ingestion. Also, there were few honeydew droplets recorded in 12 hours among the silicon treatment, suggesting a reduction of sap ingestion. A reduction in the two described parameters seemingly led to the observed reduction in the number of nymphs in the treated group.

In an indirect path, silicon could influence volatiles cues. When a plant perceives herbivory due to effectors such as a biting, wounding damage, piercing and saliva, or egg laying damage and secretions, it releases herbivore induced plant volatiles (HIPVs) that attract predators and parasitoids. The plant then benefits from the natural enemies from the reduced herbivory and the natural enemies benefit from the prey or host insects on the plant. Silicon supplemented plants are known to produce stress signals such as salicylic and jasmonic acid which in turn trigger production of HIPV's. For example, Kvedaras et al. (2010) treated cucumbers leaves with silicon, then inoculated some of the plants with second instars of cotton bollworm (*Helicoverpa armigera*). The plants were held in a lab overnight, then placed in a field harboring large numbers of the red and blue predatory beetle (*Dicranolaius bellulus*) the following day. Cards baited with *H. armigera* eggs were placed near the bollworm infested plants. There was a significantly higher removal of eggs from the group treated with silicon *and* inoculated with the pest compared to the other treatments. Kvedaras et al. conducted a similar experiment in the lab placing a silicon supplemented leaf and a control leaf at the end of each arm of a Y-olfactometer, adding a larva to each leaf and subsequently releasing a predatory beetle at the base. Results corresponded to the field studies: twice as many beetles were attracted to the arm with the Si+ pest+ leaf. The authors believe the beetle was responding to a change in the HIPV blend which was a result of the synergistic effect of the biotic stress and the application of silicon (Kvedaras et al. 2010).

Table 1.1 Natural enemies of *Stephanitis pyrioides*

Natural enemy	Reference	Notes
Anagrus takeyanus Gordh (Hymenoptera:Mymaridae)	Braman et al., 1992	Does not seem to be affected by chemical insecticides
Stethoconus japonicus Schumacher (Hemiptera:Miridae)	Henry et al. 1986	Adventive, obligate predator. Egg hatching is synchronized with the second generation allowing it to escape from pesticides used on the first generation. Only effective with high populations of *S. pyrioides* due to its obligate nature
Chrysoperla carnea Stephen (Neuroptera:Chrysopidae)	Shrewsbury and Smith-Fiola 2000	Voracious predator of *S. pyrioides*. Available through commercial supplier
Chrysoperla rufilabris Burmeister (Neuroptera:Chrysopidae)	Lee et al. 2018	Voracious predator of *S. pyrioides*. Available through commercial supplier
Rhinocapsus vanduzeei Uhler (Hemiptera:Miridae)	Shrewsbury and Smith-Fiola 2000	Generalist
Dicyphus rhododendri Dolling (Hemiptera:Miridae)	Shrewsbury and Smith-Fiola 2000	Generalist
Orius tristicolor White (Hemiptera: Anthocoridae)	Shrewsbury and Raupp 2006.	Generalist
Anyphaena celer Hentz (Araneae:Anyphaenidae)	Shrewsbury and Raupp 2006.	Abundant on azaleas
Oecanthus fultoni Walker (Orthoptera:Gryllidae)	Shrewsbury and Raupp 2006.	Generalist
Forficula auricularia Linnaeus (Dermaptera:Forficulidae)	Shrewsbury and Raupp 2006.	Generalist

Table 1.2 Effects of silicon supplementation on phytophagous insects: (1) Keeping & Kvedaras 2008, (2) Ranger et al. 2009, (3) Keeping et al. 2009, (4) Reynolds et al. 2009, (5) Klittich 2016.

Species	**Family** ***Order***	**Type of feeder** ***Crop***	**Effect ?**	**Specific effects**
Spodoptera exempta (1)	Noctuidae *Lepidoptera*	Folivore *grasses*	Yes	Feeding deterrence, reduced growth rate
Schistocerca gregaria(1)	Acrididae *Orthoptera*	Folivore *grasses*	Yes	Feeding deterrence, reduced growth rate
Sitobion avenae(1)	Aphididae *Hemiptera*	Phloem feeder *n.a.*	No	No observed effects on feeding, nor population growth performance
Spodoptera eridania(1)	Noctuidae *Lepidoptera*	Folivore *artificial diet*	Yes	Reduced ability to digest diet leading to increase consumption rate
Spodoptera frugiperda(1)	Noctuidae *Lepidoptera*	Folivore *corn*	Yes	Increased mortality, cannibalism, mandibular wear
Herpetogramma phaeopteralis(1)	Pyralidae *Lepidoptera*	Folivore *turfgrass*	No	No effect on feeding or development
Agrotis ipsilon(1)	Noctuidae *Lepidoptera*	Folivore *turfgrass*	No	No effect on feeding preference, mandibular wear, growth or survival/ turf grass
Sesamia calamistis(1)	Noctuidae *Lepidoptera*	Stem borer *maize*	Yes	Reduced larval survival, percentage pupation and adult emergence
Eldana saccharina(3)	Pyralidae *Lepidoptera*	Stem borer *sugarcane*	Yes	Reduced survival, growth rate, delayed penetration of 3rd instar into stalk
Sitobion avenae(1)	Aphididae *Hemiptera*	Phloem feeder *wheat*	Yes	Reduced infestation
Metopolophium dirhodum(1)	Aphididae *Hemiptera*	Phloem feeder *wheat*	Yes	Reduced infestation

…Continued

Species	Family *Order*	Type of feeder *Crop*	Effect ?	Specific effects
Schizaphis graminum[1]	Aphididae *Hemiptera*	Phloem feeder *wheat, sorghum*	Yes	Reduced preference , lower longevity and fecundity
Schizaphis graminum[1]	Aphididae *Hemiptera*	Phloem feeder *wheat*	No	No effect on ability to penetrate stylet, nor on ability to reach the phloem vessels. Did increase frequency of stylet withdrawal, decreased probing time
Schizaphis graminum[1]	Aphididae *Hemiptera*	Phloem feeder *wheat*	Yes	Plant defense system activated, affected preference and population growth rate, increase of three enzymes involved in defense response
Bemisia tabaci[1]	Aleyrodidae *Hemiptera*	Phloem feeder *cucumber*	Yes	Response identical to that produced by BTH (synthetic analogue of salicylate- a natural plant elicitor)
Myzus persicae[2]	Aphididae *Hemiptera*	Phloem feeder *zinnia*	Yes	Reduction in fecundity and population fitness
Liriomyza trifolii[4]	Agromyzidae *Diptera*	Folivore *chrysanthemum*	Yes	Reduction of leaf mines and stippling
Sogatella furcifera[1]	Delphacidae *Hemiptera*	Xylem feeder *rice*	Yes	Decreased food intake, growth, adult longevity, fecundity and population growth
Nilaparvata lugens[1]	Delphacidae *Hemiptera*	Xylem feeder *n.a.*	Yes	Inhibited sucking behavior
Tetranychus urticae [5]	Tetranichidae Trombidiformes	Sap feeder *rice*	Yes	Significant population reduction
Liriomyza trifolii [5]	Agromyzidae *Diptera*	Folivore *Chrysanthemum zinnia*	Yes	Significant population reduction and leaf mines

CHAPTER 2

Attracting naturally-occurring Chrysopidae with plant volatiles for lace bug control

Katerina Velasco-Graham, Man-Yeon Choi and Jana C. Lee

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Abstract

The azalea lace bug (*Stephanitis pyrioides* Scott) (Hemiptera: Tingidae) is an invasive pest of rhododendrons and azaleas (Ericaceae: *Rhododendron*) that feeds on the underside of leaves causing chlorosis, reduced photosynthesis and even plant death. While insecticides can control this pest, growers, landscape managers and homeowners have requested softer alternatives. Augmentative release of predatory green lacewing *Chrysoperla* (Neuroptera: Chrysopidae) eggs and larvae has reduced *S. pyrioides*, but large scale implementation may not be practical nor cost effective. Attracting naturally occurring Chrysopidae with herbivore-induced plant volatiles (HIPV) and floral volatiles may be an economical and less time-consuming option. In this study, we tested whether volatiles blends: 1) attracted *Chrysoperla* spp., and 2) controlled *S. pyrioides* populations on *Rhododendron* spp. in farm or urban landscapes. Experimental plots contained no lures and two or three different non-commercial multicomponent lures placed 1.2 m aboveground next to plants. Adult *Chrysoperla* were monitored in May-June in a commercial field; and adult *Chrysoperla*, *S. pyrioides* from egg to adult stages, and other natural enemies were monitored in both farm and urban landscapes from July to September for two years. Overall, sticky traps near plants baited with a blend of methyl salicylate + acetic acid + 2-phenylethanol (methyl salicylate blend) and a blend of acetophenone + acetic acid + 2-phenylethanol (acetophenone blend) captured more adult *Chrysoperla* than control traps in farm landscapes. Only the acetophenone blend in a farm landscape was associated with a reduction of *S. pyrioides*, the other multicomponent lures did not show a suppressive effect. Spiders, predatory hemipterans and predatory mites were attracted to the methyl salicylate blend and Chalcidoidea and Ichneumonidae were attracted to a blend of phenylacetaldehyde + methyl salicylate + acetic acid. The complexities of using plant volatiles to attract Chrysopidae for a biological control program for *S. pyrioides* is discussed for urban landscapes in the Willamette Valley.

Key words: azalea, biological control, *Chrysoperla*, herbivore-induced plant volatiles, Oregon rhododendron, *Stephanitis pyrioides*.

Introduction

The azalea lace bug, *Stephanitis pyrioides* Scott, 1874 (Hemiptera:Tingidae) is an invasive pest from Asia of ornamental rhododendrons and azaleas in the genus *Rhododendron* (Ericaceae) and was detected in the Pacific Northwest in 2008-9 (Rosetta 2013). *Stephanitis pyrioides* feeds on the underside of leaves causing chlorosis, decreased photosynthesis, and eventual plant death (Nair and Braman 2012). Aesthetic damage appears as yellow stippling on the upper surface of leaves and sticky residue due to frass and exuviae on the lower surface. Consumers can detect leaves with only 2% surface damage and will reject plants with 11% of leaves damaged, thereby *S. pyrioides* can substantially reduce profits in nursery and greenhouse programs (Klingeman et al. 2000). Hence, nursery and private collection growers apply systemic insecticides to control this pest (J. Lee and R. Rosetta, personal communication). Chemical insecticides are effective in controlling this pest (Balsdon et al. 1993, Shrewsbury and Smith-Fiola 2000), botanicals are partially effective (Gill and Raupp 1989), and insect growth regulators may be important ovicidal and nymphicidal tools, especially when paired with an adjuvant (Joseph 2019).

The high cost of some chemicals, difficulty of spraying contact insecticides on the underside of leaves, and insecticide persistence and resistance justify the adoption of alternative management methods. Biological control is an attractive option because managers are concerned about using insecticides in rhododendron parks frequented by people and having runoff to ponds and streams with birds and fish. Unlike the eastern U.S., specialized parasitoids and predators of *S. pyrioides* have not been detected in Oregon (Lee et al. 2019). Growers could rely on augmentative releases of the green lacewings, predators which have reduced infestations in azaleas (Shrewsbury and Smith-Fiola 2000) and in rhododendrons (Lee et al. 2018). Park managers and homeowners may favor naturally-occurring predators which were reported to influence *S. pyrioides* across habitat types (Shrewsbury and Raupp 2006). These authors suggested that sustainable low input urban landscapes with low pest pressure could be created by manipulating landscape plants according to species composition, spatial arrangement and temporal longevity while using pest resistant cultivars, cultural practices and biological control.

Pressurized water sprays coupled with Chrysopidae egg cards could suppress two overlapping generations of *S. pyrioides*, effectively reducing the population over time in

specimen gardens (Lee et al. 2018). Nonetheless, the strategy was time-consuming and may be cost-prohibitive for some. Hence, the next step in arriving at an efficient and affordable management program to control *S. pyrioides* is to investigate how to increase the abundance of naturally-occurring predators. Attacks by herbivores trigger changes in the chemicals produced and emitted by plants. These compounds, known as herbivore-induced plant volatiles (HIPV), are signals that attract natural enemies to the source of the attack. Publications worldwide have reported success in attracting naturally occurring Chrysopidae using single compound HIPV lures (reviewed by Kaplan 2012). Fewer have studied whether an increase in HIPV-recruited predators translates to efficient pest control (James and Price 2004, Woods et al. 2011, Mallinger et al. 2011). One study has tested whether using a single HIPV lure (methyl salicylate) controlled *S. pyrioides* and found no evidence of control (Flores 2016). More recently, others have reported success in attracting natural enemies using a combination of HIPVs and floral volatile compounds in other crop systems (Jones et al 2011, 2016, Koczor 2015, 2017, 2019, Lucchi et al. 2017, Toth et al. 2009). Only one study has looked at the effectiveness of the HIPV + floral volatile blend for biocontrol (Pålsson et al. 2019), a blend which has also been shown to enhance Chrysopidae oviposition (Koczor et al 2017). To date, no study has examined the effectiveness of attracting Chrysopidae with multicomponent lures to control *S. pyrioides*. Hence, it was hypothesized that multicomponent lures placed near *S. pyrioides* infested plants would successfully attract Chrysopidae adults, enhance oviposition, and hatched larvae would predate on nymphs leading to a population decrease of *S. pyrioides*. Therefore, the objectives of this study were to determine whether baiting infested plants with three different multicomponent lures would impact the abundance of Chrysopidae and control of *S. pyrioides* eggs, nymphs and adults in farm and urban landscapes in the Willamette Valley of Oregon.

Materials and Methods

Over two years, five field studies were conducted to test various HIPV and floral volatile blends. Because some sites had few *Chrysoperla* in 2018 and sampling protocols were suitable for specific plant types, sites and in-field samples were adjusted to better test hypothesis in 2019 (outlined in Fig. 2.1).

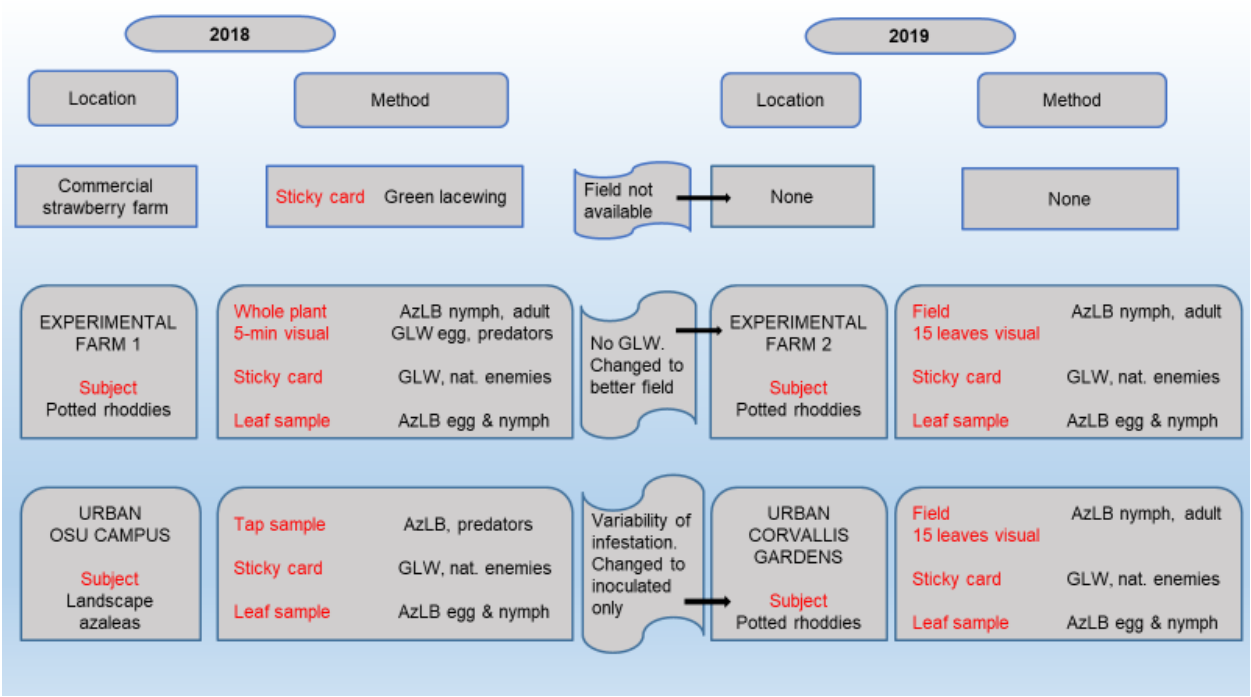

Fig. 2.1. Site location and methodology for testing Chrysopidae attractiveness and *S. pyrioides* control.

Lure preparation. Three multicomponent lures were compared: 1) methyl salicylate + acetic acid + 2-phenylethanol (MS blend, Jones et al. 2016), 2) acetophenone + acetic acid + 2-phenylethanol (AP blend, Jones et al. 2016), and 3) phenylacetaldehyde + methyl salicylate + acetic acid (PAA blend, Koczor et al. 2017). Lures were prepared by cutting polyethylene tubing (Associated Bag Company, WI) of various thicknesses (Table 2.1) into 7.5 x 5 cm sections. Each attractant in Table 2.1 was added to a 3.8 cm long dental cotton wick, placed inside tubing and heat-sealed into a pouch. Three pouches were tied together to create one multicomponent lure for the MS and AP blends. The PAA blend had all three attractants added to the *same*, smaller cotton roll/pouch. The ratios used were MS:AA:PE (4.1 : 3.1 : 1.0 g/ml), AP:AA:PE (3.1 : 3.1 : 1.0 g/ml) and PAA:MS:AA (0.1 : 0.1 : 0.1 g/ml). For the second-year study, additional acetic acid pouches were replaced in the existing lures every two weeks because the release rate drops significantly after that period (Pålsson et al. 2019). All trials included testing a control, MS and AP blend treatments; the PAA blend was included in 3 of the 5 studies when enough plants and adequately spaced sites were available.

Table 2.1. *Chrysoperla* spp. volatile attractants used for lures based on Jones et al. (2016)[1] and Koczor et al. (2017)[2].

Attractant	Pouch thickness (mm)	Cotton Roll (cm)	Release rate (mg/d)	Volume (ml)	Source
Acetic acid (AA)[1]	0.1016	3.8	50.2	3.0	Acros Organics-222140010
Acetophenone (AP)[1]	0.1016	3.8	58.7	3.0	Acros Organics-102410010
Methyl Salicylate (MS)[1]	0.1524	3.8	78.6	3.5	SAFC Supply Solutions-W274518-1 KG-K
2-phenylethanol (PE)[1]	0.0381	3.8	12.8	1.0	Sigma-Aldrich 77861-250 ml
Phenylacetaldehyde (PAA) + MS + AA [2]	0.02	1.0	n/a	100 ul each	PAA: Acros Organics-370911000

***Chrysoperla* attraction study.** In May 2018, we set up an early season attraction study in a commercial strawberry field. Many *Chrysoperla* have previously been captured in this field (Lee 2010), and no pesticide applications were applied before harvest, making this an optimal site to first test response. We could not introduce infested rhododendron plants in the commercial farm, and only *Chrysoperla* attraction was compared. Three treatments (MS blend, AP blend and control) were replicated in 5 blocks. Each treatment had a 120 cm tall wooden stake placed into the ground with a 10 x 10 cm plastic sheet and the multicomponent lure/none at the top of the stake. Distance between blocks was 100 m, and 40 m between treatments within the blocks to prevent cross-treatment overlap based on prior studies that found treatment differences (Table 2.2).

Table 2.2. Past studies on *Chrysoperla* attraction: distances between blocks and treatments

Author(s)	Distance between blocks (m)	Distance between treatments (m)
Lucchi et al., 2017	-	30
Koczor et al., 2017	20-30	5-8
Jones et al., 2016	40	20
Lee, 2010	100+	100
Toth et al., 2009	15-20	5-8

The next four studies occurred mid to late season when *S. pyrioides* were more abundant to study *Chrysoperla* and general natural enemy attraction, and subsequent *S. pyrioides* control.

Rhododendrons in farm-2018. Twenty-four rhododendrons, 12 'Boule de Niege' and 12 'Cunningham White', were re-potted with Professional Growing Mix (Sun Gro® Horticulture, MA) into 3-gal (11.35 L) pots in February 2018 and fertilized with Rhododendron, Evergreen & Azalea Food 10-5-4 (Lilly Miller, CA). Plants were held in an outdoor yard from March to June until the experiment began. Two weeks prior to data collection, rhododendrons were transported to a USDA experimental farm (44.561° N, 123.230° W), inoculated with 20 adult *S. pyrioides* from our greenhouse colony and covered with an organza bag to prevent emigration.

The experimental farm was bordered by rural backyards on two sides, and by forest or road on the other two. Potted rhododendrons were set up along the western and northern sides of the farm (Fig. 2.2). Three treatments (MS blend, AP blend and control) were replicated in 4 blocks. Each treatment had a 120 cm tall wooden stake placed into the ground with a 10 x 10 cm plastic sheet and the multicomponent lure/none at the top of the stake with two potted plants 30 cm away on either side of the stake.

Treatments were arranged in a complete randomized block design. At the southwest corner of the farm, two blocks were placed 0.6 m away from the tree-shaded western fence. On the northwest corner, two additional blocks were placed under trees located 5 m away from the fence, these locations provided shade which rhododendrons and *S. pyrioides* prefer (Trumbule et al. 1995). Distance between blocks was 100 m and 40 m between treatments to reduce volatile overlap and prevent cross-treatment movement of predators.

Azaleas in urban landscape-2018. Established landscape azalea bushes on the Oregon State University campus (44.565° N, 123.277° W) were chosen after initial scouting revealed *S. pyrioides* infestation was higher on azaleas than on rhododendrons. Before experimentation, *S. pyrioides* egg densities were determined from 20 randomly picked leaves per bush and bushes were ranked from highest to lowest. Bushes were assigned to treatments and controls such that starting egg density in each treatment were similar. Four treatments (MS blend, AP blend, PAA blend and control) were replicated 4 times in a complete randomized design. Lure placement was as described earlier.

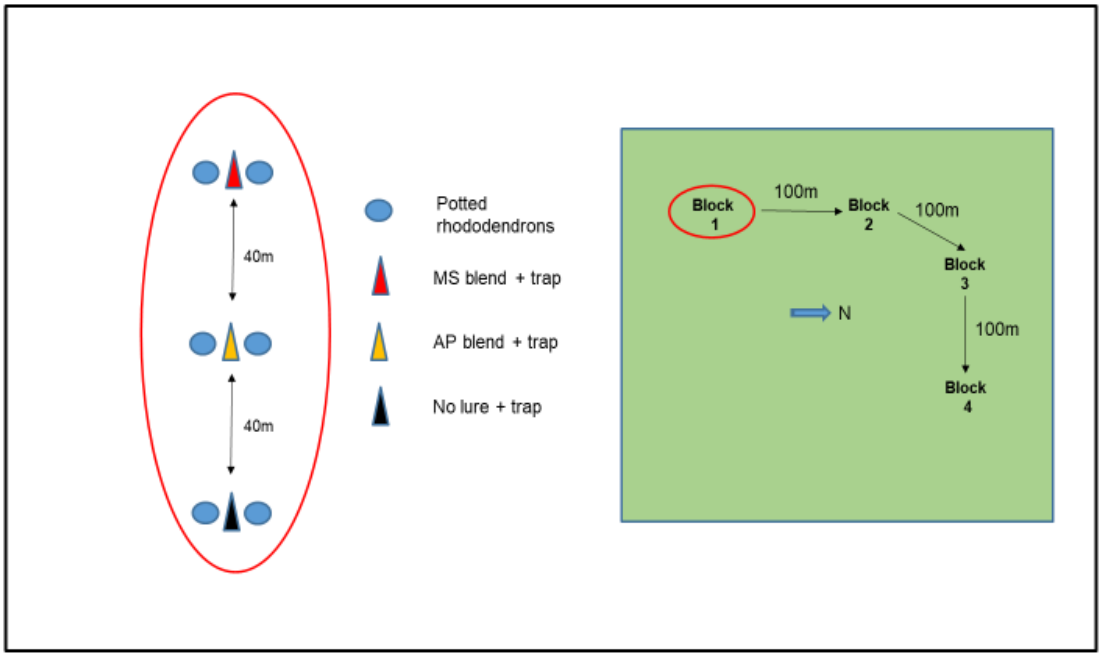

Fig 2.2. Layout of Rhododendrons in farm-2018 study.

Rhododendrons in farm or garden-2019. In the second year, 32 potted rhododendrons (Cunningham White and Boule de Niege) were fertilized in March 2019. All plants were inoculated for 3 consecutive weeks prior to the experiment with a minimum of 30 *S. pyrioides* adults per week which were collected from landscape bushes in late May and early June 2019. The experiment was set up in two distinct landscapes: a raspberry field at a second USDA experimental farm (44.551° N, 123.217° W) and four home gardens located in and around Corvallis, OR. The farm study moved in location because relatively few *Chrysoperla* were observed in the 2018 site (compared to the early season study), and *Chrysoperla* were previously seen in the 2019 site in the prior year. Four treatments (MS blend, AP blend, PAA blend and control) were replicated 4 times in a complete randomized design in both the raspberry field and home garden studies; each home was a block. Treatments consisted of one plant with one multicomponent lure affixed above the canopy of the plant. Plants were placed next to the raspberry canes in the farm and under trees in the garden to maximize shade and prevent leaf scorching. Distance between treatments was at least 23 m in the raspberry field and 30 m in the gardens. For both years, lures were deployed in fields or landscapes with no insecticide applications and rhododendrons were irrigated with ceramic watering spikes (Blumat Watering System, Austria).

Sampling

***Chrysoperla* attraction study**. A white sticky card trap (29.21 x 23.49 cm) was placed 1.2 m above ground, 0.6 m away from the lure. Sticky card traps were replaced weekly for four weeks. Our study only identified *Chrysoperla* to genus and not species for two reasons: 1) specimens were sometimes damaged on sticky traps, and 2) traditional morphology may be of limited value. There is disagreement among systematists on whether more advanced identification tools such as mating signals or ecophysiological criteria are better at distinguishing *Chrysoperla* species from each other (Henry et al. 2001).

Rhododendrons in Farm-2018. In the field, we conducted 5-minute visual counts of *S. pyrioides* and predators by raising potted plants to eye level. Three leaves from the base of each rhododendron were collected. A sticky card trap was placed 1.2 m above ground on a stake 0.6 m away from the lure to monitor natural enemies and replaced weekly. In the lab, collected leaves were first checked for *S. pyrioides* nymphs, then washed gently with warm water and a soft sponge to remove frass and sticky residue. This exposed *S. pyrioides* eggs for counting under a stereomicroscope. Leaf area was measured (LI-300C, LI-COR, Lincoln, Nebraska). Sticky cards from this and subsequent studies were screened for Cynipoidea, Mymaridae, Ichneumonidae and Braconidae families and for Chalcidoidea, Platygastroidea, Ceraphronoidea and Proctotrupoidea superfamilies. Though Mymaridae is part of the Chalcidoidea superfamily, they were analyzed separately because of their abundance. All sampling occurred weekly from July through August 2018.

Azaleas in urban landscape-2018. Vegetative tap samples were taken by dividing azalea bushes into four sections; smaller bushes were divided into three sections. Depending on the leaf density of the bush, one or several branches were randomly picked from each section and tapped vigorously 10 times with a wooden spoon into a 30 x 28.5 cm plastic funnel placed under the branches. Arthropods were tapped into the funnel and 1-gal (3.7 L) bag at the bottom. Twenty leaves were randomly removed from each bush for *S. pyrioides* egg counts. In the lab, tap samples were sifted through a 27 cm diameter screen to separate arthropods from debris. We counted spiders, predatory hemipterans, predatory mites, and coccinellids, and *S. pyrioides* adults and nymphs. Egg count and leaf area proceeded as described earlier. Tap samples were

done weekly and sticky cards were changed every two weeks from July through mid-September 2018.

Rhododendrons in farm or garden-2019. In the field, *S. pyrioides* adults were counted from 15 randomly chosen leaves, the leaves were left attached to the plant and *S. pyrioides* nymphs and predators were recorded as present or absent. Also, ten random leaves were taken to the lab, *S. pyrioides* nymph and eggs were counted and leaves measured as previously described. Natural enemies were monitored with a sticky card trap clipped onto a trellis, fence wire, or tree branch ~1.7 m above ground, and 0.6 m from the lure. Sampling occurred weekly for 10 weeks from July through September 2019.

Statistical Analysis

Analyses were conducted with R v. 3.6.2 (R Core Team, 2019).

***Chrysoperla* attraction study**. The total count of *Chrysoperla* adults was the response variable in a negative binomial generalized linear mixed model (GLMM). Treatment and week were the fixed effects, block and subject were random effects. Each trap location was the subject in a repeated measures model. There was no need for an autoregressive lag 1 correlation of errors.

Rhododendrons in farm-2018. The total count of *S. pyrioides* eggs or nymphs on leaves, or adults in the field were response variables in a negative binomial GLMM ('glmmTMB' package, Mollie et al. 2017). Treatment and week were fixed effects, and block and subject were random effects. Each pot was the subject in a repeated measures model with an autoregressive lag 1 correlation of errors if needed. Leaf area was an offset for egg counts to account for variation in leaf area. For analyzing predator and parasitoid response, either the genus, family, or superfamily count was the response variable with the same fixed and random effects as above. GLMM models were validated by visually examining a scaled residual plot ('DHARMa' package, Florian 2020) and using the sum of the squared Pearson residuals over residual degrees of freedom to check for possible overdispersion. If overdispersion occurred in the initial model, a different model was used (Tables 2.6 a & b, 2.7 a & b). Data was tested using Bayesian analysis with penalized regression via diffuse priors to deal with complete separation issues ('blme' Chung et al. 2013). Fixed factors and their interaction were checked for significance with

likelihood ratio tests fitting a full and reduced model. If an interaction was significant, treatment was tested against the full model with the interaction. If an interaction was not significant, it was removed from the model. Contrasts comparing the control with each treatment were analyzed using Dunnett's tests for post-hoc testing of the factor treatment ('emmeans' package, Lenth 2020).

Azaleas in urban landscape-2018. The GLMM, distribution and analysis for all parameters were identical to those used in 'Farm' except block was not included in the model, and the number of tap sections was used as an offset for beat samples.

Rhododendrons in farm or garden-2019. The total count of *S. pyrioides* eggs or nymphs on leaves, adults in the field, or natural enemies on sticky cards were response variables in GLMMs. A negative binomial distribution was used for most response variables; random effects, overdispersion adjustment, correlation of errors, offsets and use of penalized regression for complete separation were like the 2018 analyses. A binomial distribution was used for nymph presence/absence in the field and the overdispersion in this data set was addressed with the use of an added observation level random effect and an optimizer.

Results

The American Statistical Association expressed concern with using the main effect p-values by itself as evidence about underlying effects (Wasserstein and Lazar 2016). For this reason, main effects in Tables 2.6 and 2.7 are followed by the percent difference between treatments and the p-value associated with the contrast. In this paper, all stated differences were significant according to main effect tests, unless stated as numerical differences.

***Chrysoperla* attraction.** In the early season commercial field study, captures of *Chrysoperla* were different between treatments (lure χ^2=18.37, df=2, p<0.001, week χ^2=96.76, df=3, p<0.001, lure: week χ^2 =4.87, df=6, p=0.56). A total of 644 adults were trapped, the MS and AP blends captured 4.1 and 3.2 times more *Chrysoperla* than the control (p< 0.0001).

In the *Rhododendrons in farm* study, a total of 69 and 82 adult *Chrysoperla* were trapped on sticky traps in 2018 and 2019, respectively (Table 2.4). For adults, treated groups showed a numerical increase for 2018 and a statistical difference in abundance for 2019; baited traps caught 1.7 – 6.5 times more than the control (Table 2.6 a & b). Peak capture occurred the first two weeks in July of 2018 with the AP blend, in August 2019 with the MS blend (Fig 2.3). In 2018, there was no difference between treatments for *Chrysoperla* eggs, though it was numerically higher for both MS and AP blends compared to the control (Table 2.6). Larval *Chrysoperla* counts were too low for statistical analysis. In the *Azalea in urban landscape* and *Rhododendron in garden* studies, counts from all stages of *Chrysoperla* were too low for analysis in either year (Table 2.5, 2.7 a & b).

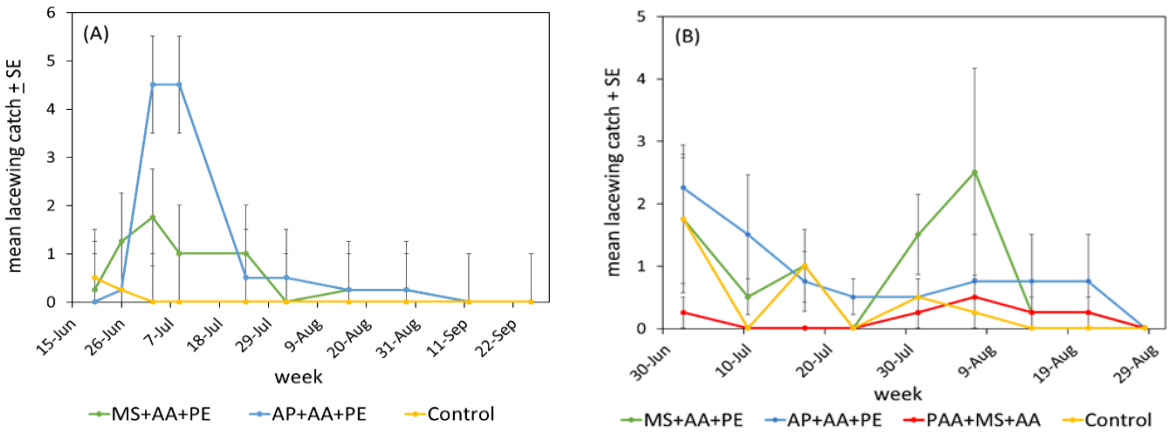

Fig. 2.3. Mean *Chrysoperla* sticky trap captures in *Rhododendrons in farm* study in (a) 2018, (b) 2019.

Stephanitis pyrioides. In the *Rhododendrons in farm-2018* study, a total of 1,061 eggs and 219 nymphs were found on leaf samples, and 294 adults in field counts. There was a difference between treatments and control for eggs and adults, but not for nymphs (Table 2.3 and 2.6a). There was a >80% reduction in eggs and adults with the AP blend (Table 2.6a). In *Rhododendrons in farm-2019*, 10,526 eggs and 1,408 nymphs were on leaf samples, and 1,051 adults from field counts. There was no difference between treated and untreated groups for eggs,

nymphs, adults or nymph presence (Table 2.3, Table 2.6b). Pest reductions with the different blends were mixed and less marked than in 2018.

In the *Azaleas in urban landscape-2018* study, a total of 18,811 eggs were found on leaf samples; nymph counts were too low to analyze. Tap-funnel samples had a total of 356 nymphs and 3,277 adults (Table 2.3). Due to an overdispersion in the statistical model which could not be addressed, we analyzed the nymphs and adults together as *mobiles*. There were no differences between treatments in eggs nor mobiles, though 51% and 60% numerically fewer mobiles were found in the MS and PAA blends than the control, respectively (Table 2.7a).

In the *Rhododendrons in garden 2019* study, a total of 8,656 eggs and 1,392 nymphs were on leaf samples, and 620 adults from field counts. Only nymphs showed an overall difference between treatments and control, with a numerical reduction of 66% with the PAA blend (Table 2.3, 2.7b).

Table 2.3. Mean ±SE for *S. pyrioides* egg, nymph and adults for 2018 and 2019 experiments. Asterisk denotes significance difference using Dunnett's tests.

	Subject	Landscape	Lure	Egg	Nymph	Adult
2018	Potted Rhododendron	FARM	MS blend	9.97 ±3.71	3.09 ±1.56	4.44 ±1.57
			AP blend	5.28 ±0.26*	0.84 ±0.42	0.81 ±0.23*
			Control	17.9 ±4.47	2.91 ±0.84	3.94 ±0.52
2018	Landscape Azalea	URBAN	MS blend	156 ±16.08	1.19 ±0.40	4.66 ±0.75
			AP blend	199 ±25.45	3.94 ±1.60	10.5 ±3.42
			PAA blend	106 ±12.72	2.16 ±0.67	4.70 ±0.80
			Control	128 ±11.29	3.84 ±1.44	8.10 ±1.12
2019	Potted Rhododendron	FARM	MS blend	68.7 ±11.83	6.04 ±2.65	5.77 ±1.05
			AP blend	51.8 ±9.50	11.4 ±4.13	5.48 ±0.97
			PAA blend	57.9 ±10.22	8.92 ±3.57	4.91 ±0.86
			Control	60.8 ±11.20	17.7 ±6.22	4.98 ±0.82
2019	Potted Rhododendron	URBAN	MS blend	54.1 ±8.02	9.17 ±2.90	2.71 ±0.54
			AP blend	34.8 ±4.71	5.24 ±1.59	4.71 ±1.11
			PAA blend	53.9 ±8.36	8.58 ±3.54	3.78 ±1.36
			Control	54.0 ±8.39	8.69 ±3.19	2.92 ±0.50

Natural enemy attraction. In the *Rhododendrons in farm-2018* study, predators were mostly encountered during a visual five-minute inspection of potted rhododendrons. There were no differences between the lure baited plants and the control plants (Table 2.6a). Among sticky traps, a total 5,441 natural enemy specimens were collected. Of these, 16.8% belonged to eight predator groups and 83.2% to six families and three superfamilies of parasitoids. The most abundant predators were spiders, predatory thrips (Aeolothripidae) and rove beetles whereas the most abundant parasitoids were the Plastygastroidea, Chalcidoidea and Mymaridae (Table 2.4). There was no difference between treatments in abundance for either predators or parasitoids though positive trends were evident on traps near lures (Table 2.6a).

In the *Rhododendrons in farm-2019* study, sticky traps had a total of 4,213 specimens of which 29% were predators and 71% were parasitoids. The most abundant predators were predatory thrips, minute pirate bugs (*Orius* spp.) and the coccinellid fungus-eating *Psyllobora* spp. The most abundant parasitoids were Chalcidoidea, Mymaridae and Platygastroidea (Table 2.4). Chalcidoids differed between treatments and control, sticky traps near the PAA blend had numerical higher catches (Table 2.4, 2.6b).

In the *Azaleas in urban landscape-2018* study**,** a total of 1,022 predators were captured during tap samples: 669 spiders, 195 predatory hemipterans, 110 predatory mites, 39 coccinellids and 9 *Chrysoperla* larvae. There was a difference between treatments and control for total predators and spiders (Table 2.5, 2.7a). Predatory mites and predatory hemipterans did not result in an overall difference, yet mites and hemipterans showed numerical increases with all blends (Fig. 2.4, Tables 2.5 and 2.7a). From sticky traps, a total of 1,256 natural enemies were captured, 7.5% were predators and 84.5% were parasitoids. The most abundant predators were spiders, the coccinellids *Psyllobora* spp. and *Stethorus* spp. whereas the Chalcidoidea, Plastygastroidea and Ceraphronoidea were the most abundant parasitoids (Table 2.5). Overall there were no differences between HIPV baited and non-baited azalea bushes (Table 2.7a).

Table 2.4. Total natural enemies on sticky traps near multicomponent lures in two farm landscapes in 2018 and 2019. MS = methyl salicylate, AA = acetic acid, PE = 2-phenylethanol, AP = acetophenone, PAA = phenylacetaldehyde, Ctr = control.

	2018 North Farm				2019 Lewis Brown				
Predators	MS+AA+PE	AP+AA+PE	Ctr	Total	MS+AA+PE	AP+AA+PE	PAA+MS+AA	Ctr	Total
Aeolothripidae	57	44	40	141	150	241	122	156	669
Chrysoperla	23	43	3	69	31	31	6	14	82
Coccinellidae	46	37	47	130	53	63	43	52	211
***Orius* spp.**	0	0	0	0	45	25	48	34	152
Spider	172	156	120	448	27	20	17	17	81
Staphylinidae	41	49	38	128	4	10	8	5	27
Total	**339**	**329**	**248**	**916**	**310**	**390**	**244**	**278**	**1222**
Parasitoids									
Braconidae	163	153	133	449	73	113	90	73	349
Ceraphronoidea	94	107	111	312	71	58	79	48	256
Chalcidoidea	339	334	242	915	167	170	282	209	828
Cynipidae	13	43	27	83	20	26	25	20	91
Ichneumonidae	30	43	47	120	20	25	36	10	91
Mymaridae	230	333	326	889	123	184	251	234	792
Platygastroidea	588	552	431	1571	117	122	133	127	499
Proctotrupoidea	91	45	50	186	21	17	31	16	85
Total	**1548**	**1610**	**1367**	**4525**	**612**	**715**	**927**	**737**	**2991**

In the *Rhododendrons in gardens-2019* study**,** a total of 2,000 natural enemies were captured from sticky traps, 11.75% were predators and 88.25% were parasitoids. The most abundant predators were the coccinellids *Psyllobora* spp., spiders and predatory thrips; the Chalcidoidea, Mymaridae and Platygastroidea were the most abundant parasitoids (Table 2.5). There were no overall differences, but the MS and PAA blend baited traps had numerically more *Psyllobora* spp. and the MS and AP blends more Chalcidoidea (Table 2.5, 2.7b).

Table 2.5. Total natural enemies on sticky traps near multicomponent lures during 2018 and 2019 in two urban landscapes.

	2018 OSU campus					2019 Corvallis Gardens				
Predators	MS+AA+PE	AP+AA+PE	PAA+MS+AA	Ctr	Total	MS+AA+PE	AP+AA+PE	PAA+MS+AA	Ctr	Total
Aeolothripidae	1	1	2	5	9	7	3	11	5	26
Chrysoperla	1	2	1	0	4	1	0	0	0	1
Coccinellidae	11	12	30	14	6	95	12	15	15	10
***Orius* sp.**	8	2	1	1	12	0	0	0	0	0
Spider	12	30	23	26	91	19	13	15	21	68
Staphylinidae	4	2	1	5	12	1	0	2	0	3
Total	**37**	**49**	**58**	**51**	**134**	**123**	**28**	**43**	**41**	**108**
Parasitoids										
Braconidae	14	21	18	12	65	6	5	1	4	16
Ceraphronoidea	33	25	22	32	112	38	25	25	43	131
Chalcidoidea	62	130	123	179	494	255	195	90	112	652
Cynipidae	2	2	0	0	4	0	0	0	0	0
Ichneumonidae	2	2	0	2	6	1	1	1	2	5
Mymaridae	15	17	21	15	68	222	105	144	148	619
Platygastroidea	49	76	59	92	276	84	45	108	79	316
Proctotrupoidea	16	8	3	9	36	6	4	6	10	26
Total	**193**	**281**	**246**	**341**	**1061**	**612**	**380**	**375**	**398**	**1765**

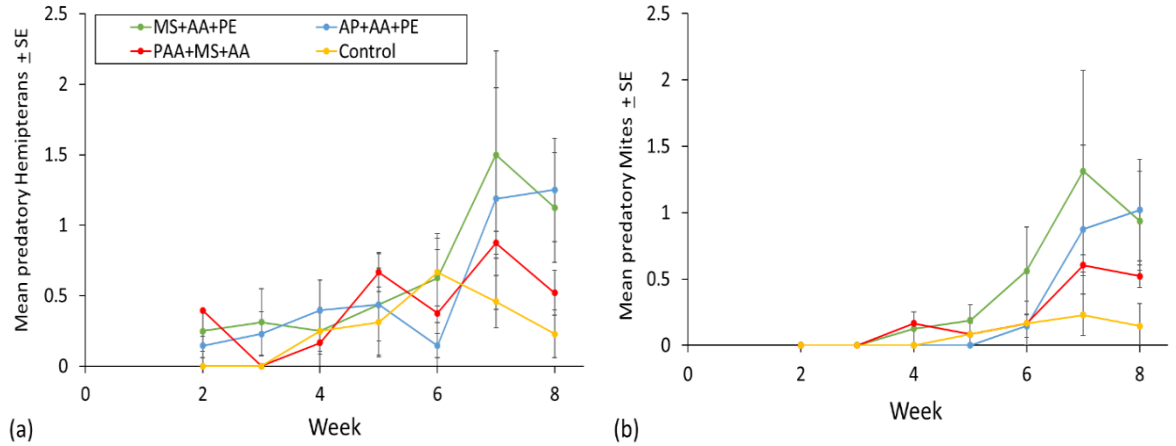

Fig. 2.4. Mean predatory hemipterans (a) and predatory mites (b) for three multicomponent lure treatments and control in 2018 tap samples of azalea bushes in an urban setting.

Table 2.6a. Analyses of *Rhododendrons in farm-2018* study for insect counts from leaf samples, in-field counts, or sticky traps. Percent improvement is followed by p-value for the specific contrast, and asterisks denote significant differences using Dunnett's test.

Sample	Response variable	Stat. Test (Distribution)	Fixed Factor/s	χ^2	df.	p-value	% Improvement
							Reduction
Leaf samples	*S. pyrioides* eggs	GLMM (NB)	Lure formulation	9.34	2	0.009*	MS 43 [p= 0.6]
			Week	29.24	7	<0.001	AP 88 [p=0.004]*
			Formulation: Week	22.56	14	0.068	
	S. pyrioides nymphs	GLMM (NB)	Lure formulation	3.84	2	0.15	MS 81 [p= 0.37]
			Week	24.10	7	0.001	AP 87 [p= 0.25]
			Formulation: Week	8.64	14	0.85	
Field counts	*S. pyrioides* adults	GLMM (NB)	Lure formulation	6.18	2	0.045*	MS 65 [p= 0.16]
			Week	24.68	7	<0.001	AP 90 [p= 0.006]*
			Formulation: Week	6.35	14	0.96	
							Increase
	Chrysoperla eggs	GLMM (Poisson)	Lure formulation	2.14	2	0.34	MS 170 [p= 0.53]
			Week	42.84	7	<0.001	AP 277 [p= 0.22]
			Formulation: Week	8.9	14	0.83	
	Other predators	GLMM (Poisson)	Lure formulation	1.32	2	0.51	MS 2 [p= 0.98]
			Week	11.64	7	0.11	
			Formulation: Week	10.02	14	0.76	
Sticky traps	*Chrysoperla* adults	GLMM (Poisson)	Lure formulation	5.55	2	0.06	MS 650 [0.15]
			Week	62.92	7	<0.001	AP 548 [0.22]
			Formulation: Week	177.2	14	1	
	Spider	GLMM (NB)	Lure formulation	2.72	2	0.26	MS 44 [p= 0.07]
			Week	45.34	9	<0.001	AP 29 [p= 0.21]
			Formulation: Week	15.06	18	0.66	
	Predatory thrips	GLMM (NB)	Lure formulation	1.35	2	0.51	MS 44 [p= 0.48]
			Week	69.03	9	<0.001	AP 12 [p=0.91]
			Formulation: Week	10.87	18	0.89	
	Rove beetle	GLMM (NB)	Lure formulation	1.41	2	0.49	MS 8 [p= 0.93]
			Week	85.88	9	<0.001	AP 35 [p= 0.48]
			Formulation: Week	16.58	18	0.55	
	Platygastroidea	GLMM (NB)	Lure formulation	0.99	2	0.61	MS 25 [p= 0.31]
			Week	89.02	9	<0.001	AP 14 [p= 0.55]
			Formulation: Week	19.65	18	0.35	
	Chalcidoidea	GLMM (NB)	Lure formulation	4.17	2	0.124	MS 40 [p= 0.047]*
			Week	33.59	9	<0.001	AP 40 [p= 0.045]*
			Formulation: Week	19.81	18	0.343	

Table 2.6b. Analyses of *Rhododendrons in farm-2019* study for insect counts from leaf samples, in-field counts or sticky traps. Percent improvement is followed by p-value for the specific contrast; asterisk denotes significant difference using Dunnett's test.

Sample	Response variable	Stat. Test (Distribution)	Fixed Factor/s	χ^2	df.	p-Value	% Improvement
							Reduction
Leaf samples	*S. pyrioides* eggs	GLMM (NB)	Lure formulation	2.34	3	0.503	AP 17 [p= 0.47]
			Week	254	10	<0.001	
			Formulation: Week	29.72	30	0.48	
	S. pyrioides nymphs	GLMM (NB)	Lure formulation	1.21	3	0.75	MS 46 [p= 0.66]
			Week	2911	7	<0.001	AP 17 [p= 0.96]
			Formulation: Week	22.35	21	0.38	PAA 46 [p= 0.68]
Field counts	*S. pyrioides* adults	GLMM (NB)	Lure formulation	0.56	3	0.904	
			Week	71.82	10	<0.001	PAA 10 [p=0.72]
			Formulation: Week	26.27	30	0.661	
	S. pyrioides nymph presence	GLMM (Binomial)	Lure formulation	0.471	3	0.925	MS 37 [p= 0.49]
			Week	129.9	7	<0.001	AP 18 [p= 0.77]
			Formulation: Week	12.56	21	0.92	PAA 19 [p=0.76]
							Increase
Sticky traps	*Chrysoperla* adults	GLMM (NB)	Lure formulation	9.27	3	0.026*	MS 177 [p= 0.67]
			Week	34.39	8	<0.001	AP 237 [p= 033]
			Formulation: Week	20.62	24	0.66	MS:PAA 477 [p=0.24]
							AP:PAA 642[p=0.1]
	Predatory thrips	GLMM (NB)	Lure formulation	3.33	3	0.343	AP 47 [p=0.21]
			Week	166.2	8	<0.001	
			Formulation: Week	22.85	24	0.528	
	Orius sp.	GLMM (NB)	Lure formulation	3.7	3	0.29	MS 45 [p=0.67]
			Week	31.35	8	<0.001	PAA 49 [p=0.62]
			Formulation: Week	32.57	24	0.11	
	Chalcidoidea	GLMM (NB)	Lure formulation	8.46	3	0.037*	PAA 34 [p=0.14]
			Week	68.79	32	<0.001	
			Formulation: Week	42.01	24	0.013	
	Ichneumonidae	GLMM (NB)	Lure formulation	6.34	3	0.09	MS 180 [p=0.54]
			Week	52.1	8	<0.001	AP 220 [p=0.30]
			Formulation: Week	14.75	24	0.93	PAA 324 [p=0.05]

Table 2.7a. Analyses of *Azaleas in urban landscape-2018* study for insect counts from leaf samples, tap samples or sticky traps. Percent improvement is followed by p-value for the specific contrast and asterisk denotes significant difference using Dunnett's test.

Sample	Response variable	Stat. Test (Distribution)	Fixed Factors	χ^2	df.	p-Value	% Improvement
							Reduction
Leaf samples	*S. pyrioides* eggs	GLMM (NB)	Lure formulation	0.15	3	0.985	
			Week	57.78	7	<0.001	n/a
			Formulation: Week	27.04	21	0.17	
	S. pyrioides mobiles	GLMM (NB)	Lure formulation	2.36	3	0.501	MS 51 [p= 0.24]
			Week	13.01	7	0.071	AP 36 [p= 0.47]
			Formulation : Week	16.5	21	0.741	PAA 60 [p= 0.14]
							Increase
Tap samples	Total predators	GLMM (NB)	Lure formulation	33.52	21	0.041*	AP 5 [p=0.83]
			Week	69.28	24	<0.001	PAA 20 [p=0.51]
			Formulation : Week	32.83	18	0.017	
	Spiders	GLMM (NB)	Lure formulation	3663	21	0.018*	AP 2 [p=0.88]
			Week	49.1	24	<0.001	PAA 14 [p=0.49]
			Formulation : Week	32.8	18	0.017	
	Predatory mites	GLMM (Poisson)	Lure formulation	4.95	3	0.17	MS 335 [p=0.12]
			Week	146.2	6	<0.001	AP 237 [p=0.37]
			Formulation : Week	13.62	18	0.75	PAA 181 [p=0.65]
	Predatory hemipterans	GLMM (Poisson)	Lure formulation	32.57	21	0.05	MS 212 [p=0.36]
			Week	109.7	24	<0.001	AP 168 [p=0.64]
			Formulation : Week	30.24	18	0.035	PAA 119 [p=0.96]
Sticky traps	Spider	GLMM (Poisson)	Lure formulation	4.56	3	0.21	AP 13 [p=0.96]
			Trap	34.28	4	<0.001	
			Formulation : Trap	2.98	12	0.99	
	Psyllobora	GLMM (Poisson)	Lure formulation	26.92	15	0.03*	AP 117 [p=0.97]
			Trap	33.44	16	0.006	PAA 285 [p=0.26]
			Formulation : Trap	21.43	12	0.04	
	Chalcidoidea	GLMM (NB)	Lure formulation	2.88	3	0.41	n/a
			Trap	2.60	3	0.455	
			Formulation : Trap	8.21	9	0.513	
	Platygastroidea	GLMM (NB)	Lure formulation	1.49	3	0.684	n/a
			Trap	4.98	3	0.172	
			Formulation : Trap	9.03	9	0.434	

Table 2.7b. Analyses of *Rhododendrons in garden-2019* study for insect counts from leaf samples, in-field counts or sticky traps. Percent improvement is followed by p-value for the specific contrast.

Sample	Response variable	Stat. Test (Distribution)	Fixed Factors	χ^2	df.	p-Value	Improvement %
							Reduction
Leaf counts	*S. pyrioides* eggs	GLMM (NB)	Lure formulation	4.13	3	0.247	MS 10 [p=0.77]
			Week	213.4	10	<0.001	AP 45 [p=0.07]
			Formulation: Week	29.66	30	0.483	
	S. pyrioides nymphs	GLMM (NB)	Lure formulation	40.73	24	0.017*	AP 47 [p= 0.70]
			Week	119.3	28	<0.001	PAA 66 [p= 0.35]
			Formulation: Week	38.03	21	0.013	
Field counts	*S. pyrioides* adults	GLMM (NB)	Lure formulation	0.99	3	0.80	PAA 17 [p=0.80]
			Week	45.47	10	<0.001	
			Formulation: Week	42.26	30	0.068	
	S. pyrioides nymph presence	GLMM (Binomial)	Lure formulation	1.62	3	0.655	AP 15 [p=0.85]
			Week	105.4	7	<0.001	PAA 43 [p= 0.53]
			Formulation: Week	-	-	-	
							Increase
Sticky traps	Psyllobora	GLMM (Poisson)	Lure formulation	2.05	3	0.56	MS 311 [p=0.57]
			Week	93.11	8	<0.001	PAA 123 [p=0.98]
			Formulation: Week	30.27	24	0.17	
	Spider	GLMM (Poisson)	Lure formulation	2.03	3	0.56	n/a
			Week	18.02	8	0.021	
			Formulation: Week	37.41	24	0.04	
	Chalcidoidea	GLMM (NB)	Lure formulation	4.81	3	0.185	MS 78 [p=0.18]
			Week	15.48	8	0.052	AP 61 [p=0.26]
			Formulation: Week	31.67	24	0.133	
	Mymaridae	GLMM (NB)	Lure formulation	1.97	3	0.576	MS 30 [p=0.47]
			Week	103	8	<0.001	
			Formulation: Week	20.09	24	0.69	

Discussion

***Chrysoperla* spp. attraction**. As expected, the lures attracted *Chrysoperla* in the farm test sites compared to the control. There was no significant difference in *Chrysoperla* captures between the MS and AA blends, but the PAA blend attracted fewer than the other two blends. Interestingly, European studies have repeatedly attracted *Chrysoperl*a *carnea* using the PAA blend (Toth et al. 2009, Koczor et al. 2015, 2017, 2019, Pålsson et al. 2019), some of them suggesting this synthetic blend can be even used to manipulate oviposition. In Washington, a

study comparing multiple blends which included the PAA blend reported *Chrysoperla* spp. were too low to analyze statistically, but raw catches suggested they had responded well to it (Jones et al. 2016). The difference in attractiveness to the PAA blend in our study and those reported in the literature could be due to lure selectivity by different Chrysopidae (Jones et al. 2011, Salamanca et al. 2017). For example, a difference in capture rates was reported for two genus and three species of Chrysopidae in apple orchards in Washington state: *Chrysopa oculata* was mainly attracted to iridodial, *Chrysopa nigricornis* to squalene and *Chrysoperla plorabunda* to benzaldehyde (Jones et al. 2011). Conversely, an electroantennogram analysis showed *Chrysoperla carnea's* response to benzaldehyde was no different from water (Toth et al. 2009). In a vineyard study in Italy, the MS blend was found to be selective to Chrysopidae of the genus *Chrysoperla* but not to the genus *Pseudomallada* (Lucchi et al. 2017). Some authors posit the response to volatiles may be a highly conserved genetic trait within the *Chrysoperla* species complex given the observed lack of location effect in their study (Jones et al. 2016). Though not as specific as pheromones, the specificity of Chrysopidae's odorant binding proteins to attractants is an understudied aspect in its recruitment for biocontrol purposes. Whether specificity exists at the family, genus or species level needs to be determined; recent publications are beginning to shed some light on this question (Li et al., 2015, Li et al., 2018). Our study did not identify *Chrysoperla* to species due to the consensus that species identification may involve mating signals or ecophysiological criteria (Henry et al. 2001). Nonetheless, this factor should be accounted for when possible in future studies.

Overall, lures attracted *Chrysoperla* in both years in the farm, but not urban settings where all treatments combined only caught 5 *Chrysoperla* adults. This difference may have been due to site differences. Our experimental farms were surrounded by a complex landscape with a mix of farmscapes and tree groves capable of supporting many diverse natural enemies, and thus, the synthetic volatiles were more likely to work the intended way (Mallinger et al., 2011). This is the case in Sweden where successful recruitment of *C. carnea* in barley fields was achieved within a landscape with wild inter-field vegetation where *Chrysoperla* should have access to alternate prey, floral resources and overwintering sites (Pålsson et al. 2019). Lures seemingly failed to attract *Chrysoperla* in both urban studies. A detailed analysis of the effects of landscape composition were beyond the scope of this study; however, it is possible that large numbers of ornamental species in urban settings may have the unintended consequence of excluding natural

enemies. Many ornamentals are non-native and may have the same negative effect on native specialized herbivores: they are unpalatable or undetectable due to the lack of shared evolutionary history (Tallamy 2007).

***Stephanitis pyrioides* control**. Besides a significant reduction in *S. pyrioides* oviposition and adults associated with the AP blend in *farm 2018* (88% and 90% respectively) and an overall difference between treatments relative to the control with nymphs in *gardens-2019*, no other statistical differences took place in farm 2018, 2019 or urban 2018, 2019 experiments. A 50% reduction in damage could be a reasonable threshold, which if not met, would trigger a park manager or homeowner to use chemical sprays or replace a bush. Thresholds have been determined for nursery managers (Klingeman et al. 2000), but not for park managers or homeowners. Thus, a more comprehensive study should work with stakeholders to develop a damage index that determines an economic threshold to switch from an alternative control to insecticide use. Interestingly, a bigger reduction of pests was observed when considering data from only 22 out 24 plants that remained consistently in the shade in *farm 2018* and 15 out 16 in *urban 2018* (Appendix A). This factor is important to consider for future field work and for park managers' and homeowners' decisions on placement of *Rhododendron* spp. within a landscape (Neal and Douglas 1988, Trumbule et al. 1995).

Natural enemy attraction. Our study found some noticeable differences, though not always significant, between treated and untreated groups for natural enemies. Generally, the MS and AP blends attracted more predators, such as predatory hemipterans and predatory mites in *Azaleas in urban landscapes-2018.* The PAA blend attracted more parasitoids, such as Chalcidoidea and Ichneumonidae in *Rhododendrons in farm-2019*. In past studies the MS blend has captured relatively few Syrphidae and Ichneumonidae (Jones et al 2016, Lucchi et al. 2017), and Braconidae (Lucchi et al. 2017), the AP blend has captured few Ichneumonidae while the PAA blend has captures more Chalcidoidea and Scelionidae (Jones et al. 2016). In our study, we did not see significant differences relative to the control with Scelionidae (analyzed within superfamily Platygastroidea) despite a high number of captures. The attraction of parasitic Hymenoptera to PAA may be explained by PAA's presence in floral volatiles, hence it may be an important cue for adult wasps that feed on floral resources (Jones et al. 2106).

Predator-prey dynamics. Overall *S. pyrioides* reductions were not large nor consistent enough to draw strong correlations with the use of plant volatile blends. Increases in some predators did not match reductions of *S. pyrioides*; parasitoids in this study were not responsible for control since only *A. takeyanus* is a parasitoid of the lace bug but it is not present in Oregon (Flores et al. 2016). Few studies have looked at the relationship between plant volatile use and predator-prey abundance. Decreases in spider mites paired with increases in several key spider mite predators using MeSA in hopyards were reported by James and Price (2004) and Woods et al. (2011). Recently, *C. carnea* adults were recruited using the PAA blend in an apple orchard and barley fields with ensuant increases in eggs and larvae and significant decreases of two aphid species (Pålsson et al. 2019).

Though not in the scope of this study, the asynchrony of phenologies of *S. pyrioides* and *Chrysoperla* in the Willamette Valley may have been a factor. Laboratory observations have shown *Chrysoperla* spp. larvae feed infrequently on *S. pyrioides* adults (Lee et al. 2018) but feed readily on nymphs (Shrewsbury and Raupp 2006). In Oregon, nymphal *S. pyrioides* are most abundant relative to other life stages in May, and then present in smaller proportions for the remainder of the season (Flores 2016). Thus, for effective control, larval stages of *Chrysoperla* spp. should overlap with the first generation of *S. pyrioides* nymphs. Few eggs were laid in our attraction study conducted in May (data not reported).

Conclusion. Our study demonstrated that the MS and AP blends attracted *Chrysoperla* in farm settings. Likewise, there were some reductions in *S. pyrioides* infestations with the AP blend. While promising, our study did not show a consistent benefit to develop recommendations at this time. Future studies elucidating the temporal dynamics of *S. pyrioides* and *Chrysoperla* spp. or other important natural enemies would be useful for recruiting natural enemies on demand for effective biological control.

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CHAPTER 3

Silicon supplementation of rhododendrons to enhance resistance to *Stephanitis pyrioides*

Katerina Velasco-Graham and Jana C. Lee

Abstract

The azalea lace bug, *Stephanitis pyrioides* (Scott) (Hemiptera: Tingidae) is a major pest of azaleas and rhododendrons (*Rhododendron* spp.). Feeding injury reduces photosynthesis and leads to chlorosis; extreme infestations result in plant death. *Rhododendron* are popular in landscapes across the United States making this plant important for the nursery industry, landscape managers and homeowners. To maintain marketability of nursery stock, systemic insecticides are used to keep *S. pyrioides* populations below economic thresholds. However, with the potential negative effects of systemics on the environment, pollinators and people, landscape managers and homeowners prefer alternative controls. Increasing host plant resistance by supplementing plants with elemental silicon has enhanced the defense systems of monocots and some dicots. In this study, two no-choice and two choice experiments tested whether calcium silicate supplementation improved the resistance of rhododendron plants. Either whole plants or detached leaves were exposed to *S. pyrioides* in the greenhouse or field. A control was compared to three treatments: calcium silicate delivered via soil, calcium silicate delivered as a foliar spray, and calcium carbonate delivered via soil to test for a possible calcium effect. Plants were supplemented for 8 weeks in the spring and 4 weeks in the fall. After *S. pyrioides* were released, populations were monitored after 7-d or 30-d by counting frass spots, eggs and adults. From greenhouse choice experiments, there was a reduction in frass deposition, oviposition and adults on whole plants supplemented with calcium silicate compared to the control; and a reduction in frass deposition and oviposition on detached leaves. We did not observe an effect of calcium. It remains unclear if calcium silicate supplementation would also reduce pest activity in no-choice conditions because confined *S. pyrioides* seldom moved onto any leaves in greenhouse and field studies. While silicon supplementation appears promising, further studies are needed before incorporating into an IPM program for *S. pyrioides*.

Keywords: alternative controls, calcium silicate, Oregon, *Rhododendron*, silicon, *Stephanitis pyrioides*.

Introduction

The azalea lace bug, *Stephanitis pyrioides* Scott, 1874 (Hemiptera: Tingidae) is an invasive pest of rhododendrons and azaleas in the genus *Rhododendron* (Ericaceae). The pest was detected in the Pacific Northwest in 2008-9 (Rosetta 2013). Feeding by *S. pyrioides* damages plants by causing chlorosis, decreasing the photosynthesis rate, and creating yellow stippling on the upper surface of leaves. The pest also leaves a sticky residue of frass and exuviae on the lower surface (Nair and Braman 2012). Left untreated, damage may lead to plant death. However, even with only 2% surface damage on 11% of leaves, *S. pyrioides* economically impacts the nursery and greenhouse industry since consumers will reject these plants (Klingeman et al. 2000).

Insecticides, including neonicotinoids and pyrethroids, have been used to manage *S. pyrioides* infestations in nurseries and landscapes. Insect growth regulators (IGRs) have recently shown promising results for reducing population pressure via transovarial activity in adults in addition to ovicidal and nymphicidal effects, though an efficient delivery method needs further research (Joseph 2019 a, b). Although not as effective as chemical pesticides, botanical and natural compounds have shown promise and are preferred by homeowners and landscapers due to safety concerns (Nair and Braman, 2012). Biological control has appeared more promising in the northeastern U.S., partly owing to presence of the parasitoid *Anagrus takeyanus* Gordh, (Hymenoptera: Mymaridae) and the predator *Stethoconus japonicus* Shcumacher, (Hemiptera: Miridae); such natural enemies have not been detected during surveys in the Pacific Northwest (Lee et al. 2019). Augmentative biocontrol using commercially available *Chrysoperla carnea-group* (Neuroptera: Chrysopidae) can result in 97% reduction of *S. pyrioides* when predator larvae were tapped onto azaleas (Shrewsbury and Smith-Fiola 2000). Suppression was variable when tapped onto rhododendrons, though 88-99% of tapped predator larvae landed on rhododendrons (Flores 2016; Lee et al. 2018). A combination of pressurized water sprays followed by *Chrysoperla* egg cards resulted in a reduction *of S. pyrioides* on rhododendrons at the end of a 9-week study (Lee et al. 2018). However, the use of *Chrysoperla* spp. as an augmentative biocontrol may be costly for some growers (Flores et al. 2016) and water sprays may not be practical in larger landscapes. The use of herbivore-plant volatiles to recruit naturally-occurring Chrysopidae to control *S. pyrioides* have shown modestly promising results (Chapter 2). In summary, none of the alternatives researched have been effective enough to

recommend broad changes in management of *S. pyrioides*; another cost-effective and conveniently applied method would be helpful to growers.

Silicon supplementation may be potentially a convenient management tool since it is a non-essential element that influences host plant resistance to stem borers, and foliar, phloem and xylem feeders (Klittich and Parella 2014, Goussain et al. 2005, Dos Santos et al. 2015, Han et al. 2015, Pereira et al. 2010, Keeping and Kvedaras 2008, Reynolds et al. 2009). The biologically available form of silicon, silicic acid, is translocated from the soil to a plant's roots, stems and leaves. As it travels upward, silicic acid begins to concentrate due to transpiration and once it enters developing epidermal cells, the silica deposits and are known as pytholiths (Alhousari and Greger, 2018). Pytholiths are immobile once deposited, and more silicon accumulates in older tissues (Ma and Yamaji, 2006). Over two decades, studies looking at silicon supplementation have elucidated physical and chemical modes of action in protecting plants from herbivory. The hypotheses supporting the physical mode of action suggest pytholiths could be a) increasing the strength of cell walls, enhancing host resistance to penetration of sucking insects or wearing out mandibles of chewing insects (Keeping and Kvedaras 2008, Keeping et al. 2009), or b) damaging the insect's midgut, leading to a decrease in food digestibility in chewing insects (Dos Santos et al. 2015, Han et al. 2015). A chemical mode of action seems plausible for borers that hatch inside a leaf and never encounter the epidermal layer or for sucking insects. Indeed, soluble silicon may mediate enzymatic reactions which trigger changes in leaf chemistry or defense volatiles involved in tri-trophic interactions (Reynolds et al. 2009, Kvedaras et al. 2010). The change in leaf chemistries could involve: the synthesis of lignin and suberin, the production of chemicals with antibiotic properties, the catalysis of compounds that reduce nutritional quality and digestibility of food, or the production of phenolic compounds with deterrent and toxic properties (Epstein 2009, Reynolds et al. 2009). For example, aphids withdrew their stylets more often in silicon treated plants (Goussain et al. 2005), decreased probing time and sap ingestion and produced fewer honeydew droplets (Pereira et al. 2010). Silicon may also affect plants by inducing plants to generate defenses that act on the third trophic level. Volatiles emitted by a silicon-supplemented plant in response to herbivore attack may aid predators and parasitoids to locate the plant (Reynolds et al. 2009). Twice as many predators were attracted to Si+ cucumbers with pests than to Si- cucumbers with pests in a y-tube olfactometer. The authors believe the

predator beetle (*Dicranolaius bellulus* Guérin-Méneville) (Coleoptera: Melyridae) was responding to a change in the HIPV blend from the application of silicon (Kvedaras et al. 2010).

Though most dicots are known to be low accumulators of silicon, supplementation has been investigated in some pest-dicot plant systems. This includes sweet potato weevil (*Cylas formicarius* Fabricius) (Coleoptera: Brentidae) and sweet potatoes (Singh et al. 1993), leaf miners (*Liriomyza trifolii* Burgess) (Diptera: Agromyzidae) and chrysanthemums (Klittich and Parella 2004), whiteflies (*Bemisia tabaci* Gennadius) (Hemiptera: Aleyrodidae) and cucumbers (Correa et al. 2005), green peach aphid (*Myzus persicae* Sulzer) (Hemiptera: Aphididae) and zinnias (Ranger et al. 2009). To date, no studies have determined if Ericaceae and specifically *Rhododendron* spp. can accumulate silicon. Hence, the first objective of this study was to examine the ability of silicon supplementation to increase silicon content in *Rhododendron*. Because there was prior evidence that both soil and foliar application were effective (Reynolds et al. 2009), both application methods were tested. The second objective was to test the effects on feeding and egg laying by *S. pyrioides* using no-choice and free-choice experiments in the greenhouse and in the field.

Material and methods

Plants. In March 2019, 20 'Boule de Niege' and 20 'Cunningham white' rhododendrons were re-potted in Professional Growing Mix (Sun Gro® Horticulture, MA) in 3-gal (11.35 L) pots, fertilized with 1/3 cup (80 mL) Rhododendron, Evergreen & Azalea Food 10-5-4 (Lilly Miller, CA) and subsequently moved into a greenhouse for supplementation.

Each experiment consisted of four treatments: 1) calcium silicate foliar application, 2) calcium silicate soil application, 3) calcium carbonate soil application, and 4) control. A calcium carbonate soil treatment was used to clarify whether calcium also had a deterrent effect on *S. pyrioides.* Due to logistical constraints and that the product was labelled only for soil application, a calcium carbonate foliar application was not included. Rhododendrons, 10 per treatment, were supplemented weekly for 8 weeks. Controls were watered only. A solution concentration of 10 mL per 7.57 L of water was prepared for calcium silicate (Ca 10%, SiO_2 22%,) and calcium carbonate (Ca 20%,) (Mainstay Si and Mainstay Calcium, Redox Chemicals, Burley, Idaho).

Plants in soil application groups were given 1 L per plant of the respective solution whereas leaves of plants in the foliar application group were soaked using the cone and fan settings of a 2-gal (7.57 L) sprayer (Scott's, Ohio). Solutions in the sprayer were constantly agitated to prevent solute precipitation. Plants from all treatments received a total of 2-gal (7.57 L) of water per week in the greenhouse and kept moist in the field using ceramic water spikes (Blumat, Austria).

No-choice: field. Rhododendrons were transported to an experimental farm (44.55° N, 123.23° W) in July 2019. Plants were set up along the shaded southern fence in a complete randomized design and separated by 10 m to minimize interplant semiochemical communication (Reynolds et al., 2009). Plants were inoculated with *S. pyrioides* adults harvested from landscape rhododendrons on Oregon State University. *Stephanitis pyrioides* were confined to a leaf inside a circular leaf micro-cage with a thin spongy ring at the rim. The micro-cage made of clear plastic (3 cm in diameter and 2 cm in height) and covered on one side with lightweight mesh, was affixed to the underside of the leaf with barrettes (Fig. 3.1). The spongy rim was to prevent *S. pyrioides* from escaping by creating a seal to the leaf underside. Five *S. pyrioides* were added per cage (3 female: 2 male) using a camel hairbrush with two caged leaves per plant, and 10 replicate plants per trial. Each trial lasted one week. At the beginning of each trial, plants were inoculated with new *S. pyrioides*.

(a) (b) (c)

Fig. 3.1. Top view of circular leaf cage with thin spongy ring at the rim (a) used to confine *S. pyrioides* on rhododendron. Cages were affixed to the underside of the leaf (b) using barrettes (c).

All experiments were assessed by counting *S. pyrioides* at the end of the study period. Counts included live adults, frass spots as an indicator of feeding, and eggs on collected leaves. Frass was removed by gently washing leaves with warm water to view eggs embedded in the epidermis under a stereomicroscope. Leaf area was measured using a leaf area meter (LI-300, LI-COR, Nebraska). Counts were divided by leaf area to obtain frass and egg density.

Eight plants were moved to a different area due to logging near the original location. Because few *S. pyrioides* were observed to move from the cage across the spongy ring and onto the leaf, the second trial was modified. Six *S. pyrioides* (4 females: 2 males) were placed inside a 25.4 x 114.3 cm organza sleeve. The sleeve was carefully slipped over a leaf and the cuff fastened around the leaf petiole with a hair clip with two caged leaves per plant. After the second trial, the field experiment was terminated due to continued logging near the fence line.

No-choice: greenhouse. Rhododendrons used in the field were supplemented again with the same treatments in the greenhouse in September 2019 in preparation for greenhouse studies. Supplementation was done weekly for four weeks using the same protocol as in the spring. Plants were inoculated with *S. pyrioides* harvested from OSU landscape azaleas. To reduce handling, *S. pyrioides* (4 females: 2 males) were moved from field collection bags to small containers and transferred to a leaf by placing an uncapped container on its side at the bottom of an organza sleeve. The sleeve was slipped onto a leaf located on a rosette beneath the new growth and the cuff shut tight with a drawstring (Fig. 3.2) with two caged leaves per plant, ten replicate plants per trial, and three trials. Each trial lasted a week. Adults, frass and eggs were counted as previously described.

Fig. 3.2. Side view of rhododendron leaf encased in an organza bag. A container holding *S. pyrioides* was uncapped and placed at the bottom of the bag, near the leaf.

Choice studies. We set up two experiments: one with detached leaves and one with whole plants. We expected similar outcomes between the two if silicon was mediating resistance solely at the individual plant level. Alternatively, the outcomes might be different if silicon was not only mediating resistance at the plant level but was also triggering "plant crosstalk", leading to changes in plant chemistries in all treatments including the control.

Choice: detached leaf. In the greenhouse, the forty potted rhododendrons used for the no-choice experiments above had leaves collected for a four trial, choice study. For each trial, plants had one leaf clipped from the rosette located below the new growth. To set up all 40 leaves (10 leaves per treatment, four treatments), two tents were used per trial (20 leaves per cage). Each leaf was placed individually into a small cup of water and arranged in an 18 cm radius circle inside each 60 x 60 x 60 cm insect rearing cage in a complete randomized design (Fig. 3.3). Eighty *S. pyrioides* were released at the center of the circle and allowed free movement for one week; then adults, frass and eggs were counted.

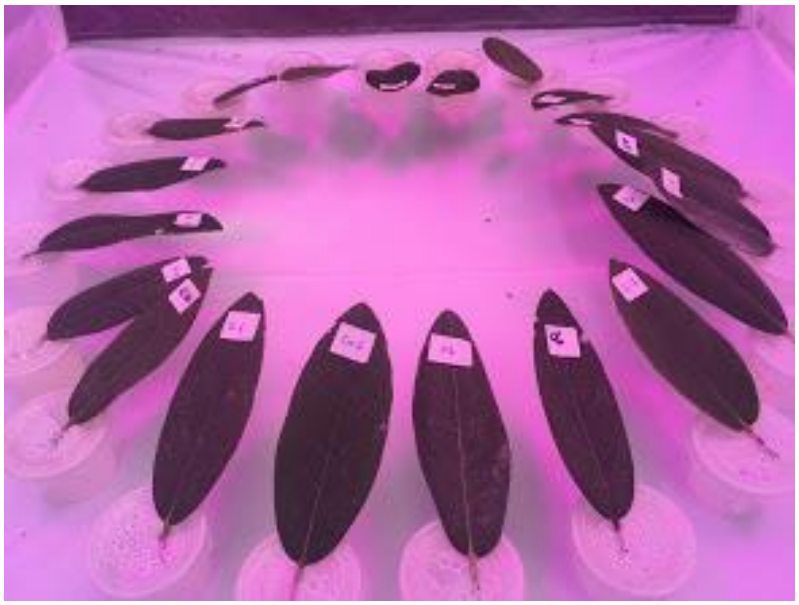

Fig. 3.3. Top view of insect cage with leaves from 20 rhododendron plants in a complete randomized design, *S. pyrioides* were released the day before in the center.

Choice: potted plant. Rhododendrons plugs (‘Boule de Niege’) were re-potted into 4 x 4-inch pots, fertilized and supplemented once weekly for four weeks following the same protocols as described for the field trial. Calcium silicate and calcium carbonate soil application groups received 0.227 L per plant; the calcium silicate foliar application plants were soaked using a sprayer. All plants were kept moist thereafter using trays to allow water uptake as needed. At the end of the four-week supplementation period, 36 rhododendrons were divided among nine 47.5 x 47.5 x 93 cm insect rearing cages. Four treatments per cage were arranged in a square in complete randomized design; plants were rotated counterclockwise weekly to avoid positional effects. One hundred *S. pyrioides* were released at the center of the cage and allowed to move freely for four weeks (Fig. 3.4). Ten leaves per plant were randomly collected and adult, frass and egg counts were collected as previously described.

(a) (b) (c)

Fig. 3.4. Arrangement of four rhododendron plants post-supplementation. Nine insect cages had four treatments each (a, b); *S. pyrioides* were allowed free movement for 4 weeks post-release (c).

Silicon content. Tissue samples were taken from plants in the spring on week 0 pre-supplementation and weeks 3 and 9 post-supplementation. In the fall, pre- and post-supplementation samples were taken. Three leaves growing beneath a newly expanded rosette were clipped, dried at 60°C for 48 hr and ground in a Wiley mill. Silicon concentrations were

measured by inductively couple plasma-optical emission spectroscopy (ICP-OES) (Optima 3000; Perkin Elmer, Wellesley, MA) after microwave digestion in HNO_3.

Statistical analyses. Analyses were conducted with RStudio v. 1.1.463 and R software v. 3.6.1 (2019). The silicon data was examined visually for normality. Given that the residuals were symmetrical, silicon content was analyzed with a linear mixed model ('nlme' package, Pinheiro et al. 2019). The model included Si-% dry weight as the response variable, treatment and week as fixed factors, and subject as a random effect. A linear mixed model with a correlation structure with the lowest AIC value was used to account for plant correlations across sampling.

For no-choice and choice studies, a generalized linear mixed model ('glmmTMB' package, Mollie et al. 2017) with a negative binomial or Poisson distribution was used because all values were count data with discrete, non-negative integers and typically right-skewed distribution (Stroup 2015). Models included frass, eggs and adults analyzed separately as response variables. Fixed factors were treatment and week for the no-choice study, treatment and tent for the choice-leaf study, and treatment for the choice-plant study. Subject was a random effect for studies. Area was an offset with the frass and egg data to infer the mean number per area. Tent was initially meant to be a random effect, but it had only two levels and was switched to a fixed effect. Random effects must have at least three levels.

Fixed factors and their interaction were checked for significance with likelihood ratio tests fitting a full and reduced model. If an interaction was significant, treatment was tested against the full model with the interaction. For post-hoc testing, contrasts comparing the control with each treatment were analyzed with Dunnett's test and multiple comparisons with Tukey's test ('emmeans' package, Lenth 2020). GLMM models were validated by visually examining a scaled residual plot ('DHARMa' package, Florian 2020) and using the sum of the squared Pearson residuals over residual degrees of freedom to check for possible overdispersion. Data was tested using penalized regression via diffuse priors to deal with complete separation ('blme' Chung et al. 2013) and an optimizer to help the models converge.

Results

Silicon content. Supplementation of silicon via foliar or soil did not significantly increase the content of silicon in the leaves of treated rhododendron plants (treatment $F_{3, 155}$ =1.15, p=0.331, sample $F_{4, 155}$ =57.86, p<0.001, no interaction). Plants had 0.026% Si in all groups at the onset of the study. Silicon content peaked but did not differ considerably between treated and untreated groups after 8 weeks of application (3rd leaf sampling: range 0.036 - 0.040% of Si) (Table 3.1), dropped slightly after the summer season and did not recover to previous maximum levels even after 4 weeks of additional applications (5th sampling) (Fig. 3.5, Table 3.1). Calcium via soil did not increase significantly the context of calcium in treated plants (treatment $F_{3,143}$=1.86, p=0.138, week $F_{4,143}$ = 120.12, p,0.0001, treatment: week $F_{12,143}$=2.13, p=0.018).

Table 3.1. Mean percent silicon by dry weight (±SEM) of rhododendrons for five samples from April to September. Sample 1 was based on 10 leaves from ten different plants combined into one sample. Silicon (Si), calcium (Ca).

season **week** **sample**		**Spring** **0** **1**	**Spring** **3** **2**	**Spring** **9** **3**	**Fall** **0** **4**	**Fall** **4** **5**
	Control	0.026	0.027 ±0.002	0.040 ±0.001	0.036 ±0.001	0.035 ±0.001
	Si Foliar	0.026	0.026 ±0.001	0.038 ±0.001	0.035 ±0.001	0.035 ±0.001
	Si Soil	0.026	0.027 ±0.001	0.036 ±0.002	0.036 ±0.001	0.034 ±0.001
	Ca Soil	0.026	0.026 ±0.002	0.040 ±0.001	0.035 ±0.001	0.034 ±0.002

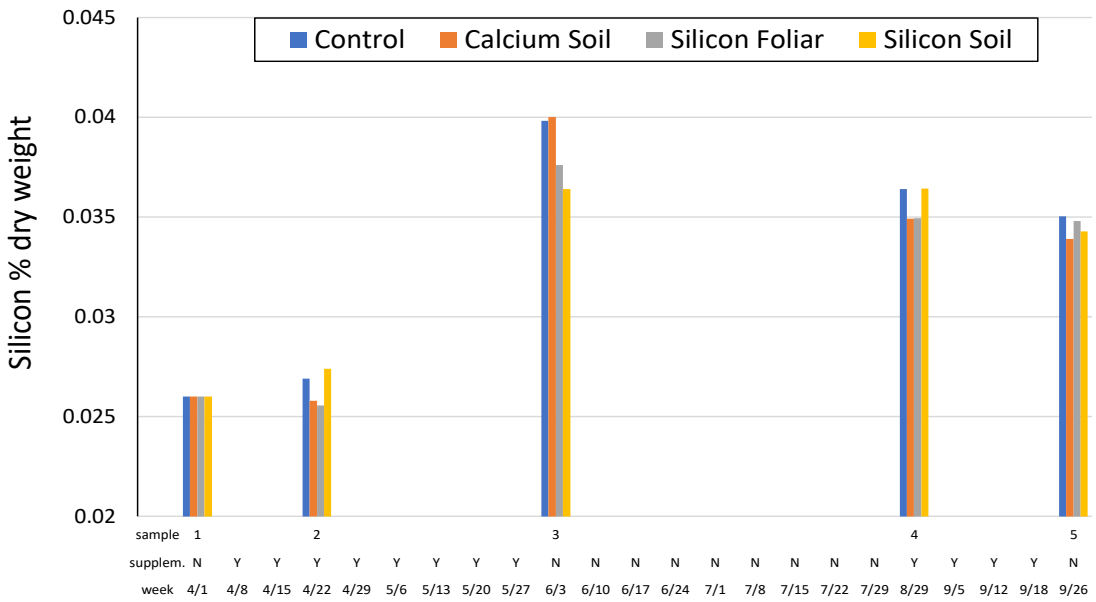

Fig. 3.5. Mean percent silicon by dry weight of rhododendron leaf tissue for five sampling dates, 1 pre- and 4 post-supplementation. Plants were supplemented weekly for 8 weeks prior to the no choice field experiment and weekly for 4 weeks before the free choice detached leaf experiment.

No choice test: field and greenhouse. In the field and for the first trial, *S. pyrioides* were initially confined in micro-cages pressed to a leaf with a barrette, but most escaped through a narrow gap. For the second field trial, when cages were modified with a foam rim pressed to the leaf, most *S. pyrioides* stayed in the cage and did not move to the leaf. For the third field trial, when we used an organza sleeve to confine insects, many *S. pyrioides* stayed on the sleeve and did not move to the leaf. In all three trials, since few *S. pyrioides* were found on leaves, they had not fed or laid eggs in any treatment including the control, thus trials were not analyzed.

In the greenhouse, when we used a container to add *S. pyrioides* to the organza sleeve to encourage them to move, we saw improved mobility (Fig. 3.2). There was no overall difference between treatments for frass spots (χ^2=5.35, df=3, p=0.147) though silicon foliar showed a 46% reduction relative to the control (Dunnett test, P<0.05). Egg counts were too low for statistical analysis.

Choice: detached leaf. After a 7-day exposure to *S. pyrioides*, there was a difference in frass deposition between the treated and untreated groups with all three treatments. The number of eggs was also different for all three treatment groups relative to the control. There was no difference between groups in the number of adults (Table 3.2). Numerical reductions in oviposition occurred in the silicon treatments relative to the calcium treatment (Table 3.3).

Table 3.2. Mean egg and frass density, and number of adults (±SEM) on detached rhododendron leaves exposed to *S. pyrioides* for 7 days. Significant values in bold; asterisks indicate significant difference between treatment and control.

Treatment	**Frass Density (deposition/cm^2)**	**Egg Density (egg/cm^2)**	**Adults**
Control	1.63 ± 0.19	0.262 ± 0.042	1.92 ± 0.40
Silicon foliar	1.18 + 0.27*	0.132 ± 0.037*	1.20 ± 0.25
Silicon soil	1.11 ± 0.16*	0.127 ± 0.040*	1.12 ± 0.25*
Calcium soil	1.03 ± 0.15*	0.198± 0.058*	1.23 ± 0.21
Distribution	(GLMM) NB	(GLMM) NB	(GLMM) Poisson
Treatment	χ^2=11.9, df=3, **p=0.007**	χ^2=15.3, df=3, **p=0.001**	χ^2= 5.6, df=3, p=0.130
Tent	χ^2=40.1, df=7, p<0.001	χ^2=76.8, df=28, p<0.001	χ^2= 20.6, df=7, p=0.004
Trtmnt: Tent	χ^2=23.8, df=21, p=0.30	χ^2=21.67, df=21, p=0.42	χ^2=27.1,df=21, p=0.167

Table 3.3. Percent reduction in frass spots, eggs and adults of *S. pyrioides* in choice-leaf and choice-plant experiments. Reduction in terms of silicon treatment relative to the control or to calcium treatment. Asterisks indicate differences (p <0.05) with Dunnett's test (1) and Tukey's HSD test (2).

Comparison	**Frass**		**Eggs**		**Adults**	
	leaf	**plant**	**leaf**	**plant**	**leaf**	**plant**
Si Foliar vs control[1]	46%*	72%*	70%*	78%*	48%*	82%*
Si Soil vs control[1]	43%*	57%*	75%*	63%*	65%	81%*
Si Foliar vs calcium[2]	-5%	38%	37%	64%*	-37%	36%
Si Soil vs calcium[2]	-11%	7%	48%	40%	8%	35%

Choice: potted plant. After a 30-day exposure to *S. pyrioides*, there was a significant difference in frass deposition between all treatments and the control. Also significant was the difference in oviposition between the silicon foliar and silicon soil treatments and the control (Table 3.4, Fig. 3.6). The greatest reduction in frass and oviposition were observed in silicon foliar though they were not significantly different from silicon soil. Numerical reductions in frass deposition, oviposition and adults occurred in the silicon treatments relative to the calcium treatment; only oviposition in silicon foliar was reduced significantly relative to calcium (Table 3.3).

Table 3.4. Mean egg and frass density and mean adults (± SEM) on potted rhododendron plants exposed to *S. pyrioides* adults for 30 days. Significant values are in bold; asterisks indicate significant difference between treatments and control.

Treatment	**Frass Density (deposition/cm^2)**	**Egg Density (egg/cm^2)**	**Adults**
Control	5.34 ± 0.61	1.03 ± 0.17	4.30 ± 0.75
Silicon foliar	1.54 ± 0.31*	0.23 ± 0.06*	0.81 ± 0.29*
Silicon soil	2.32 ± 0.49*	0.38 ± 0.09*	0.82 ± 0.32*
Calcium soil	2.48 ± 0.63*	0.64 ± 0.19	1.26 ± 0.39*
Distribution	(GLMM) NB	(GLMM) NB	(GLMM) Poisson
Statistics	χ^2=16.3, df=3, **p<0.001**	χ^2=14.4, df=3, **p=0.0023**	χ^2=24.1, df=3, **p<0.0001**

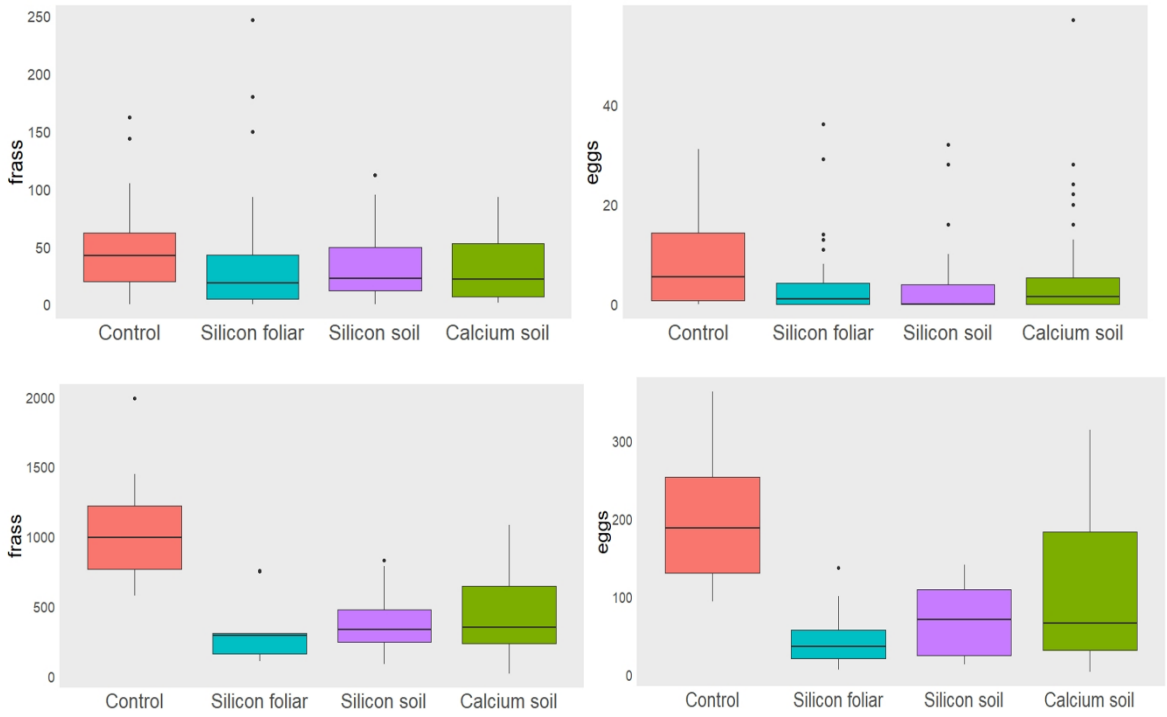

Fig. 3.6. Effects of four treatments on *S. pyrioides* on mean ± SEM of a) frass spots on detached leaf, b) eggs on detached leaf, c) frass spots on potted plant, and d) eggs on potted plant.

Discussion

Supplementation with calcium silicate resulted in no significant incorporation of silicon by *Rhododendrons* spp. whether it was soil-applied or foliar sprayed despite an 8-week spring and 4-week fall supplementation schedule. All treatments in this study, including the control, were able to incorporate small amounts of silicon present naturally in the potting soil and yet the treated groups were unable to incorporate the extra supplemented silicon.

In principle, plants lacking silicon transporter genes or homologues will not be able to efficiently accumulate silicon via active transport. However, all plants can take up silicon passively through diffusion, similar to water uptake, or through facilitated diffusion via proteinaceous channels which are energy independent and present in all plants. There are also "rejective" plants, such as tomatoes, which actively exclude silicic acid from their roots (Liang et al. 2015). Less is known about dicots than monocots about silicon uptake. Pumpkins have silicon transporter genes homologous to those in rice plants, soybeans have influx transporters but are downregulated by an increase in silicon supply, sunflowers and wax gourds have shown uptake

with an active and passive contribution, and fava beans are rejective (Liang et al. 2015). Work done in ornamental crops has shown zinnias, chrysanthemums and daisies are capable of uptake (Klittich and Parella 2014, Liang et al. 2015, Klittich 2016). Whether a monocot or a dicot, there is evidence that absorbed amounts may be proportional to the concentration of the silicon supplied to plants (Gatarayiha et al. 2010). Our results suggest that *Rhododendrons* spp. are *passive* accumulators.

Overall, the trend from testing both detached leaves and potted plants was that *S. pyrioides* had a lower preference for supplemented plants, as seen with fewer adults on leaves, frass spots as an indicator of feeding, and eggs laid. Trends were significantly lower in silicon foliar compared to the control in 6/6 cases and lower in silicon soil in 5/6 cases (Table 2). In addition, oviposition in silicon foliar was lower than calcium alone for potted plants. Similar outcomes for the choice-leaf and choice-plant experiments suggest plant communication did not play a role in mediating resistance of *Rhododendron* spp. to *S. pyrioides*. In other systems, silicon supplementation has been shown to reduce overall pest presence (Correa et al. 2005, Pereira et al. 2010, Kvedaras et al. 2010, Han et al 2015, Klittich 2016), decrease feeding by all four feeding guilds (Keeping and Kvedaras 2008, Pereira et al. 2010, Dos Santos et al. 2015, Han et al. 2015, Klittich 2016) and reduce fecundity and oviposition (Correa et al. 2005, Keeping and Kvedaras 2008). In contrast, some studies have shown no influence of silicon on the feeding, development and survival of some folivores and phloem feeders (Keeping and Kvedaras 2008).

Our study showed a slight advantage of silicon applied foliarly over soil drench. Whether silicon applied as a foliar spray increases plant resistance is not clear. Several studies have reported foliar supplementation works equally well or better than soil application (Keeping and Kvedaras 2008, Reynolds et al. 2009), and others have shown no differences between application method. For example, a foliar application of calcium silicate on cucumber leaves reduced whitefly (*Bemisia tabaci*) oviposition by more than three-fold in relation to the control whereas the reduction for soil-applied was not as marked (Correa et al. 2005). They also found that whitefly survival rate for the egg to fourth-instar nymph period was distinctly lower from foliar relative to soil application. The three-fold increase in mortality and increased developmental period of nymphal stages of the whitefly on cucumbers treated with silicon foliar led the authors to suggest that silicon had been absorbed through the leaves and may be inducing synthesis of defense chemicals. Conversely, Matlou (2006) found no increase in silicon accumulation in

sorghum, a silicon responsive crop, after foliar application from three sources, at two rates and with a wetting agent, but did see an increase with soil.

Given that our study showed no accumulation of silicon in rhododendrons with either soil or foliar application, it is tempting to agree with Guével et al.'s (2007) suggestion that foliar sprays may have a **direct** effect on plants and not an effect **mediated** by them. This "topical" effect was reported elsewhere by Lee et al. (2015) who found that treating blueberries with calcium silicate increased penetration force by 10% which translated to a 52% reduction in oviposition in the spotted wing drosophila (*Drosophila suzukii*). However, a topical effect does not explain pest reductions that we documented for rhododendrons supplemented via soil. While silicon supplementation does not result in uptake in rhododendrons, it may affect plant suitability in other ways, and should be investigated further.

Additional evaluation is needed to determine the effects of treating *Rhododendron* with silicon on the performance and population fitness of *S. pyrioides*. Future no-choice studies should address the behavior of *S. pyrioides* in confined areas before conducting new trials with micro-cages or sleeves in the field. A new no-choice experiment could set up treated and control potted plants individually in separate tents in a greenhouse. Silicon supplementation may be an alternative to chemicals or used as part of IPM program to lower pest pressure and frequency of insecticide use, but the challenge of attaining good coverage on the underside of leaves with topical sprays remains. Field studies should be conducted on landscaped *Rhododendron* spp. to determine whether foliar sprays or soil drenches provide efficient control of *S. pyrioides*.

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CHAPTER 4
General Conclusions

Katerina Velasco-Graham

Stephanitis pyrioides is a relatively new invasive pest of the genus *Rhododendron* in the Pacific Northwest. Targeting eggs, nymphs and adults is challenging because eggs are embedded into the leaf tissue and nymphs and adults feed on the underside of leaves. Contact insecticides will invariably miss a good portion of the resident *S. pyrioides* population. Hence, nurseries are compelled to use systemic insecticides. However, park managers and homeowner are looking for a safer alternative for azaleas and rhododendrons already planted in landscapes. IPM, an ecosystem-based interdisciplinary strategy, emphasizes pest management measures and strive for the least negative effect upon human health, non-target organisms and the environment but still provide satisfactory control. In this study we looked at two potential new measures, one biological and one cultural, for the control of *Stephanitis pyrioides.*

Our study deployed plant and floral volatiles blends known to attract various natural enemies, including Chrysopidae species to manipulate *Chrysoperla* spp., a voracious predator of *S. pyrioides* nymphs (Jones et al. 2011, Koczor et al. 2017). Two out of three volatile blends attracted *Chrysoperla*. Several more have been shown to attract Chrysopidae in the Pacific Northwest and could be used in future research (Jones et al. 2011). The marked difference in success for the PAA blend in the Willamette Valley relative to European results may relate to species differences (Koczor et al. 2017, Li et al. 2018, Pålsson et al. 2019). Volatile blends attracted *Chrysoperla* in farm settings, not in urban settings. This does not mean *Chrysoperla* are not present in urban settings. Given the location of the intended beneficiaries of this strategy, i.e. azaleas and rhododendrons planted in various urban landscapes, further research is needed to determine what other factors may have played a role in our results. For instance, the use of insecticides in test gardens may have interfered with the beneficial arthropod community and thus the natural regulation of pests in the *rhododendrons in garden-2019* is unknown. *Chrysoperla* were not attracted uniformly throughout the season due to natural seasonal and environmental fluctuations experienced by natural enemies. Further studies are needed to establish phenology of *Chrysoperla* in the Willamette Valley to determine predatory-prey synchrony and therefore feasibility of attracting naturally occurring *Chrysoperla* as a biological control strategy.

To test a second alternative control measure, we supplemented rhododendrons with calcium silicate to determine effect on *S. pyrioides* feeding and oviposition behavior. Past studies

have shown that various compounds, including calcium silicate, can effectively deliver silicon to plants and influence the defense systems of plants (Liang et al. 2015, Reynolds et al. 2009). Content analysis of the rhododendron leaf tissue of our study indicated no difference in silicon accumulation between treated and untreated groups. Yet, in our greenhouse choice studies, supplemented plants showed a reduction in *S. pyrioides* frass deposition and oviposition whether silicon was delivered through a soil drench or a foliar spray. Additional research is needed to confirm that silicon supplementation negatively affects *S. pyrioides* behavior, to determine how this impacts populations in the long-term and how long the effect lasts, and to devise an optimum method to supplement azaleas and rhododendrons in urban landscape settings. Regardless of the type of intervention, a separate but related line of research needs to focus on *S. pyrioides* threshold value for landscapes, especially those managed by companies, that would inform timing of intervention.

Other factors may affect *S. pyrioides* fitness and performance. Past investigations into the mechanisms of resistance, notably, leaf wax composition, leaf pubescence and physical and chemical changes in leaf chemistry in response to herbivory (Nair and Braman 2012) may explain why some cultivars are more susceptible than others (Appendix B). While alternative measures are being examined, park managers and homeowners should rely on other factors which may affect the level of damage that *S. pyrioides* inflicts on landscaped *Rhododendron* such as environmental conditions. For example, sunny habitats may lead to stress-induced changes in nutritional physiology of azaleas subsequently influencing plant host preference (Nair and Braman 2012). Sunny habitats may also provide thermal refuge for *S. pyrioides* due to lower natural enemy abundance (Trumbule and Denno 1995). Thus, planting in shady locations should help *Rhododendron* spp. better tolerate *S. pyrioides* infestations. Finally, planning structurally complex landscapes, especially overstory tree layers, will lower the risk of *S. pyrioides* outbreaks possibly due to enhanced natural enemy activity (Shrewsbury and Raupp 2000).

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APPENDICES

Appendix A: Effects of sun exposure on *S. pyrioides* populations

Despite attempts to homogenize shade conditions for all test plants, there were variations in sun exposure which led to noticeable differences in *S. pyrioides* abundance in the 2018 farm and urban experiments. For example, the highest *S. pyrioides* counts in *Rhododendrons in farm 2018* occurred in week 2, 5, 6, 7, and 8 in containerized rhododendron '1A' located at the end of the shaded fence line that received more sun than the rest. When subject 1A is removed from the data set, the use of volatile blends markedly changes the differences for the main effects (from p=0.012 to 0.0042 for eggs, p=0.15 to 0.016 for nymphs, p=0.04 to <0.01 for adults) (Table A1). Further, the AP blend results in an 87% [p=0.002] reduction for eggs in contrast with the control, the MS blend and AP blend in a 97% [p= 0.005] and 88% [p=0.03] reduction for nymphs, and the MS blend and AP blend in an 85% [p< 0.001] and 84% [p< 0.001] reduction for adults. A similar shift in statistical significance occurs for *Azaleas in urban landscape-2018* when *S. pyrioides* mobile counts for azalea bush '4B' are dropped; azalea 4B received afternoon sun and very little shade. Removal of 4B from the dataset changes the main effect from p=0.501 to 0.029 (Table A1), resulting in a higher reduction for MS and AP blends, 64% [p=0.012] and 73% [p=0.004], respectively. These results concur with Trumbule et al. (1995) and Shrewsbury and Raupp (2000) that *Stephanitis pyrioides* occurs more abundantly in sunny and exposed landscapes compared to more shaded plantings in complex landscapes due to top-down pressures exerted by natural enemies which prefer shady habitats.

Table A1. Mean ±SE for *S. pyrioides* egg, nymph and adults for 2018 (with and without subjects in sunny spots) in *rhododendrons in farm* and *azaleas in urban landscape*. Asterisk denotes significance difference using Dunnett's tests.

	Subject	Landscape	Lure	Egg	Nymph	Adult
2018	Potted Rhododendron	FARM	MS blend	9.97 ±3.71	3.09 ±1.56	4.44 ±1.57
			AP blend	5.28 ±0.26*	0.84 ±0.42	0.81 ±0.23*
			Control	17.9 ±4.47	2.91 ±0.84	3.94 ±0.52
2018	Potted Rhododendron (excludes sunny spot)	FARM	MS blend	5.83 ±2.32	0.17 ±0.09*	0.67 ±0.18*
			AP blend	5.28 ±2.26*	0.84 ±0.49*	0.81 ±0.23*
			Control	17.9 ±4.47	2.91 ±0.84	3.94 ±0.52

	Subject	Landscape	Lure	Egg	Nymph	Adult
2018	Landscape Azalea	URBAN	MS blend	156 ±16.08	1.19 ±0.40	4.66 ±0.75
			AP blend	199 ±25.45	3.94 ±1.60	10.5 ±3.42
			PAA blend	106 ±12.72	2.16 ±0.67	4.70 ±0.80
			Control	128 ±11.29	3.84 ±1.44	8.10 ±1.12
2018	Landscape Azalea (excludes sunny spot)	URBAN	MS blend	156 ±16.10	1.20 ±0.40*	4.66 ±0.75*
			AP blend	192 ±33.06	1.01 ±0.34*	3.02 ±0.50*
			PAA blend	106 ±12.72	2.17 ±0.67	4.70 ±0.80
			Control	128 ±11.29	3.85 ±1.44	8.10 ±1.12

Appendix B: Choice study of *Rhododendron* cultivars

Objective: To determine which rhododendron cultivars are most resistant to attack by *Stephanitis pyrioides.*

Rationale: Previous work has shown that cultivars from the genus *Rhododendron* are not all equally susceptible to herbivory, though most investigations have been on azalea taxa. The possible mechanisms of resistance include epicuticular waxes, stomatal characters, leaf moisture content and leaf pubescence. Studies have shown leaf wax has a definite role in *S. pyrioides* resistance in azaleas, stomatal size could not be correlated to feeding preference, and leaf water content and leaf pubescence could not be related to resistance in most azalea taxa. Also suggested for azaleas are differences in leaf physical and chemical composition combined with antibiosis (Nair and Braman 2012). In rhododendrons, leaf pubescence was correlated to *S. pyrioides* avoidance and antibiosis (Flores 2016, Komnenus and Lee 2018).

Method: Cultivars were re-potted into 1-gal pots, fertilized with Rhododendron, Evergreen & Azalea Food 10-5-4 (Lilly Miller, CA) and placed in a greenhouse. Each replicate consisted of 22 rhododendron cultivars and 1 blueberry (Table 1). One leaf per cultivar (or several leaves if the cultivar had smaller leaves) was clipped from the second or third node above the base and placed into a small plastic labeled cup filled with water. To prevent *S. pyrioides* from accidentally falling into the water, the mouth of the cup was covered by a small section of parafilm “M” (Bemis Co, Inc. Neenah, WI) secured in place by a cut out clip-on lid. Cups were arranged in a circle inside a BugDorm-2120 tent 60 x 60 x 60 cm (l x w x h, MegaView Science Co., Taiwan) in a walk-in growth chamber (22°C, 60% RH, 16:8 L:D). The bottom half of petri dish with 66 *S. pyrioides* from our colony was placed in the center. *Stephanitis pyrioides* were allowed to move freely for two weeks. Cups were rotated every 4 days to avoid positional effects. Adults on leaves were tallied every 2 days. At the end of the 2-week period, leaves were collected, gently washed with warm water and eggs counted under a stereomicroscope. Leaf area was measured (LI-300C, LI-COR, Lincoln, Nebraska). Fourteen replicates were conducted between March and August 2019.

Table B1. Rhododendrons and blueberry tested for *S. pyrioides* preference

Rhododendron cultivars	Anna Rose Whitney
	Bubblegum
	Catawbiense Album
	Catawbiense Boursalt
	Catawbiense Grandiflorum
	Cunningham's White
	English Roseum
	Florence Parks
	Hellikki
	Landmark
	Maximum Roseum
	Nova Zembla
	Olga Mezitt
	Ponticum Variegatum
	Purple Passion
	Ramapo
	Raise the Roof Slam Dunk
	Sneezy
	Sugar Puff
	Taurus
	Yaku Princess
Blueberry variety	Duke

Results.

Oviposition. There was a difference in mean oviposition between cultivars (χ^2=89.05, df=21, p<0.0001) (analysis used leaf area as an offset). The most susceptible cultivars included Cunningham White, Florence Parks, Olga Mezitt, Ramapo, Sugar Puff and Taurus with ≥ 15 fold mean increase in oviposition relative to Yaku Princess. The least susceptible cultivars included Bubblegum, Catawbiense Album, English Roseum, Hellikki, and Ponticum Variegatum with ≤ 5 fold mean increase in oviposition relative to Yaku Princess (Fig. B1 and B3).

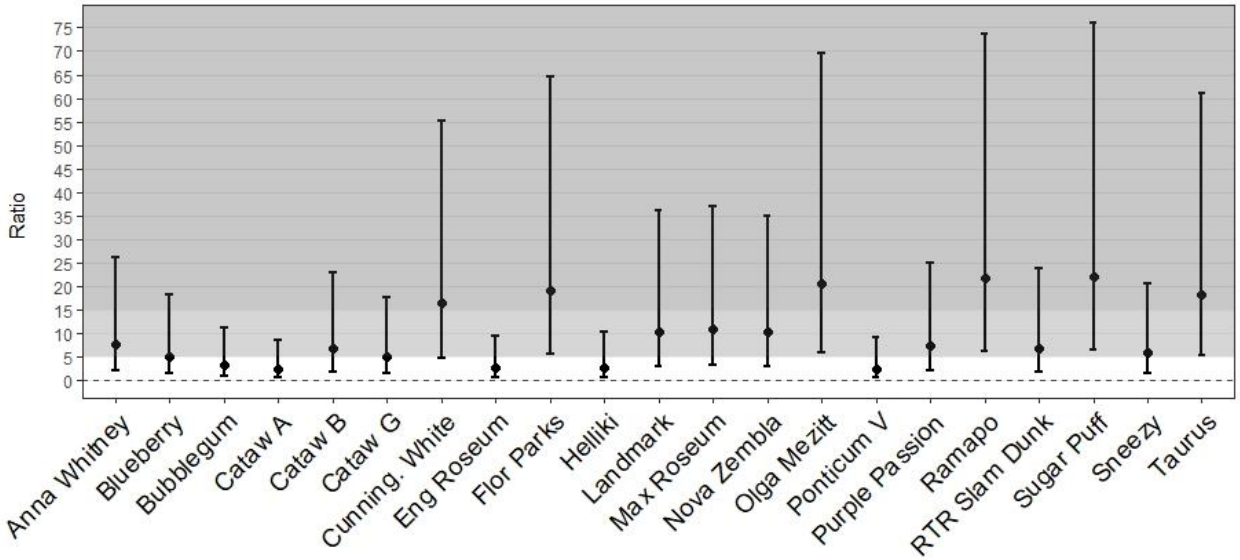

Fig. B1. Estimated ratios in mean oviposition of *S. pyrioides* between Yaku Princess (the least susceptible cultivar) and all other rhododendron cultivars. The error bars are Dunnett-adjusted 95% confidence limits. In each comparison, mean oviposition for each cultivar was divided by the mean oviposition of Yaku Princess. Values against the dark grey background indicate a minimum 15-fold increase, against light grey a 5 to 15-fold increase and against white, a 0-5 fold increase in mean oviposition relative to Yaku Princess.

Adults. There was a difference in preference for cultivars (χ^2=177, df=21, p<0.0001). *Stephanitis pyrioides* adults were more frequently found on Landmark, Ramapo, and Olga Mezitt (mean > 1.0 adult/cm^2) and found less frequently on Yaku Princess, English Rose, Catawbiense Album, Ponticum Variegatum and Hellikki (mean adults <0.2 adults/ cm^2) (Fig. B2 and B3).

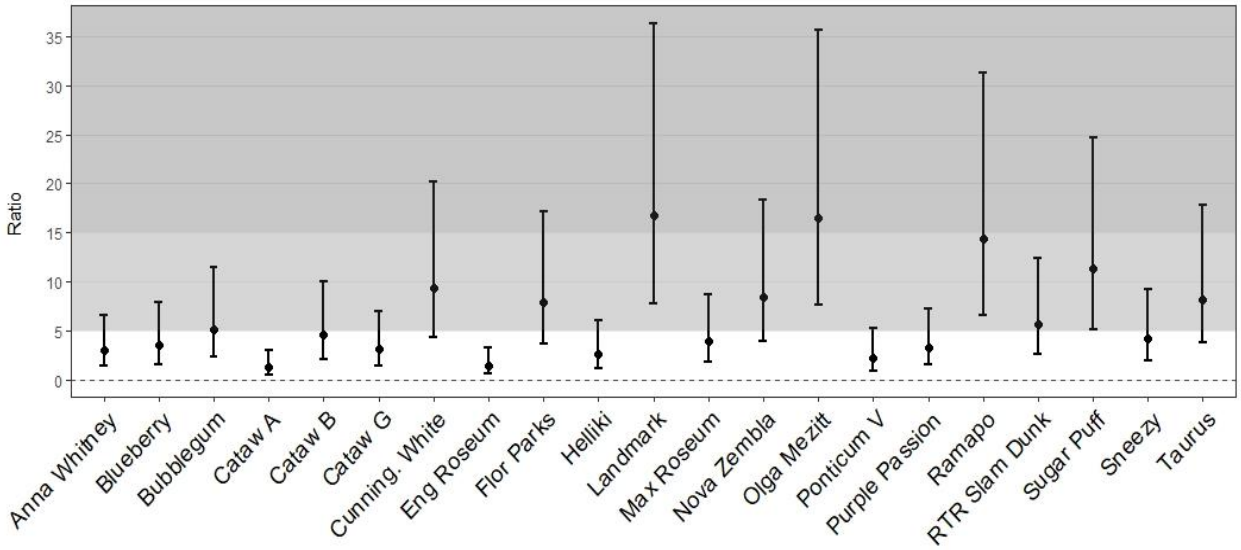

Fig. B2. Estimated ratios in mean adult *S. pyrioides* between Yaku Princess (the least susceptible cultivar) and all other rhododendron cultivars. The error bars are Dunnett-adjusted 95% confidence limits. In each comparison, mean adult for each cultivar was divided by the mean adult of Yaku Princess. Values against the dark grey background indicate a minimum 15-fold increase, against light grey a 5 to 15-fold increase and against white, a 0-5 fold increase in mean adult relative to Yaku Princess.

Discussion. Given a choice, lace bug's preference for cultivars may differ for feeding and laying eggs since adults were not always found on the cultivars which they seemingly preferred for oviposition. For example, despite finding *S. pyrioides* most often on Landmark, egg density did not place it into the "most susceptible" category listed in our results. Such mismatches were also seen with Cunningham White, Florence Parks and Sugar Puff. Consistency between higher frequency of adult presence and high egg density were only found with Olga Mezitt and Ramapo. Catawbiense Album, English Roseum, Helliki, Ponticum Variegatum, and Sneezy had lower frequency of adult presence and low egg density. Future research will need to address resistance of various Rhododendron cultivars with no choice experiments to determine the effects on feeding, oviposition and hence *S. pyrioides* population survival.

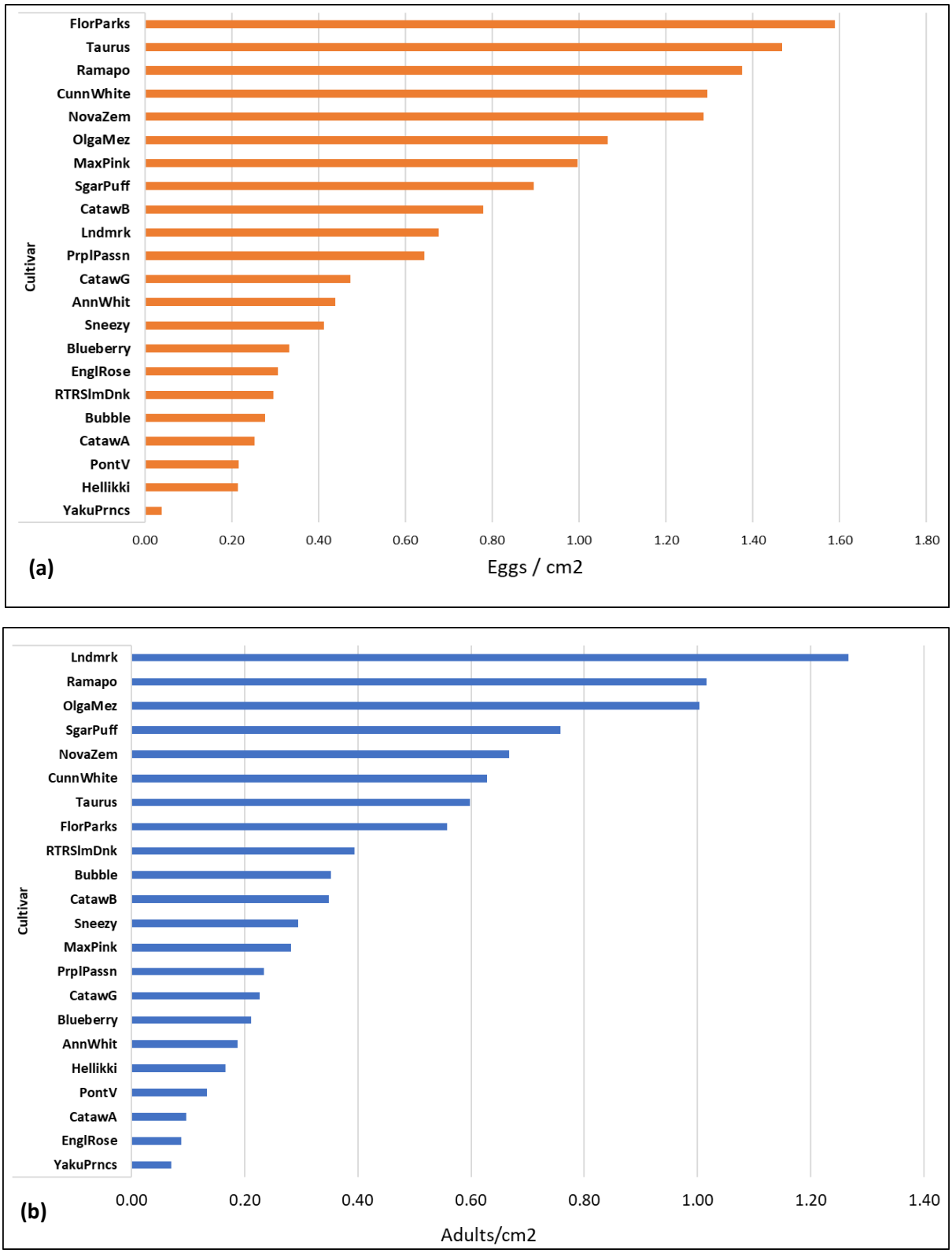

Fig. B3. Egg (a) and adult (b) densities of *S. pyrioides* on two Ericaceae genus: 21 *Rhododendron* cultivars and one *Vaccinium* variety.