STEPHANITIS LACE BUGS AFFECTING ERICACEOUS PLANTS: HOST RANGE, MECHANISMS OF RESISTANCE, AND MANAGEMENT

by

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ABSTRACT

The Andromeda lace bug *Stephanitis takeyai* Drake and Maa (Hemiptera: Tingidae) is an important pest of pieris, a popular ericaceous ornamental shrub. In the first project, over sixty *Pieris* taxa (species, cultivars and hybrids) were evaluated for their reaction to *S. takeyai* and *S. pyrioides* (Scott), the more economically important species, using no-choice, multi-choice and whole plant assays. *P. phillyreifolia* and *P. japonica* 'Variegata' were identified as resistant to both species of lace bugs while *P. japonica* 'Cavatine' was susceptible to both. *P. japonica* 'Temple Bells' was highly susceptible to *S. takeyai*, but resistant to *S. pyrioides*. Oviposition by *S. takeyai* was noted in various *Pieris* taxa, whereas *S. pyrioides* did not oviposit in any of the *Pieris* taxa. In the second project, some of the potential mechanisms of resistance in selected *Pieris* taxa to *S. takeyai* were examined. Assays with extracts of *Pieris* leaf-surface wax revealed that they did not have a role in resistance. Resistance in *Pieris* taxa to *S. takeyai* appeared to be a combination of different factors like leaf toughness, moisture and stomatal characters. The resistant P. phillyreifolia leaves were tougher, lower in moisture and had smaller stomata than susceptible taxa. The acceptability of ten ericaceous hosts to S. takevai was assessed in the third study. In no-choice tests maximum leaf damage was recorded on P. japonica and Rhododendron calendulaceum, while slight but non-significant damage was noted on Vaccinium arboreum and Rhododendron 'Hampton Beauty'. Nymph emergence was recorded on P. japonica, R. calendulaceum and Rhododendron 'Hampton Beauty'. In multi-choice tests maximum leaf damage was recorded on P. japonica whereas R. calendulaceum suffered only slight damage. This showed that several plants, which may not be favorable hosts, could still act as reservoirs for the pest. In the fourth project effectiveness of insecticides available to the homeowner, supplemented with a natural enemy *Chrysoperla carnea* (green lacewing) in suppressing the azalea lace bug was compared. Treatments using traditional insecticides (acephate and imidacloprid) were significantly more effective in controlling the lace bugs than alternative methods like oil and soap. C. carnea did not contribute significantly to the lace bug suppression.

INDEX WORDS:Stephanitis takeyai, pyrioides, Pieris japonica, phillyreifolia,Ericaceae, resistance mechanisms, lace bug, no choice test, multichoice test, leaf surface lipids, leaf wax, leaf toughness, stomata,management, acephate, imidacloprid, Chrysoperla carnea

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То

Purushothaman and Padmini Nair, my parents

Bhavani Amma, my grandmother

and

Padmanand, my husband

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

"Lace bug" is the generic term used to refer to members of the Hemipteran family Tingidae. These small bugs usually 2 to 5 mm in length, are characterized by reticulate outgrowths of the pronotum and wings in the adults that resemble lace and give the family its common name. The Tingidae are cosmopolitan in distribution and over 2,000 species in about 250 genera are recorded worldwide (Slater 1982; Stonedahl et al. 1992). Lace bugs are a well studied group of insects owing to their phytophagous nature, which often takes on economic importance. Their fragile appearance belies the devastation they can inflict on their host plants. Some workers consider tingids to be of little economic importance, unless their host or near relative is cultivated extensively (Froeschner 1988). However others report Tingidae as the most important family in the order Hemiptera in relation to injury on ornamental trees and shrubs (Johnson and Lyon 1991). Under normal circumstances damage may be insignificant, but severe infestations can even cause death of plants (Froeschner 1995; Klingeman et al. 2000b). With changes in cropping systems worldwide and minor crops gaining importance, there have been reports of different species of lace bugs attaining major status and causing economic damage (Neal and Schaefer 2000). One genus of lace bugs that has always attracted attention due to its association with cultivated plants, tropical and temperate, is Stephanitis Stål.

The genus Stephanitis in North America

There are over 60 species of lace bugs in the genus *Stephanitis*, some of which are well known as pests of tropical and temperate fruit and ornamental trees and shrubs worldwide (Howard 2001). In North America, the three common species are the azalea lace bug *Stephanitis pyrioides* (Scott), the rhododendron lace bug *S. rhododendroni* Horváth and the Andromeda lace bug *S. takeyai* Drake and Maa. They are all generally known to attack woody ornamentals, especially azaleas, rhododendrons and related plants of the family Ericaceae (Alverson et al. 1994). The differences among these three species have been described for identification purposes (Bailey 1950; Dunbar 1974). Of these, *S. rhododendroni* is believed to be indigenous to North America (Johnson 1936), but *S. pyrioides* and *S. takeyai* were both introduced from Japan which is believed to be their country of origin (Neal and Schaefer 2000). Froeschner (1988) also reported a fourth species, *S. blatchleyi* Drake, but it was last collected in 1927 and is believed to be extinct (Drake 1925; Oliver et al. 1990).

S. rhododendroni is the species that has been associated with flowering rhododendrons, as well as the other ericaceous hosts preferred by the genus *Stephanitis*. Though it is believed to have originated in North America, there have been several disputes about this, as well as the placement of the species in the genus *Stephanitis* instead of *Leptobyrsa* (Neal and Schaefer 2000), mainly due to the differences in the number of generations (one or two, as opposed to three or four in other *Stephanitis* spp.) and also in the number of instars (four, as opposed to five in other *Stephanitis* spp.) (Johnson 1936). Another point of dispute is the discontinuous presence of the species as reported by various workers, which suggests poor establishment (Neal and Schaefer

2000), in spite of the absence of natural enemies (Johnson 1936). These observations question the origin, distribution and taxonomy of this species.

The major economic, cosmopolitan tingid species is the azalea lace bug, *S. pyrioides* (Scott), as it causes severe economic damage to landscape and cultivated azaleas in most countries where azaleas (*Rhododendron* L. spp.) are grown (Shrewsbury and Smith-Fiola 2000, Klingeman III et al. 2001b). Its notoriety has increased concurrently with the increasing popularity of azaleas as ornamental plants and therefore it has been extensively studied (Neal and Douglass 1988). Currently *S. pyrioides* is widely distributed over several countries in Asia, Europe and the eastern United States (Neal and Schaefer 2000). Apart from azaleas, it is known to infest other ericaceous hosts like kalmia (*Kalmia latifolia* L.), pieris (*Pieris ovalifolia* (Wall.) D. Don) (Drake and Ruhoff 1965) and lyonia (*Lyonia nezikii* Nakai & H. Hara) (Takeya 1963).

Several aspects of the life history of *S. pyrioides* have been studied in great detail over the past century providing significant information with respect to the biology, damage and management of the pest. Development times and thermal requirements for nymphs at constant temperatures were determined (Neal and Douglass 1988; Braman et al. 1992) as well as degree-day accumulations for generation development (Braman et al. 1992). The feeding mechanism of *S. pyrioides* was observed as inserting stylets through the stomata on the lower surface of the leaves and depleting the chlorophyll content from the upper palisade parenchyma cells, thereby affecting the photosynthetic capacity of leaves (Ishihara and Kawai 1981). This was confirmed by Buntin et al. (1996) who also reported that frass spot numbers provided an index of the amount of feeding. This serves as a useful method to quantify the intensity of feeding by lace bugs. Oliver et al. (1985)

studied the setal exudates of S. pyrioides nymphs and their possible role in defense against predators. Chemical control using insecticides has been successfully used against S. pyrioides (Johnson 1960; Raupp et al. 1992). However rising concern about use of chemicals has directed research on the use of other pest management strategies including biological control. A few predators have been identified, notably; the Japanese mirid Stethoconus japonicus Schumacher which is an obligate and highly aggressive predator of S. pyrioides, but its activity is limited by various factors (Neal and Haldemann 1992). However naturally occurring and augmentatively released parasitoids and predators can complement chemical management programs (Balsdon et al. 1993; Shrewsbury and Smith-Fiola 2000). Klingeman et al. (2000a) evaluated grower, landscape manager and consumer perceptions of S. pyrioides feeding injury on azaleas and found that using aesthetic injury levels to determine treatment thresholds significantly affected the amount of pesticides being used to control the pest. The existence of resistance to S. pyrioides has been reported among both evergreen (Schultz 1993) and deciduous azaleas (Braman and Pendley 1992; Wang et al. 1998). The potential role of leaf epicuticular lipids in resistance was examined by Balsdon et al. (1995) and Wang et al. (1999). Chappell and Robacker (2006) found definite influence of epicuticular leaf wax in resistance to S. pyrioides among deciduous azaleas. The effects of stomatal size on feeding preference of S. pyrioides was also evaluated on evergreen azaleas but they were not found to be correlated (Kirker et al. 2008). The mechanisms of resistance in azaleas to S. pyrioides are yet to be understood completely.

Reciprocal crosses between congenerics *S. pyrioides* and *S. takeyai* resulted in a unidirectional asymmetric sexual hybrid female from S. *pyrioides* females and *S*.

takeyai males. This was the first report of hybridization among sympatric congeneric species in Tingidae. The hybrids were also confirmed using two physical methods (Neal and Oliver 1991).

The andromeda lace bug *Stephanitis takeyai* Drake and Maa is considered to be one of the most important pests of *Pieris* D. Don spp. (Johnson and Lyon 1991). It is believed to be indigenous to Japan (Tsukada 1994b), but has now spread to various other parts of the world.

S. takeyai is known to be polyphagous in Japan as well as the various countries where it has spread and established, attacking host plants of different unrelated families (Tsukada 1994b). Damage caused by foliage feeders like *S. takeyai* is most pronounced on ornamental plants like pieris which are valued for their foliage as well as flowers. The affected plants show chlorotic blotches on leaves, which may shed prematurely, leading to drying up of twigs or even the whole plant (Schread 1968).

Despite its importance as a polyphagous pest, relatively few studies have been done on *S. takeyai*. Polyphagy is suggested to be an ancestral character in the genus *Stephanitis*, and monophagous species are supposed to have developed later (Tsukada 1994c). This finding suggests the importance of more studies on *S. takeyai* and its relation to other members of the genus.

Origin, distribution and spread of S. takeyai

The bug was first described in 1873 from specimens collected in Madras, India and given the name *Tingis globulifera* (Walker 1873). It was also described in Japan (Matsumura 1905), who again named it *Tingis globulifera*. On realizing the homonymy, it was shifted to the genus *Stephanitis* and named *S. globulifera* (Matsumura 1908;

Horváth 1912). Later it was renamed again as *S. takeyai*, owing to the preoccupation of *globulifera* (Drake and Maa 1955).

Although the earliest records of the pest are from India, *S. takeyai* is generally considered to be native to Japan (Takeya 1963; Tomokuni 1987; Tsukada 1994b). Its preferred host is the Japanese andromeda *Pieris japonica* (Thunb.) D. Don ex G. Don, along with which it is believed to have evolved (Tsukada 1994b). The earliest reports of its spread to other parts of the world also mention that the source of the pest was probably plant material shipped from Japan (Bailey 1951).

S. takeyai was first reported in North America in 1950 on *P. japonica* plants in Connecticut, and there were speculations even at that time that this recently introduced species may become a serious pest of pieris and other ornamental Ericaceae (Bailey 1950). The plants were part of nursery stock shipped from Japan (Bailey 1951). Later, it spread to several other states like Delaware, Maryland, Massachusetts, New Jersey, Pennsylvania, Rhode Island, New York, Virginia and Ohio (Dunbar 1974), and West Virginia (Torres-Miller 1989). By 1997, *S. takeyai* was reported as a major pest of andromeda in southern New England and southward (Nielsen 1997). There have been unpublished reports of the pest from the southeastern United States recently.

In Europe, the earliest report of *S. takeyai* was from a single *P. japonica* plant in a private garden in Boskoop, Province of Zuid-Holland in 1994. The population was reportedly eradicated by destruction of the infested plant (Aukema 1996a). However in 1999, two samples with high numbers of nymphs and adults of *S. takeyai* were collected from *P. japonica* in another private garden in Boskoop. A limited survey in private gardens in this area showed that the vast majority of *P. japonica* plants were infested, but

the insect was not found on other plant species. Some *P. japonica* plants were heavily damaged due to the sap-feeding activities of large populations. This suggested that the pest was present for a number of years in the area (EPPO 2001a). A compilation of the database on Dutch Heteroptera also includes *S. takeyai* among 22 additional species discovered since 1989. It mentions *S. takeyai* among Heteroptera recorded from The Netherlands since 1980. The pest was reportedly brought in along with its host *P. japonica* from Japan, and so its arrival is categorized as passive dispersal, by a transport related pathway (Aukema 2001).

S. takeyai was also reported from the United Kingdom around the same time (EPPO 1998). It was noted at the time, that the risk of spread was limited since there was no plant propagation. However, a pest risk analysis (PRA) initiated later that year (MacLeod 1998) revealed that the pest was found on established plants of pieris which were imported from the Netherlands in 1996. Later interceptions of the pest in 2000 also involved plants from the Netherlands and Italy. An outbreak of *S. takeyai* was also reported in the United Kingdom (Halstead and Malumphy 2003).

In Poland, *S. takeyai* was reported from a commercial nursery, on *P. japonica* plants (Soika and Labanowski 1999; Labanowski and Soika 2000). It was reported as established in Poland in 1999, possibly introduced on andromeda stock from Germany in 1998 (Soika and Labanowski 1999). The presence of *S. takeyai* was also reported in Italy (Colombo and Limonta 2001). It was revealed on inspection of a nursery which sells plant products from all over the world, again suggesting the inadequacy of proper quarantine measures (EPPO 2001b).

The lace bug is also presumed to have spread to Germany from the Netherlands. It was recorded for the first time in Germany in a sample taken from a *P. japonica* shrub in a public park in Bremen. The sudden yellowing of leaves in some *P. japonica* shrubs in the garden prompted a closer examination of the plants, which confirmed the infestation to be that of *S. takeyai* (Hommes et al. 2003). Later investigations revealed its occurrence in house gardens, horticultural farms and public parks. This confirmed the suspicions of *S. takeyai* to have spread to a greater extent than expected in Germany (Hommes and Westhoff 2004).

Thus it is clear that the increase in commercial trade of plants has considerably increased the risk of spread of *S. takeyai* to newer regions, where it could potentially attack different plant species and take on major status.

Biology

The first observations on *S. takeyai* after its discovery in Connecticut showed that the bug had at least four nymphal instars whose measurements were also made. The smallest nymph, without apparent spines, was 0.7 mm long. Others, which had prominent spines on the dorsal side of the head, thorax, and abdomen, measured 1.2 mm, 1.8 mm and 2.4 mm in length. Adults found with them measured 3.9 to 4.2 mm (Bailey 1951).

Later studies confirmed that the lace bug had four to five generations a year (Schread 1953; Dunbar 1974) in most locations, but two generations have also been reported (Soika and Labanowski 1999). The eggs and instars and their development times were described and measured by several workers in different locations (Bailey 1974; Dunbar 1974; Kawakami 1983).

When *S. takeyai* was first discovered in Connecticut, USA the late appearance of adults suggested that it overwintered in the egg stage (Bailey 1950). However, when adults and nymphs were found to be numerous and active even after exposure to low temperatures of $40 - 50^{\circ}$ F, it was suggested that they may overwinter in these stages, contradicting the earlier suggestion, and also that it laid overwintering eggs which were scattered on the leaf, as well as summer eggs which were deposited next to the midrib (Schread 1953). The position of oviposition on the leaf surface also varied with the host. On the evergreen azalea *Rhododendron* sp., eggs are laid on the midrib (Neal Jr 1988). Some studies also showed that overwintered eggs were not cold tolerant (Schread 1968; Dunbar 1974). The general understanding however is that eggs of *S. takeyai* are laid on the leaves like the majority of other Tingids, where they overwinter (Tsukada 1994a; Soika and Labanowski 1999).

The average duration of the life cycle from egg to adult at ambient temperature (25° C) was found to be about 23 days with the preoviposition and oviposition periods 6.6 and 14.8 days, respectively. Average longevity of adult females and males was found to be 44 days and 63 days, respectively. Duration of the egg stage was 9 to 14 days, and the nymphal stage lasted 12 to 15 days (Dunbar 1974). Studies on the development of *S. takeyai* in Japan, the country of its origin showed similar results. A comparison of the development times on its two preferred hosts, *P. japonica* and *Lyonia elliptica* (Siebold & Zucc.) Hand.-Mazz. showed that development took longer on *L. elliptica*, and adult longevity was also greater on this host as compared to *P. japonica*. Thus it is clear that the host has a significant effect on the development of *S. takeyai* in addition to other factors (Tsukada 1994a).

Eggs are laid embedded into tissue on the undersides of leaves and the oviposition sites are covered by varnish-like spots of excrement. Both nymphs and adults can be found feeding on leaf undersides. Feeding injury is displayed on the upper leaf surface as severe yellow stippling (Childs 2000). Lace bug damage results from the nymphs and adults piercing the epidermis with their mouthparts and drawing out cell contents. This causes reduction in photosynthesis and chlorophyll content due to damage of palisade parenchyma of leaves, and restriction of stomatal gas exchange, leading to the characteristic chlorotic flecks, which are visible on the upper side of the leaf (Buntin et al. 1996).

Host Range

The common name "Andromeda lace bug" is itself based on the major, reproductive host of *S. takeyai* which is the Japanese andromeda *Pieris japonica*. It belongs to the family Ericaceae (Watson and Dallwitz 1992), which also includes several important cultivated plants like azalea, rhododendron, cranberry, blueberry and heather.

In, Japan, *S. takeyai* is known to exhibit non-obligate seasonal host alternation between its two main hosts, both of which belong to the family Ericaceae, feeding on *P. japonica* during the winter and the deciduous shrub *L. elliptica* during the summer. If *L. elliptica* is scarce, *S. takeyai* may continue to feed on *P. japonica* (Tsukada 1994b). The persimmon tree (*Diospyros kaki* Thunb.) of the family Ebenaceae, the camphor tree (*Cinnamomum camphora* (L.) J. Presl, Family Lauraceae), the Chinese onise (*Illicium religiosum* Siebold and Zucc., Family Illiciaceae) and other useful plants have been reported as hosts of *S. takeyai* in Japan (Takeya 1963). Watanabe (1983) stated the occurrence of *S. takeyai* in Japanese pine stands, on *Pinus densiflora* Siebold and Zucc.

and *P. thunbergii* Parl. In a pest risk analysis MacLeod (1998) reported this occurrence as a possibility of it becoming a pest of forestry.

The presence of *S. takeyai* has been recorded in the United States from several other hosts like *Andromeda* sp., *Aperula* sp., *Cinnamomum* sp., *Lindera* sp., *Lyonia* sp., *Pieris* sp. and *Salix* sp. which are members of different plant families and not closely related (Drake and Ruhoff 1965). It has also been recorded on the rhododendron, *R. calendulaceum* (Michx.) Torr., when the branches of *P. japonica* and *R. calendulaceum* were contiguous (Bailey 1974). Another study reported spicebush (*Lindera benzoin* (L.) Blume) and sassafras (*Sassafras albidum* (Nutt.) Nees) both belonging to family Lauraceae, as hosts of *S. takeyai* (Wheeler Jr. 1977).

Plants within the Hippocastanaceae, Magnoliaceae, Rosaceae, Saxifragaceae and Styracaceae are also reported to be hosts for *S. takeyai* in Poland (Soika and Labanowski 1999).

These reports clearly suggest the phytophagous nature of *S. takeyai* making it all the more a cause for concern, in the light of the increasing commercial trade in plant material worldwide. Several plants, which may not be favorable hosts, could still serve as reservoirs for the pest. Hence a proper understanding of the host plant utilization of different plants by *S. takeyai* would be an indispensable part of formulating management strategies for the pest.

Pieris – the preferred host

Pieris, which belongs to the plant family Ericaceae, are handsome evergreen shrubs recognized by their glossy green leaves and clusters of urn-shaped flowers colored red, pink or white. They are particularly noted for the magnificent colors displayed by

their young leaves. The colors of the new spring foliage vary from deep red to bronze in various cultivars. These features make pieris a popular choice as landscape or foundation plants, shrub borders or incorporated with other evergreens. Early bloom time is another desirable character of this plant.

Among the various cultivated species of pieris, Japanese pieris (*P. japonica*) native to Japan, is the most common and considered the most beautiful (Heriteau 2006). *P. japonica* is believed to be native to Japan (and Taiwan and parts of eastern China) (Ohwi 1984) and the pest is believed to have originated together with its host. Although it is known to be polyphagous, the preference of *S. takeyai* for *P. japonica* is also confirmed (Schread 1968).

The species native to North America is Mountain pieris *P. floribunda* (Pursh) Benth. and Hook. f. It is an underutilized, ornamental shrub indigenous to the Appalachian mountains of the U.S. extending from Virginia southward into Georgia (Starrett et al. 1996). *P. floribunda* is less attractive, but more tolerant to stresses like alkaline soil and pests.

Himalaya pieris (*P. formosa* (Wall.) D. Don) which is found mainly in the Pacific Northwest and England, has larger leaves and good shape but needs an even, cool climate (BTRL 1999). The hardy late bloomer Dwarf pieris (*P. taiwanensis* Hayata) is ideal for small gardens. *P. phillyreifolia* (Hook.) DC. (Climbing fetterbush) is native to North America and is common in the Southeastern states of Alabama, Florida, Georgia, Mississippi and South Carolina (USDA ARS 2011). *P. nana* (= *Arcterica nana* Maxim.) in *Pieris* subg. *Arcterica*, is currently placed as sister to *Pieris* subg. *Pieris*, as supported by molecular and morphological analyses (Kron and Judd 1997). Other cultivated

species of *Pieris* include *P. cubensis* (Grisebach) Small. in Cuba (Judd 1995) and *P. swinhoei* Hemsley in Southeastern China (FOC 2011).

Like other temperate plants of the family Ericaceae pieris is adapted to partial shade, but also grows well in full sun in cooler climates. It is not tolerant of wind. Rich, moist, well-drained, and acidic soils with a pH of 5 to 6 are preferred. Being a calcifuge plant, nutrient deficiencies and root disease commonly occur on soils that are alkaline and poorly drained. Pieris is known to form ericoid mycorrhizae with certain ascomycete fungi, and this symbiotic relationship is considered crucial to the success of members of the family in edaphically stressed environments (Cairney and Meharg 2003).

Pieris (also known as Andromeda) is infested by a number of pests; foliage feeders like lace bugs and mites being the most important. They not only weaken the plant but also reduce the attractiveness of the foliage by causing yellowing and premature defoliation. Scales and borers can attack branches and stems, affecting flow of nutrients and water in the plant. Among diseases, *Phytophthora* root rot is the most destructive and causes wilting and death of plants. Plants in moisture stressed soils are more prone to such root diseases. Diseases like canker, causing dieback of stems and branches are also noted in stressed environments like low temperature or drought. In wet weather, leaf spots can be widespread. Nematodes are also known to cause considerable damage to the root system, which can result in plant decline and also predispose the plants to other damage. Damage by animals like deer and rodents is also reported. Being evergreen, animals may feed on bark below the snow or mulch line which can result in girdling and death of plants (BTRL 1999). However pieris are generally considered deer resistant

(Burroughs and Dudek 1994; Horton and Edge 1994; Jull 2001; Perdomo et al. 2004; Kisanuki et al. 2008).

Resistant/susceptible varieties

S. takeyai is a serious pest of *P. japonica* in particular on cultivars 'Select', 'Debutante', 'Cupido' and to a lesser degree on 'Flaming Silver', Variegata' and 'Redmill' (Labanowski and Soika 2000). *P. floribunda* is believed to be resistant to the pest, and the hybrid *P. floribunda* x *japonica* less favorable (Dunbar 1974).

Management of S. takeyai

Different management strategies have been tried for keeping lace bugs under control, but they are considered hard to manage due to their many adaptations. The undersurface of leaves, where the adults and nymphs are usually found, is often difficult to reach using conventional pest control equipment and chemicals. The eggs, with their natural cover of varnish-like frass deposited by the female, are also protected to some extent (Livingstone and Yacoob 1987; Nalepa and Baker 1994). Tingids are also comparatively free from parasitism and predation (Wheeler Jr et al. 1975; Gordh and Dunbar 1977; Livingstone et al. 1982; Livingstone and Yacoob 1983; Henry et al. 1986; Livingstone and Yacoob 1986; Sathiamma et al. 1998; Sathiamma et al. 1999).

However, as with other pests, proper management measures become necessary when the damage caused by lace bugs causes major economic concern. Although *S. takeyai* is an important pest of pieris, only few workers have studied it from the aspect of pest management (Johnson and Lyon 1991). Regular and thorough inspection of plants is always recommended for presence of overwintering lace bug adults, eggs, and newly hatched nymphs. In case of mild infestations causing slight damage, the bugs can be

washed off with a stream of running water and use of chemicals can be avoided. However, in case of heavy infestations, chemical control may be required (Sparks et al. 2002).

Recommended chemicals for lace bug control include carbaryl, acephate, dimethoate, malathion and bifenthrin. Systemic insecticides like imidacloprid have been reported to be effective in controlling infestations (Hommes and Westhoff 2004). Chemical insecticides are also recommended in places with high risk of lace bug attack, and early spraying of plants is advisable, so that further generations of the pest are not allowed to develop. This also reduces the amount of insecticides used and thereby plant protection costs. Repeated applications of insecticidal soaps (M-Pede®) or horticultural oils are also reported to be effective in controlling lace bug populations (Sparks et al. 2002).

Use of botanical insecticides is a relatively less explored area in pest control; because they are generally more expensive and less available commercially as compared to synthetic insecticides. This may be due to problems in production of a consistent product and lack of sales. In general, data on their effectiveness and long term toxicity is insufficient. However, high toxicity and rapid degradation are two factors which make botanical insecticides highly suited for pest control, especially in urban situations. The botanical insecticides that are commercially available include pyrethrin/pyrethrum, rotenone, sabadilla, ryania, nicotine, citrus oil extracts, and neem (Cloyd 2004). Botanical insecticides have been reported to be effective in the control of lace bugs (Coffelt 1994; Peet 1996; Drees 1997; Buss and Short 2001; Cloyd 2004; Layton 2006).

Biological control

Although they appear to be delicate and fragile, the adults especially being weak fliers, the eggs and nymphs are well protected by several unique mechanisms. The eggs of lace bugs are usually inserted into plant tissues and covered in excrement (Livingstone and Yacoob 1987) in the form of a greasy, varnish-like spot. The exoskeleton of the nymphs is often covered with spines or bristles that are secretory in nature, and are seen to exude droplets of fluid which may have a protective function (Neal 1988). These secretions are also known to deter birds from feeding (Mason et al. 1991), and to be toxic to some microorganisms and nematodes (Neal et al. 1995). The nymphs are also seen to occur in large aggregations on the same host plant and are usually free of parasitism or predation (Neal and Schaefer 2000). There may be several other protective mechanisms in these apparently vulnerable insects that are yet to be discovered.

To encourage potential biological control agents, it is always recommended to look for parasitized or dead lace bugs during inspection of plants prior to initiating a chemical control program (Sparks et al. 2002). Two important biological control agents that have been recognized to control *S. takeyai* are the predatory mirid bug *Stethoconus japonicus* Schumacher and the specialist egg parasitoid *Anagrus takeyanus* Gordh. The predatory Japanese plant bug *S. japonicus*, also native to Japan is a potentially useful, adventive, obligate predator of *Stephanitis* lace bugs (Henry et al. 1986). It was also found associated with *S. takeyai* in Japan (Yasunaga et al. 1997).

Earlier workers collected certain parasitoids from overwintering eggs of *S*. *takeyai*, which were then thought to be *Anaphes* sp. (Schread 1968). Later, this mymarid species was identified as *Anagrus takeyanus* (Gordh and Dunbar 1977). It was collected

from the eggs of *S. takeyai*, and also from eggs of *S. pyrioides* (Braman et al. 1992; Balsdon et al. 1996). *A. takeyanus* is thelytokous, which is different from other *Anagrus* species of North America, which led to the speculation that it was also introduced along with *S. takeyai* from Japan. Besides, *Stephanitis* species in North America are not known to be parasitized. This speculation was confirmed when *A. takeyanus* was identified from Japan (Tsukada 1992). It was also found that parasitization by the wasp is the main mortality factor of the eggs, and frass cover on the egg mass functions as a parasitization avoidance mechanism (Tsukada 2000b). Corresponding to the different life cycles in *S. takeyai*, i.e., with seasonal host alternation and without, *A. takeyanus* showed different life cycles. It was understood that the aestivation of the wasp was appended to overwintering as an adaptation to the evolution of seasonal host-plant alternation in the lace bug, which was secondarily lost by the bug in the absence of seasonal host-plant alternation. The different life cycles in the bug and the wasp significantly affected the rate of parasitism and the hatchability of *S. takeyai* eggs (Tsukada 2000a).

Chemistry

The unusual acetogenins secreted by nymphs and adults of lace bugs (Tingidae) are some of the most interesting groups of defensive compounds from true bugs (Millar 2005). These compounds are active against bacteria, fungi, and nematodes (Neal et al. 1995) and they also have a possible role in defense against predators (Oliver et al. 1990; Mason et al. 1991).

The individual species of the genus *Stephanitis* are chemotaxonomically distinct and are characterized by the oxidation states of the secreted compounds in their setal exudates. Four major components of the setal exudate of nymphs of *S. takeyai*, were

identified (Oliver et al. 1990). The synthesis of one of these components was also undertaken to confirm the structural assignment and to provide sufficient material to evaluate its properties (Oliver et al. 1988).

Mechanisms of resistance in host plants

The presence of varying degrees of resistance to lace bugs among *Pieris* species and cultivars is indicative of different mechanisms of resistance. These may involve the physical and chemical characters of the leaf surface. Possible mechanisms of resistance in pieris have not been reported earlier but several other ericaceous plants have been studied for their mechanisms of resistance to herbivores. The resistance exhibited by certain azalea cultivars to *S. pyrioides* has been investigated by several workers (Balsdon et al. 1995; Wang et al. 1998; Wang et al. 1999; Chappell and Robacker 2006). Results indicated that antibiosis combined with physical characteristics could be involved in observed *S. pyrioides* resistance. Antibiosis is suggested by lower survivorship of adults and nymphs, significantly reduced feeding, plant damage and growth rate of *S. pyrioides* (Wang et al. 1998).

The cuticle which is the first line of defense in plants against any external invasion is composed of a mixture of epicuticular lipids. Although the primary function of these epicuticular lipids is prevention of water loss, they also function ecologically in the mediation of interactions between plants and their insect herbivores. Insect herbivore attachment and movement on the plant surface may also be affected by the physical structure of plant surface lipids (Eigenbrode and Espelie 1995). In studies on composition and variability of epicuticular lipids in azaleas and their relationship to lace bug resistance, the predominant surface-lipid components from the tested taxa were

found to be *n*-alkanes and triterpenoids (Balsdon et al. 1995; Wang et al. 1999). The lipid components were seen to vary according to the season and were significantly correlated to the azaleas' reaction to the azalea lace bug, which was determined by parameters like leaf area damaged, oviposition, egg and nymph development and nymphal survival (Wang et al. 1999). Ursolic acid, *n*-hentriacontane, and *n*-nonacosane were the high in the resistant azalea genotypes whereas α -amyrin, β -amyrin, and *n*-nonacosane were seen in higher amounts in the susceptible ones (Wang et al. 1999). 'Hino Crimson', a cultivar highly susceptible to the azalea lace bug was also found to be low in the triterpenoid components, α - and β -amyrin (Balsdon et al. 1995). Conversely, other studies have shown α - and β -amyrin to be associated with feeding and oviposition deterrence in some plants (Chapman 1977; Robertson et al. 1991; Yang et al. 1993). Thus, acceptance or rejection of the host by the herbivore may be elicited by a combination of sensory signals sent out by varying levels of these chemicals.

In a more recent study, leaf wax extracted from *S. pyrioides* resistant genotypes and applied to susceptible genotypes conferred a high level of resistance to both feeding and oviposition by *S. pyrioides* in the treated susceptible genotypes. Conversely, leaf wax extracted from susceptible genotypes and applied to resistant genotypes conferred susceptibility to the treated resistant genotypes, although the effect was less substantial. These results indicated that leaf wax serves as a primary mechanism of resistance of deciduous azalea to *S. pyrioides* (Chappell and Robacker 2006).

Another mechanism that may affect herbivore activity is pubescence on plant surfaces. Pubescence may also serve other essential physiological functions like prevention of desiccation. Hanley et al. (2007) extensively reviewed numerous studies

about the effects of pubescence and other structural plant defense mechanisms on herbivores. Azalea cultivars have been observed to vary considerably in the amount of leaf pubescence. In evaluations of azalea resistance to *S. pyrioides*, leaf water content and leaf pubescence were significantly different among taxa. However insufficient evidence was available to conclude that leaf pubescence was involved in azalea lace bug resistance (Wang et al. 1998). Leaf toughness is a major source of mechanical defense in plants against insect herbivores and in some cases may be more influential on herbivores than plant chemical contents (Lowman and Box 1983). Toughness affects the establishment of first instars of insects with chewing mouthparts, increasing costs of food acquisition and impeding food assimilation, thereby affecting, survival, size, weight, and performance of successive larval stages (Slansky 1990). The role of leaf toughness in resistance of *Vaccinium* L. spp. (family Ericaceae) to leaf hoppers has been examined and possible correlations were identified in some species like *V. crassifolium* Andrews (Meyer and Ballington 1990).

Other ultrastructural features of the plant like size of stomata may have a role in feeding by herbivores, especially those with piercing and sucking mouthparts. It has been observed earlier that lace bugs feed by inserting their proboscides through leaf stomata and draw out cell contents (Buntin et al. 1996). The effects of stomatal size on feeding preference of *S. pyrioides* was studied on selected cultivars of evergreen azalea. However, although the tested taxa were found to vary in the size of their stomata, the variation did not appear to be correlated to feeding by *S. pyrioides* (Kirker et al. 2008).

Plant chemistry may also influence insect feeding. Production of chemicals that deter or injure herbivores is one of the most common defense mechanisms in plants. The

defensive chemicals produced by plants (also called secondary compounds or allelochemicals) are usually unique to the plant or its family, and can elicit varying responses in herbivores. The knowledge of insect herbivore responses to these chemicals is an important step in formulating sustainable pest management strategies (Despres et al. 2007). Many ericaceous plants contain diterpenic substances which constitute a unique group of toxins. The acute toxicity of 36 samples of ericaceous toxins and their congeners to mice has been determined (Hikino et al. 1976). Several toxic compounds have been identified from *Pieris* sp., like grayanoids from *P. formosa* which possess antifeedant and insecticidal properties (Ding et al. 1998, Wang et al. 1998a) and asebotoxins III, a diterpenoid from the leaves of *P. japonica* 'Asebi' (Hikino et al., 1971a, b; Takeya et al. 1981). Examples of other ericaceous toxins are grayanotoxins, leucothols, and grayanols from *Leucothoe grayana* Miximowicz (Hikino et al., 1973; Fushiya et al., 1974), rhodojaponins from Rhododendron japonica Springer (Hikino et al., 1970b; Hikino et al., 1972b), and lyonia toxin from Lyonia ovalifolia var. elliptica (Siebold & Zucc.) Hand.-Mazz. (Hikino et al., 1970a).

The concentration of water and mineral nutrients in plants may also influence insect herbivory since they derive significant amounts of moisture and nutrients from their host plants. The role of water in all forms of life is widely recognized and water may be a more critical limiting factor than even protein in the growth of several foliage feeding insects (Barbehenn et al. 1999). However in one study with azaleas, leaf water content was not found to be significantly correlated with azalea lace bug performance (Wang et al. 1998). Various activities of insects are directed by nutritional needs (Slansky 1982) and thus nutrients essentially direct energy flow at all trophic levels

(Mattson 1980). Mineral nutrient requirements of most insects have not been studied but essential elements have been identified for some (House 1961). The nutrient contents in different *Pieris* species or their correlation with herbivory have not been reported earlier.

The resistance shown by some *Pieris* taxa to *S. takeyai* may be a combination of different factors. However these have not been studied before.

Research Objectives

Project 1. Gradients in susceptibility of *Pieris* taxa to *Stephanitis* lace bugs

The Andromeda lace bug *Stephanitis takeyai* is one of the most important pests of *Pieris* sp. which is a popular choice for landscape or foundation plants, shrub borders or incorporating with other evergreens. Very few workers have studied it from the aspect of pest management (Johnson and Lyon 1991) and the range of susceptibility among cultivated *Pieris* taxa has not been studied before. Mountain pieris, *P. floribunda* is believed to be resistant to the pest, and the hybrid *P. floribunda* x *japonica* less favorable (Dunbar 1974). Therefore this study was carried out to evaluate *Pieris* taxa for their resistance to the two species of lace bugs *S. takeyai* and *S. pyrioides*. Since the azalea lace bug, *S. pyrioides* is the major economic, cosmopolitan tingid species which is also known to infest other ericaceous hosts like Kalmia (*Kalmia latifolia*) and Pieris (*Pieris ovalifolia*) (Drake and Ruhoff 1965), we included *S. pyrioides* in our study.

Project 2. Resistance mechanisms in Pieris taxa to Stephanitis takeyai

The presence of varying degrees of resistance to lace bugs among *Pieris* species and cultivars is indicative of different mechanisms of resistance and these mechanisms are not known. These may involve the physical and chemical characters of the leaf

surface. Possible mechanisms of resistance in azaleas to S. pyrioides have been investigated earlier (Balsdon et al. 1995, Wang et al. 1998, Wang et al. 1999, Chappell and Robacker 2006). Results indicated that antibiosis combined with physical characteristics could be involved in observed S. pyrioides resistance. Antibiosis is suggested by lower survivorship of adults and nymphs, significantly reduced feeding, plant damage and growth rate of S. pyrioides (Wang et al. 1998). Leaf wax extracted from S. pyrioides resistant genotypes and applied to susceptible genotypes conferred a high level of resistance to both feeding and oviposition by S. pyrioides in the treated susceptible genotypes. Conversely, leaf wax extracted from susceptible genotypes and applied to resistant genotypes conferred susceptibility to the treated resistant genotypes, although the effect was less substantial. These results indicated that leaf wax serves as a primary mechanism of resistance of deciduous azalea to S. pyrioides (Chappell and Robacker 2006). The different mechanisms of resistance in Pieris taxa need to be studied further. The first objective of this study was to evaluate the effects of leaf-surface lipids on feeding, oviposition and survival of S. takeyai on Pieris leaves. Other objectives included evaluation of other possible mechanisms of resistance like leaf toughness, leaf nutritional parameters and leaf ultrastructure.

Project 3. Host plant utilization within family Ericaceae by the Andromeda lace bug *Stephanitis takeyai*

The preferred and major reproductive host of *S. takeyai* is the Japanese Andromeda *Pieris japonica*, from which it derives its common name "Andromeda lace bug". However even in its country of origin Japan, *S. takeyai* is known to exhibit non-

obligate seasonal host alternation between its two main hosts, both of which belong to the family Ericaceae, feeding on *P. japonica* during the winter and the deciduous shrub *Lyonia elliptica* during the summer. If *L. elliptica* is scarce, *S. takeyai* may continue to feed on *P. japonica* (Tsukada 1994b). It is also reported to attack the persimmon tree (*Diospyros kaki*) of the family Ebenaceae, the camphor tree (*Cinnamomum camphora*, Family Lauraceae), the Chinese onise (*Illicium religiosum*, Family Illiciaceae) and other useful plants (Takeya 1963). *S. takeyai* is also reported in forestry, on *Pinus densiflora* and *P. thunbergii* (Watanabe 1983) where it can presumably become a pest.

The presence of *S. takeyai* has been recorded in the United States from several other hosts like *Andromeda* sp., *Aperula* sp., *Cinnamomum* sp., *Lindera* sp., *Lyonia* sp., *Pieris* sp. and *Salix* sp. which are members of different plant families and not closely related (Drake and Ruhoff 1965). It has also been recorded on the rhododendron, *R. calendulaceum*, when the branches of *P. japonica* and *R. calendulaceum* were contiguous (Bailey 1974). Another study reported spicebush (*Lindera benzoin*) and sassafras (*Sassafras albidum*) both belonging to family Lauraceae, as hosts of *S. takeyai* (Wheeler Jr 1977).

Plants within the Hippocastanaceae, Magnoliaceae, Rosaceae, Saxifragaceae and Styracaceae are also reported to be hosts for *S. takeyai* in Poland (Soika and Labanowski 1999).

Several plants, which may not be favorable hosts, could still serve as reservoirs for the pest. Hence a proper understanding of the host plant utilization of different plants by *S. takeyai* would be an indispensable part of formulating management strategies for

the pest. This study was undertaken to confirm some of the earlier reports of host plant utilization by *S. takeyai* within the family Ericaceae.

Project 4. Integration of chemicals available to the home owner with natural enemy *Chrysoperla carnea* for management of Azalea Lace Bug

The current recommendations for ALB management focus on the use of chemical insecticides. However, there have always been concerns about the environmental effects of these chemicals. Many chemical formulations designated for use on ornamental plants are not suited for plants that are used for consumption due to higher persistence and toxicity. With increasing public awareness about these issues, there is a great demand for alternative pest management strategies which are cost effective and efficient at the same time. Especially from the home owners' point of view, safer, yet effective management measures are always sought after.

An earlier study (Balsdon et al. 1993) compared the efficacies of nine insecticidal materials in controlling the ALB and among these; acephate provided the most cost effective and long-term suppression of ALB. The study also examined the potential of the parasitoid wasp *Anagrus takeyanus* to establish populations on treated plants and *A. takeyanus* parasitism was not affected by any of the compounds. This indicates a possibility of integrating this or other natural enemies with chemical control for effective ALB suppression. Shrewbury and Smith-Fiola (2000) examined augmentative biological control as an alternative approach for managing azalea lace bug in production nurseries using the green lacewing, *Chrysoperla carnea. C. carnea* was chosen because of its effectiveness as a biological control agent for azalea lace bugs as well as its ease of

availability from commercial suppliers. This study was based on these earlier studies and aimed at evaluating the effectiveness of chemicals available to the home owner, supplemented with a natural enemy (*C. carnea*) in suppressing the azalea lace bug.

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CHAPTER 2

GRADIENTS IN SUSCEPTIBILITY OF PIERIS TAXA TO STEPHANITIS LACE BUGS

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ABSTRACT The Andromeda lace bug *Stephanitis takeyai* Drake and Maa (Hemiptera: Tingidae) is one of the most important pests of *Pieris* D. Don spp. which is a popular choice for landscape or foundation plants, shrub borders or incorporating with other evergreens. Few workers have studied S. takeyai from the aspect of pest management and the range of susceptibility among cultivated pieris taxa has not been studied before. No-choice Petri dish assays evaluated pieris taxa for their resistance to the two species of lace bugs S. takeyai and S. pyrioides (Scott). The azalea lace bug, S. pyrioides was included in the study because it is the major economic and cosmopolitan tingid species which is also known to infest other ericaceous hosts. Over 60 pieris taxa were evaluated for their susceptibility to the two species of lace bugs based on leaf damage, adult survival on the leaves and emergence of nymphs. Results showed that the taxa P. phillyreifolia and P. japonica 'Variegata' were consistently resistant to both species of lace bugs while P. japonica 'Cavatine' was consistently susceptible to both. P. japonica 'Temple Bells' and was notable in being highly susceptible to S. takeyai, but resistant to S. pyrioides. Oviposition was noted only with S. takeyai on some Pieris taxa, whereas S. pyrioides did not oviposit on any of the Pieris taxa. Choice assays (with 10 Pieris taxa) and whole plant assays (with 5 Pieris taxa) using S. takeyai alone were also conducted, confirming the resistance of *P. phillyreifolia* and susceptibility of *P. japonica* 'Temple Bells'.

KEY WORDS: *Pieris phillyreifolia, P. japonica, Stephanitis takeyai, S. pyrioides*, assay, susceptibility, resistance

The Ericaceous plant pieris (*Pieris* D. Don spp.) is a popular choice for landscape or foundation plants, shrub borders or incorporating with other evergreens. Glossy green leaves and clusters of urn-shaped flowers colored red, pink or white give the plant its striking beauty, and the magnificent colors displayed by its young leaves are particularly notable. Foliage feeders like lace bugs and mites cause the most visible damage to pieris. They not only weaken the plant but also reduce the attractiveness of the foliage, which affects the aesthetic value and marketability. The Andromeda lace bug *Stephanitis takeyai* Drake and Maa (1955) is one of the most important pests of *Pieris* spp. (Johnson and Lyon 1991) along with which it is believed to have originated (Tsukada 1994). The damaged plants show yellowish white stipples or blotches on the upper surfaces of the leaves and on the under surface, lace bug adults and nymphs can be seen along with their oily, black frass spots. Lace bug damage may lead to premature leaf shedding, drying up of twigs or even the whole plant (Schread 1968). This type of damage is serious on ornamental plants like pieris which are valued for their foliage as well as flowers.

The USDA ARS GRIN Online Database lists 13 species records for the genus *Pieris* which include synonyms as well (ARS, USDA 2011). In a taxonomic revision of the genus *Pieris*, six species were recognized and a seventh one, *P. nana* Maxim. was recognized as *Arcterica nana*. The *Pieris* group was divided into two subgroups: *Pieris*, comprising *P. japonica* (Thunb.) D. Don ex G. Don, *P. formosa* (Wall.) D. Don and *P. floribunda* (Pursh) Benth. and Hook. f. and *Phillyreoides*, comprising *P. phillyreifolia* (Hook.) DC, *P. cubensis* (Grisebach) Small. and *P. swinhoei* Hemsley (Judd 1982). Later, both molecular and morphological analyses supported placement of *Pieris* subg. *Arcterica*, i.e., *P. nana*, as sister to *Pieris* subg. *Pieris* (Kron and Judd 1997).

The most common *Pieris* species is Japanese pieris (*P. japonica*). It is native to Japan and is considered the most beautiful (Heriteau 2006). Although there are reports of its being polyphagous, the preference of S. takeyai for P. japonica has been recognized (Schread 1968). Mountain pieris P. floribunda, native to North America, is an underutilized, ornamental shrub indigenous to the Appalachian mountains of the U.S. extending from Virginia southward into Georgia (Starrett et al. 1996). P. floribunda is more tolerant to stresses like alkaline soil and pests but is considered less attractive than *P. japonica* (Heriteau 2006). *P. phillyreifolia* (Climbing fetterbush) is also native to North America and is common in the Southeastern states of Alabama, Florida, Georgia, Mississippi and South Carolina. Lesser cultivated species of pieris include Himalaya pieris and dwarf pieris. *P. formosa*, which has larger leaves and good plant shape, prefers cooler climates and is mainly found in the Pacific Northwest and England (BTRL 1999). P. taiwanensis is ideal for small gardens and is a hardy late bloomer. Other cultivated species of *Pieris* from different countries include *P. cubensis* (Cuba) (Judd 1995) and *P.* swinhoei (Southeastern China) (FOC 2011). Among the various species of Pieris, P. *japonica* is the most widely cultivated and popular among nurserymen and home owners and it is believed to have over 100 known cultivars (van Santvoort 2008).

Although *S. takeyai* is an important pest of *Pieris*, few workers have studied it from the aspect of pest management (Johnson and Lyon 1991). Of the known *Pieris* taxa, mountain pieris, *P. floribunda* is believed to be resistant to the pest, and *P. floribunda* x *japonica* hybrids less favorable (Dunbar 1974). In Poland, particularly severe damage was observed on cultivars: 'Select', 'Debutante', 'Cupido' and in a lesser degree on 'Flaming Silver', Variegata' and 'Redmill' (Labanowski and Soika 2000). However the range of susceptibility among cultivated *Pieris* taxa has not been studied before.

The objective of our study was to evaluate available *Pieris* taxa for their susceptibility to the two species of lace bugs *S. takeyai* and *S. pyrioides*. Since the major economic, cosmopolitan tingid species is the azalea lace bug, *S. pyrioides*, which is also known to infest other ericaceous hosts like kalmia (*Kalmia latifolia* L.) and pieris (*Pieris ovalifolia* (Wall.) D. Don) (Drake and Ruhoff 1965), we included *S. pyrioides* in our study.

Materials and Methods

PLANT MATERIALS. Leaves from 61* *Pieris* taxa (species or cultivars) were obtained from the Department of Horticulture *Pieris* collection located at the UGA Horticulture Farm in Watkinsville, GA. The plants were grown in three gallon (11.356 liter) and one gallon (3.785 liter) pots and maintained in a screen house with regular irrigation. Pesticides were not used in the screen house.

LACE BUGS. *S. pyrioides* colonies were established and periodically replenished using adult azalea lace bugs collected from natural populations found near Griffin, GA. The colonies were housed in 1.0-m³ screen cages in the entomology insect rearing facility at Griffin, GA. These colonies were reared on several cultivars of evergreen azaleas under conditions of $27 \pm 1^{\circ}$ C and a photoperiod of 14:10 (L:D) h. *S. takeyai* colonies were initiated from a population obtained from a location in New York in April 2009.

^{*} the number of varieties varied slightly in some assays according to availability of leaves. The collection was broadly divided into two groups, HFS (Horticulture Farm Stock) and O (Oregon).

The colonies were housed in plastic containers and maintained on several cultivars of *Pieris* under conditions of $27 \pm 1^{\circ}$ C and a photoperiod of 14:10 (L:D) h. For the actual testing, known numbers of adults were first collected in plastic tubes using an aspirator and then transferred into the testing Petri dishes using a brush.

NO- CHOICE PETRI DISH ASSAYS – SINGLE SPECIES. No-choice tests are useful tools to assess feeding, survival and acceptance of host plants for oviposition by insects. They are especially useful when testing a large number of potential host plants to which the test insect has not been exposed before. In this case, the use of no-choice tests prior to choice tests or field assays justifies the classification of plants that are not attacked as non-hosts (Van Driesche and Murray 2004). Our first assay was initiated using S. pyrioides in March 2008. Three leaves of a variety placed in a Petri dish with their petioles covered with moist tissue paper constituted one replication. Each variety was replicated four times. Ten adult lace bugs were released into each Petri dish. The dishes were arranged in a randomized complete block design and placed under conditions of $27 \pm 1^{\circ}$ C and a photoperiod of 14:10 (L:D) h. Observations on number of bugs alive were taken at 48 hour intervals. After the exposure period the surviving adults were removed and the leaves were scored for percent leaf area damaged using the scoring chart based on Klingeman et al. 2000 (Fig. 2.1). After scoring, the leaves were maintained under the same conditions as during the exposure period and observed daily for emergence of nymphs. This assay was repeated in May 2008.

A similar assay was conducted using *S. takeyai* during July 2009 and repeated in August 2009. In these assays, two adult lace bugs were released into each Petri dish. Observations on number of bugs alive were taken at 2, 7, 9 and 13 days and leaf damage

scoring was done on the 13th day. After scoring, the leaves were maintained under the same conditions as during the exposure period and observed daily for emergence of nymphs.

NO- CHOICE PETRI DISH ASSAYS – COMBINED ASSAYS. Following the single species assays, a combined assay was conducted simultaneously using both species of lace bugs. This was started in September 2009 and repeated in October 2009. In these assays we used 20 *Pieris* taxa (Tables 2.4 and 2.5) which were a mix of susceptible and tolerant varieties and selected based on the results of the previous assays. We maintained two sets of dishes, one for each species with twenty varieties each replicated four times. Two adult lace bugs were released into each Petri dish. Observations on number of bugs alive were taken at 2, 7, 9 and 13 days and leaf damage scoring was done on the 13th day.

MULTI -CHOICE ASSAY. The feeding or oviposition by a herbivore on a host plant in a no-choice situation may be considered as an unnatural behavior due to starvation or impending death. They may also be considered induced in confined conditions such as inside a container because it brings the insects in such close proximity to the plant which may never occur in nature (Van Driesche and Murray 2004). To overcome bias due to these reasons, we conducted choice assays, exposing *S. takeyai* adults to different *Pieris* taxa simultaneously. For these assays, ten *Pieris* taxa (Tables 2.6 and 2.7), which were a mix of susceptible and tolerant varieties, were selected based on the results of the previous assays. Plants of these varieties were obtained from nurseries near Griffin, GA. Leaves were collected from these plants as and when required for the assays. *S. takeyai* colonies were grown in plastic containers and maintained on several cultivars of *Pieris* under conditions of $27 \pm 1^{\circ}$ C and a photoperiod of 14:10 (L:D) h. Adult lace bugs were

collected from these colonies as required for the assays. For conducting the multi-choice assays, three leaves of each variety were placed as a group with their bases covered with moist tissue paper. Ten such groups of leaves placed in a circular pattern inside a large 30 cm Petri dish constituted one replication and there were three such replications. The leaf groups were arranged randomly within the circular pattern in each replication. Twenty adult lace bugs were released into each Petri dish. All the Petri dishes were placed inside a growth chamber under conditions of $27 \pm 1^{\circ}$ C and a photoperiod of 14:10 (L:D) h. Observations on number of bugs present on each of the leaves was recorded one hour after releasing the bugs, and then again at the start of each observation on the 2^{nd} , 7th, 9th and 13th day. After the 13th day the surviving adults were removed and the leaves were assessed for leaf damage by counting the number of frass spots left by the bugs (Buntin et al. 1996). After damage assessment, the leaves were placed back in their positions and maintained under the same conditions as during the exposure period and observed daily for emergence of nymphs. Nymphs were removed when they were observed.

WHOLE PLANT ASSAY. Excised whole leaves or leaf discs are the most common substrates for testing the feeding or ovipositional preferences of phytophagous insects because it is often not feasible to use whole plants (Lewis 1984). However it is widely understood that physical damage can cause significant changes in the plant's chemistry which may in turn influence the behavior of herbivores feeding on it (Rhoades 1983). Hence we conducted no-choice assays using *S. takeyai* on whole potted plants. We used five *Pieris* taxa (Tables 2.8 and 2.9) which were again a mix of susceptible and tolerant varieties and selected based on the results of the previous assays. Healthy potted plants

that had at least 5 branches each were selected. One branch with at least 100 leaves was selected on each plant and this was enclosed in a sleeve cage (BugDorm). Ten adult lace bugs (5 male and 5 female) were released into each sleeve cage and this constituted one replication. Each variety was replicated six times during the period from July – August 2010. The plants were placed under conditions of $27 \pm 1^{\circ}$ C and a photoperiod of 14:10 (L:D) h. They were watered as required and observed on the 2nd, 7th, 9th and 13th day for survival of the lace bugs. After the 13th day, the branches were cut at the base beyond the cage and the leaf damage was assessed. The number leaves that were damaged out of 100 leaves was counted in each of the sleeve cages. A 'damaged' leaf was one with frass spots left by the lace bugs (Buntin et al. 1996). From the damaged leaves, five leaves were selected at random and the average number of frass spots was recorded. After this, each of the entire cut branches (with their cut ends covered in moist tissue paper) was placed inside a large Petri dish or container and maintained under conditions of $27 \pm 1^{\circ}$ C and a photoperiod of 14:10 (L:D) h to observe for nymph emergence. Nymphs were removed when they were observed.

STATISTICAL PROCEDURES. The experiments used a randomized complete block design. The replications were considered as the block factor. Treatment means were analyzed separately for each kind of trial. Meansof the variables (adult survival, leaf damage and nymph emergence in no-choice assays; adult presence on leaves, leaf damage and nymph emergence in choice assays; and adult survival, number of damaged leaves, average leaf damage and nymph emergence in whole plant assays) were subjected to analysis of variance (ANOVA) using the general linear model procedure (SAS Institute 2003). Means were separated with Fisher protected least significant difference (LSD) test.

Results

NO- CHOICE PETRI DISH ASSAYS – SINGLE SPECIES. The assays with *S. pyrioides* (Table2.1) as well as *S. takeyai* (Table 2.2) revealed significant differences among the taxa evaluated with respect to number of live bugs as well as leaf damage.

In the first assay with S. pyrioides (Table 2.1), the highest leaf damage was observed in *P. japonica* 'Mountain Fire' (F = 6.50, df = 60, P < .0001). High survival of adults was also noted on this taxon (F = 2.25, df = 60, P < .0001). Highest adult survival was noted in P. taiwanensis 'Snow Drift'. Other P. japonica taxa like 'Shojo' and 'Scarlet O'Hara' were comparable to these two varieties. No leaf damage or adult survival was observed in several taxa notably P. phillyreifolia, P. japonica 'Variegata', P. japonica 'Pygmaea' and P. japonica 'Wada'. On repeating this assay we got a different range in susceptibility (Table 2.1). The highest leaf damage was observed on P. *japonica* 'White Cascade' (F = 4.81, df = 60, P < .0001) and the highest adult survival was seen in the hybrid P. floribunda x P. japonica 'Brower's Beauty' (F = 2.47, df = 60, P < .0001), both of which showed low leaf damage and adult survival in the earlier assay. However, some similarities were observed viz., the *P. japonica* taxa 'Mountain Fire' and 'Shojo' exhibited high susceptibility, while *P. phillyreifolia* and the *P. japonica* taxa 'Variegata', 'Pygmaea' and 'Wada' showed no damage. P. japonica 'Temple Bells' also showed low damage.

The most notable difference seen in the assay with *S. takeyai* (Table 2.2) was the high susceptibility of *P. japonica* 'Temple Bells' indicated by the significantly high leaf damage (F = 1.66, df = 52, P = 0.0142), as well as adult survival (F = 2.30, df = 52, P = 0.0002). Conversely, *P. japonica* 'Shojo' showed significant resistance to *S. takeyai*.

Other susceptible taxa included the hybrids *P. japonica* x *P. floribunda* 'Spring Snow' and *P. floribunda* x *P. japonica* 'Brower's Beauty', *P. japonica* var. *amamiana* and *P. japonica* 'Dodd's Crystal Cascade Falls'. Resistant taxa included *P. phillyreifolia*, *P. japonica* 'Variegata' and *P. japonica* 'Pygmaea', which was consistent with the previous assays using *S. pyrioides*.

S. pyrioides did not deposit eggs in test leaves, whereas *S. takeyai* oviposited in the *Pieris* leaves. Counts of emergednymphs appeared related to adult survival and leaf damage. The highest number of nymphs was observed on *P. japonica* 'Scarlet O'Hara', and this was similar to *P. japonica* var. *amamiana,* while the lowest numbers were observed on resistant taxa like *P. phillyreifolia, P. japonica* 'Variegata' and *P. japonica* 'Pygmaea' (F = 1.77, df = 52, P = 0.0069). A significantly lower number of nymphs than expected emerged from *P. japonica* 'Temple Bells'. We repeated this assay with consistent results. *P. japonica* 'Temple Bells' again showed highest leaf damage (F = 2.57, df = 61, P < .0001) and adult survival (F = 2.90, df = 61, P < .0001), while *P. phillyreifolia, P. japonica* 'Pygmaea' were again resistant and also showed low or no nymph emergence (F = 1.34, df = 61, P = 0.0737). *P. japonica* 'Temple Bells' did not show nymph emergence in this assay.

NO- CHOICE PETRI DISH ASSAYS – COMBINED ASSAYS. The combined assays using both species of lace bugs simultaneously confirmed our observations in the single species assays. The two lace bug species differed significantly with respect to adult survival and leaf damage (Table 2.3, Fig. 2.2, 2.3). In the first round of the combined assay (Table 2.4), highest leaf damage by *S. pyrioides* was noted on *P. japonica* 'Cavatine', which was on par with that on *P. japonica* x *P. floribunda* 'Spring Snow' and *P. formosa* var.

forestii (F = 2.21, P = 0.011). These two taxa also showed some adult survival, which was in general low on all taxa (F = 0.89, P = 0.60). With S. takeyai, the highest damage was again seen on P. japonica 'Cavatine', which was similar to several other taxa like P. taiwanensis 'Snow Drift', P. j x P. formosa var. forestii 'Forest Flame', P. japonica var. amamiana, P. japonica 'Temple Bells' and P. japonica x P. floribunda 'Spring Snow' (F = 2.75, P = 0.002). S. takeyai adults showed better survival on all Pieris taxa (F = 2.75, P = 0.002). 1.76, P = 0.051). Lowest leaf damage as well as adult survival of both species of lace bugs was observed on *P. phillyreifolia* and *P. japonica* 'Variegata'. In the second round of combined assays (Table 2.5), S. pyrioides again caused most damage on P. japonica 'Cavatine', P. japonica x P. floribunda 'Spring Snow' and P. formosa var. forestii (F = 2.55, P = 0.003), whereas the highest damage by S. takeyai was noted on P. japonica var. amamiana, which was on par with the damage on *P. japonica* 'Temple Bells', P. *japonica* 'Cavatine' and *P. japonica* 'Scarlett O'Hara' (F = 4.3, P < 0.0001). S. *pyrioides* adult survival was generally low (F = 1.51, P = 0.116) while S. *takeyai* adults survived better on the *Pieris* taxa (F = 2.31, P = 0.008) in this assay also. P. phillyreifolia and P. japonica 'Variegata' showed the lowest leaf damage as well as adult survival with both species of lace bugs. Overall, P. phillyreifolia and P. japonica 'Variegata' were consistently resistant to both species of bugs in both the combined assays, while *P. japonica* 'Temple Bells' and *P. japonica* var. *amamiana* were notable in being highly susceptible to S. takeyai, but resistant to S. pyrioides.

CHOICE ASSAYS. Of the ten Pieris taxa used in the multi-choice assays (Table 2.6), *P. japonica* 'Temple Bells' exhibited the highest number of frass spots on all four days of observation (8.33, 17.11, 21.89 and 31.00 respectively on day 2, 7, 9 and 13

respectively). *P. japonica* 'Cavatine' also proved to be highly susceptible and was not significantly different from *P. japonica* 'Temple Bells'. The two *P. phillyreifolia* taxa 'Little Leaf' and 'Baldwin' showed the least amount of damage (0.22 spots each on the 13th day) and these were significantly different from all the other taxa, except *P. taiwanensis* 'Snow Drift' and *P. japonica* 'Valley Rose'. The other varieties that appeared to be less preferred were *P. taiwanensis* 'Snow Drift' and *P. japonica* 'Valley Rose'. Significantly higher number of nymphs was observed on *P. japonica* 'Temple Bells' than on all the other taxa. This was followed by *P. japonica* 'Dorothy Wycoff' and *P. japonica* 'Cavatine' which were not significantly different from each other. The results of the multichoice assays were consistent with those of the no-choice assays conducted earlier.

The observations on the presence of adults on the leaves (Table 2.7) show that the lace bugs did not show marked preference for any taxon at the beginning of the experiment (1 hour after release). Even on the second day after release, there was still no significant difference between the taxa with respect to the adults' presence on the leaves. However, from the 7th day onwards there were clear indications about the adults' preferences. Towards the end of the exposure period, maximum number of adults was seen on 'Temple Bells' and this was also the most damaged variety.

WHOLE PLANT ASSAY. Table 2.8 shows adult survival on the different varieties in the whole plant assay. Highest adult survival was noted on *P. japonica* 'Cavatine' with an average of 6 adults surviving on the 13th day. This was on par with *P. japonica* 'Temple Bells' which had an average of 5.5 adults on the 13th day. Lowest survival was recorded on *P. phillyreifolia*.

The *Pieris* taxa also differed significantly with respect to leaf damage, both in terms of number of damaged leaves out of 100 leaves as well as in the average leaf damage, recorded on 5 leaves (Table 2.9). The highest number of damaged leaves was noted in *P. japonica* 'Temple Bells' (75.67) which was significantly higher than all the other taxa. The highest average leaf damage (indicated by frass spots) was also noted in *P. japonica* 'Temple Bells' (78.67) and this was on par with *P. japonica* 'Cavatine' with 74.5 spots. Nymph emergence was highest in *P. japonica* 'Cavatine' with 73.5 nymphs, which was significantly higher than all the other taxa, except *P. japonica* 'Temple Bells' with 55.33. The results of the whole plant assays were consistent with both no-choice and multi-choice assays.

Discussion

We evaluated over 60 *Pieris* taxa (species, cultivars and hybrids) for their response to the two species of lace bugs *S. pyrioides* and *S. takeyai* in no-choice assays. This information has not been reported previously. Our experimental design was similar to that used in previous studies that examined susceptibility of host plants to lace bugs (Wang *et al.* 1998). We conducted no-choice, choice and whole plant assays confirming our observations on susceptibility of the *Pieris* taxa.

The no-choice assays gave an estimate of the broadest range of *Pieris* taxa that might be possible hosts for the two species of lace bugs. Such tests can yield valuable information on the extent to which food or oviposition site deprivation can induce broadening of host utilization in the field (Withers 1997). Another advantage of nochoice tests is that even least preferred taxa that may have escaped attack in a choice situation are revealed (Withers 1999), but this can also result in overestimation of the

host range. Hence, choice tests are essential since they better resemble the natural situation and may reveal more accurately which less- or non- preferred hosts are accepted in presence of the preferred host (Murray *et al.* 2010). A disadvantage of conducting only choice assays is that less preferred hosts may be ignored in presence of the preferred one and therefore the assay may not reveal the actual host range. Insects may actually present contradictory responses in no-choice and choice tests, and a susceptible cultivar from a no-choice test may prove resistant in a choice test (Tingey 1986). Thus, both kinds of assays have their strengths and drawbacks and therefore we conducted both nochoice and choice containerized tests for a more accurate prediction of potential host range. The results from such containerized studies are often used to deduce insect feeding preferences on whole plants in the field, which may be erroneous (Risch 1985). These results may vary with the testing method used and also influenced by physical damage to the plant part. For example, in experiments with leaf discs, the disc size influences insect feeding behavior because of changes in the ratio of chemical signals from the cut leaf edge to those of the intact leaf center (Jones and Coleman 1988). Therefore to reduce the bias from using containerized study results, we also conducted assays using whole plants instead of excised leaves. We noted that the survival of adult lace bugs was higher on the whole plants, as compared to single leaves inside Petri dishes. Also, there was notable damage in the form of frass spots on leaves of P. phillyreifolia, which was never noticed in the Petri dish assays. This could be due to higher moisture content and longer freshness of leaves of whole plants as compared to excised leaves. This may have prompted the lace bugs to explore the leaves for a longer time and make more attempts to feed than they would have done in a Petri dish situation.

In the single species assays, the number of adults used was different (ten *S*. *pyrioides* and two *S*. *takeyai*) due to differences in availability of adults. However even the higher number of adults of *S*. *pyrioides* did not cause as much damage as the lower number of *S*. *takeyai*. The combined assays were uniform in all respects and clearly revealed the differences in the activity of the two species. Comparing *S*. *takeyai* and *S*. *pyrioides*, it was clear that the former favored *Pieris* as its preferred host, as indicated by its better adult survival, higher leaf damage and most importantly, nymph emergence. Among the different *Pieris* species, the greatest preference was for *P*. *japonica* as reported earlier (Schread 1968). *P. taiwanesis* and *P. formosa* were less preferred and *P. phillyreifolia*, showed least preference among the four species.

We evaluated 51 *P. japonica* taxa in our assays. Among them *S. takeyai* showed clear preference for certain *P. japonica* taxa viz., 'Temple Bells' and 'Cavatine', whereas others like 'Variegata' and 'Prelude' were less preferred. The lesser preference of 'Variegata' has been mentioned earlier (Labanowski and Soika 2000), but some other observations in that report are different from our results. For example in our assays the cultivars 'Flaming Silver' and 'Red Mill' were more damaged than 'Debutante' and 'Cupido', whereas Labanowski and Soika (2000) observed severe damages on cultivars: 'Select', 'Debutante', 'Cupido' and in a lesser degree on 'Flaming Silver', Variegata' and 'Redmill'. In further screening experiments, it might be useful to ascertain the identity of the taxa being tested to ensure uniformity of results. We could not include *P. floribunda* which is reported to be resistant to *S. takeyai* in our screening due to lack of availability of plants at the time. However the hybrid *P. floribunda* x *P. japonica* 'Brower's Beauty' seemed to be less preferred, as mentioned in an earlier report (Dunbar 1974), and so did

the hybrids *P. japonica* x *P. floribunda* 'Spring Snow' and *P. japonica* x *P. formosa* var. *forestii* 'Forest Flame'. This may indicate that lesser preferred species like *P. floribunda* and *P. formosa* may be sources of resistant genes.

Our studies have revealed the gradients in susceptibility of cultivated *Pieris* taxa to the two species of lace bugs, *S. pyrioides* and *S. takeyai*. It is also clear that *S. takeyai* is capable of causing significant damage to several *Pieris* cultivars. Since very little information is available regarding its potential host range among Ericaceae and other related families, it would be worthwhile to conduct further studies along these lines. *S. takeyai* was first reported in North America in 1950 on *P. japonica* plants in Connecticut, and there were speculations even at that time that this recently introduced species may become a serious pest of *Pieris* and other ornamental Ericaceae (Bailey 1950). It has been reported from several other states (Dunbar 1974, Torres-Miller 1989, Nielsen 1997) and there have been unpublished reports of the pest from the southeastern United States recently.

Leaf parameters like toughness, pubescence, moisture content, epicuticular wax content, and stomatal size and density have been studied as possible mechanisms of resistance to lace bugs and other sucking pests ((Meyer and Ballington 1990, Braman and Pendley 1992, Balsdon et al. 1995, Wang et al. 1998, Wang et al. 1999, Chappell and Robacker 2006, Kirker et al. 2008). The exact reasons for the preferences exhibited by *S. takeyai* are not yet known, but the wide variability in leaf shape, size, texture, color and growth habit among the *Pieris* taxa, even within the *japonica* cultivars suggest that different mechanisms may be involved, which need to be studied further.

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Code	Pieris taxa	No. live	Т-	% leaf	Т-	Tukey	No. live	T-	% leaf	Т-	Tukey
No.		bugs	group	area	group	group	bugs	group	area	group	group
				damaged					damaged		
		March 2008					May 2008				
HFS-39	<i>P. floribunda</i> x <i>P. japonica</i> 'Brower's Beauty'(B)	0.0	e	0.0	j	f	2.25	а	4.67	d-g	a-g
HFS-40	<i>P. japonica</i> 'Cavatine' (B)	0.0	e	0.34	h-j	d-f	0.0	f	0.0	р	g
HFS-41	<i>P. japonica</i> 'Cavatine' (C)	0.0	e	0.0	j	f	0.25	ef	2.58	f-p	c-g
HFS-42	P. japonica 'Compacta' (B)	0.0	e	0.34	h-j	d-f	1.5	a-d	3.0	f-n	c-g
HFS-43	P. japonica 'Dodd's Crystal Cascade Falls' (C)	0.0	e	0.67	f-j	c-f	0.0	f	0.17	op	fg
HFS-44	P. japonica 'Dodd's Pearl Falls' (C)	0.0	e	0.0	j	f	1.25	a-e	2.0	g-p	d-g
HFS-45	P. japonica 'Dodd's Sugar Run Falls' (C)	0.0	e	0.34	h-j	d-f	0.25	ef	1.34	k-p	e-g
HFS-46	P. japonica 'Dorothy Wycoff' (FF)	0.0	e	1.34	c-f	b-f	0.25	ef	1.0	l-p	e-g
HFS-47	P. japonica 'Flaming Silver' (B)	0.0	e	0.0	j	f	1.0	b-f	2.83	f-o	c-g
HFS-48	P. japonica 'Flaming Silver' (FF)	0.0	e	0.17	ij	ef	1.75	a-c	5.0	c-f	a-g
HFS-49	P. japonica 'Karenoma' (B)	0.25	de	1.25	c-g	b-f	1.25	a-e	2.75	f-o	c-g
HFS-50	<i>P. japonica</i> 'Little Heath' (B)	0.25	de	0.0	j	f	0.25	ef	2.5	f-p	d-g
HFS-52	P. japonica 'Mountain Fire' (B)	0.75	b-d	4.25	а	а	2.0	ab	4.34	d-i	a-g
HFS-54	P. japonica 'Prelude' (B)	0.5	c-e	0.0	j	f	0.0	f	0.0	р	g
HFS-57	P. japonica 'Pygmaea' (FF)	0.0	e	0.0	j	f	0.0	f	0.34	n-p	fg
HFS-58	P. japonica 'Sarabonde' (B)	0.25	de	0.34	h-j	d-f	1.25	a-e	3.58	d-l	b-g
HFS-59	<i>P. japonica</i> 'Scarlett O'Hara' (B)	0.75	b-d	1.84	b-d	b-e	0.5	d-f	1.58	j-p	e-g
HFS-60	<i>P. japonica</i> 'Shojo' (B)	1.0	bc	2.42	b	bc	1.5	a-d	6.17	b-d	a-e
HFS-62	P. japonica 'Valley Fire' (FF)	0.0	e	0.75	f-j	b-f	0.25	ef	1.09	k-p	e-g
HFS-63	P. japonica 'Valley Rose' (B)	0.0	e	0.17	ij	ef	0.5	d-f	1.5	j-p	e-g
HFS-64	P. japonica 'Valley Rose' (FF)	0.0	e	0.0	j	f	0.25	ef	1.67	i-p	d-g
HFS-65	P. japonica 'Valley Valentine' (B)	0.5	c-e	2.5	b	ab	0.75	c-f	1.0	l-p	e-g
HFS-67	P. taiwanensis 'Snow Drift' (B)	1.75	а	2.09	bc	b-d	0.0	f	0.17	op	fg
HFS-68	P. taiwanensis 'Snow Drift' (FF)	0.0	e	0.17	ij	ef	0.25	ef	1.09	k-p	e-g
HFS-69	<i>P. japonica</i> 'Temple Bells'	0.0	e	0.59	f-j	d-f	0.0	f	1.42	k-p	e-g
HFS-70	P. phillyreifolia	0.0	e	0.0	j	f	0.0	f	0.0	p	g
O-1	P. japonica 'Benihaja'	0.0	e	0.0	i	f	0.5	d-f	2.25	g-p	d-g
O-2	P. japonica 'Bisbee Dwarf'	0.0	e	0.83	f-j	b-f	0.0	f	1.75	h-p	d-g
O-3	P. japonica 'Bolero'	0.0	e	0.17	ij	ef	0.25	ef	3.33	e-m	b-g
					-		•				

Table 2.1. No-choice assays with S. pyrioides for adult survival and percent leaf area damaged on Pieris taxa

O-4	P. japonica 'Bonsai'	0.25	de	0.17	ij	ef	0.25	ef	4.42	d-h	a-g
O-5	P. japonica 'Chaconne'	0.5	c-e	0.0	j	f	0.0	f	2.08	g-p	d-g
O-6	P. japonica 'Christmas Cheer'	0.0	e	0.67	f-j	c-f	0.5	d-f	2.5	f-p	d-g
O-7	P. japonica 'Coleman'	0.0	e	0.0	j	f	0.25	ef	1.42	k-p	e-g
O-8	P. japonica 'Crimson Compact'	0.0	e	0.5	f-j	d-f	0.25	ef	3.67	d-l	b-g
0-9	<i>P. japonica</i> 'Cupido'	0.0	e	0.0	j	f	0.5	d-f	4.17	d-j	b-g
O-10	P. japonica 'Daisen'	0.0	e	0.44	g-j	d-f	0.0	f	1.75	h-p	d-g
0-11	P. japonica 'Debutante'	0.0	e	0.17	ij	ef	0.0	f	2.42	f-p	d-g
O-12	P. japonica 'Firecrest'	0.25	de	1.08	d-h	b-f	1.5	a-d	5.09	c-f	a-g
O-13	P. japonica 'Flamingo'	0.0	e	0.0	j	f	0.0	f	1.58	j-p	e-g
O-14	<i>P. japonica</i> 'Havila'	0.25	de	0.75	f-j	b-f	0.0	f	2.58	f-p	c-g
O-15	P. japonica 'Iseli Cream'	0.0	e	0.0	j	f	0.5	d-f	3.67	d-l	b-g
O-17	P. japonica 'La Rocaille'	0.0	e	0.34	h-j	d-f	1.75	a-c	8.92	а	ab
O-18	P. japonica 'Nocturne'	0.0	e	0.0	j	f	0.0	f	0.83	m-p	e-g
O-19	<i>P. japonica</i> 'PI 418 531'	0.0	e	0.42	g-j	d-f	0.5	d-f	8.33	ab	a-c
O-20	<i>P. japonica</i> 'Purity'	0.67	b-d	0.22	h-j	ef	1.0	b-f	3.75	d-k	b-g
O-22	<i>P. japonica</i> 'Red Mill'	0.0	e	0.75	f-j	b-f	0.25	ef	1.67	i-p	d-g
O-23	P. japonica 'Sinfonia'	0.67	b-d	0.0	j	f	1.0	b-f	5.92	b-e	a-f
O-24	P. japonica 'Stockman'	0.0	e	0.0	j	f	0.25	ef	3.75	d-k	b-g
O-25	P. japonica 'T40-82A'	0.0	e	0.0	j	f	0.25	ef	1.58	j-p	e-g
O-26	P. japonica 'T44-82U'	0.0	e	0.17	ij	ef	0.0	f	3.75	d-k	b-g
O-27	P. japonica 'UNH'	1.25	ab	0.92	e-i	b-f	0.0	f	3.08	f-m	c-g
O- 28	P. japonica 'Valentine's Day'	0.0	e	0.0	j	f	1.5	a-d	7.42	a-c	a-d
O-29	P. japonica 'Valley Valentine' x 'Kubas'	0.0	e	0.17	ij	ef	0.0	f	1.92	h-p	d-g
O-30	P. japonica 'Variegata'	0.0	e	0.0	j	f	0.0	f	1.0	l-p	e-g
0-31	P. japonica 'Wada'	0.0	e	0.0	j	f	0.0	f	0.75	m-p	e-g
O-32	P. japonica 'White Caps'	0.0	e	0.34	h-j	d-f	0.25	ef	3.67	d-l	b-g
O-33	P. japonica 'White Cascade'	0.0	e	0.56	f-j	d-f	1.25	a-e	10.0	а	а
O-35	P. japonica var. amamiana	0.25	de	0.17	ij	ef	0.5	d-f	3.0	f-n	c-g
O-36	P. japonica x P. floribunda 'Spring Snow'	0.5	c-e	0.33	h-j	d-f	0.0	f	2.17	g-p	d-g
O-37	P. japonica x P. formosa var. forestii 'Forest Flame'	0.5	c-e	1.75	b-e	b-f	0.0	f	3.75	d-k	b-g
O-38	P. formosa var. forestii	0.25	de	0.17	ij	ef	0.25	ef	1.17	kp	e-g
	F	2.25		6.5			2.47		4.81		
	Р	< 0.0001		< 0.0001			< 0.0001		< 0.0001		

Means in the same column followed by different letters are significantly different ($\alpha = 0.05$) (B), (C) and (FF) indicate the nurseries Briggs, Cofer's and Forest Farm.

Code No.	Pieris taxa	No. live	% leaf area	# nymphs	No. live bugs	% leaf area	No. nymphs	
	-	bugs	damaged		damaged			
			(July, 2009)		(August 2009)			
HFS-39	P. f. x P. j. 'Brower's Beauty' (B)	1.33 a-c	5.89 a-h	1.67 e-g	0.75 c-f	4.42 d-o	0.0 c	
HFS-40	P. j. 'Cavatine' (B)	1.0 a-d	5.78 a-h	0.67 g	1.75 ab	9.92 a	7.5 bc	
HFS-41	P. j. 'Cavatine' (C)	1.0 a-d	5.11 b-i	5.0 d-g	1.5 a-c	9.75 ab	0.0 c	
HFS-42	P. j. 'Compacta' (B)	0.33 cd	3.89 d-j	6.0 d-g	0.75 c-f	5.58 c-l	2.75 c	
HFS-43	P. j. 'Dodd's Crystal Cascade Falls' (C)	1.67 ab	6.67 a-g	2.33 e-g	1.5 a-c	6.58 a-h	3.0 c	
HFS-44	P. j. 'Dodd's Pearl Falls' (C)	-	-	-	1.25 a-d	5.5 c-l	2.25 c	
HFS-45	P. j. 'Dodd's Sugar Run Falls' (C)	1.0 a-d	6.0 a-h	11.33 c-g	1.5 a-c	6.17 a-j	0.0 c	
HFS-46	<i>P. j.</i> 'Dorothy Wycoff' (FF)	1.0 a-d	2.78 e-j	0.0 g	0.5 d-f	5.0 d-m	0.0 c	
HFS-48	P. j. 'Flaming Silver' (FF)	1.67 ab	6.22 a-h	0.0 g	1.75 ab	7.83 a-e	2.75 c	
HFS-49	P. j. 'Karenoma' (B)	1.67 ab	7.11 a-f	0.0 g	1.0 b-e	6.75 a-h	3.75 c	
HFS-50	<i>P. j.</i> 'Little Heath' (B)	1.0 a-d	6.22 a-h	0.0 g	0.5 d-f	3.67 f-p	0.0 c	
HFS-52	P. j. 'Mountain Fire' (B)	0.67 b-d	5.11 b-i	5.67 d-g	1.0 b-e	4.83 d-m	0.0 c	
HFS-54	P. j. 'Prelude' (C)	1.33 a-c	9.33 ab	1.0 fg	1.0 b-e	8.08 a-d	4.5 c	
HFS-57	P. j. 'Pygmaea' (FF)	0.33 cd	2.89 e-j	0.0 g	0.0 f	0.5 op	0.0 c	
HFS-58	<i>P. j.</i> 'Sarabonde' (B)	0.33 cd	6.22 a-h	5.0 d-g	1.25 a-d	4.92 d-m	3.5 c	
HFS-59	<i>P. j.</i> 'Scarlett O'Hara' (B)	1.0 a-d	5.89 a-h	28.67 a	1.75 ab	7.0 a-h	0.0 c	
HFS-60	<i>P. j.</i> 'Shojo' (B)	0.33 cd	1.78 g-j	1.33 e-g	0.75 c-f	5.92 a-j	1.25 c	
HFS-62	P. j. 'Valley Fire' (FF)	0.33 cd	2.78 e-j	6.67 d-g	1.25 a-d	8.09 a-d	0.0 c	
HFS-63	<i>P. j.</i> 'Valley Rose' (B)	0.67 b-d	3.0 e-j	3.0 d-g	1.5 a-c	3.67 f-p	8.0 bc	
HFS-64	<i>P. j.</i> 'Valley Rose' (FF)	0.0 d	1.45 h-j	0.67 g	1.75 ab	4.42 d-o	0.0 c	
HFS-65	P. j. 'Valley Valentine' (B)	0.0 d	0.67 ij	0.0 g	1.25 a-d	3.0 h-p	0.0 c	
HFS-67	<i>P. t.</i> 'Snow Drift' (B)	0.67 b-d	4.56 b-j	13.0 c-g	1.5 a-c	6.08 a-j	5.75 bc	
HFS-68	<i>P. t.</i> 'Snow Drift' (FF)	0.33 cd	4.55 b-j	2.67 e-g	1.75 ab	6.0 a-j	0.0 c	
HFS-69	<i>P. j.</i> 'Temple Bells' (FF)	1.67 ab	10.22 a	4.33 d-g	2.0 a	9.33 а-с	0.0 c	
HFS-70	P. phillyreifolia	0.0 d	0.0 j	0.0 g	0.5 d-f	0.17 p	0.0 c	
O-1	P. j. 'Benihaja'	1.0 a-d	4.0 c-j	5.33 d-g	0.25 ef	2.42 i-p	0.0 c	
O-2	<i>P. j.</i> 'Bisbee Dwarf'	-	-	-	0.0 f	0.67 n-p	0.0 c	
O-3	P. j. 'Bolero'	0.67 b-d	5.78 a-h	15.67 a-e	1.0 b-e	5.67 c-k	7.0 bc	
O-4	P. j. 'Bonsai'	0.33 cd	3.11 e-j	3.67 d-g	0.25 ef	2.33 ј-р	0.0 c	
O-5	P. j. 'Chaconne'	0.33 cd	4.78 b-j	4.0 d-g	0.0 f	1.17 m-p	1.25 c	
O-6	P. j. 'Christmas Cheer'	1.33 a-c	4.78 b-j	4.0 d-g	0.5 d-f	1.84 k-p	1.5 c	
O-7	P. j. 'Coleman'	1.0 a-d	6.45 a-g	7.0 d-g	0.75 c-f	4.42 d-o	0.0 c	

Table 2.2. No-choice assays with S. takeyai for adult survival and percent leaf area damaged on Pieris taxa

O-8	P. j. 'Crimson Compact'	0.67 b-d	4.0 c-j	0.0 g	0.25 ef	1.84 k-p	0.0 c
O-9	P. j. 'Cupido'	1.33 a-c	5.56 a-i	6.67 d-g	0.5 d-f	4.67 d-n	7.75 bc
O-10	P. j. 'Daisen'	1.0 a-d	2.22 f-j	15.33 a-f	0.75 c-f	3.08 h-p	4.25 c
O-11	P. j. 'Debutante'	1.33 a-c	4.56 b-j	15.67 а-е	1.0 b-e	3.17 h-p	0.0 c
O-13	P. j. 'Flamingo'	1.67 ab	5.45 a-i	10.33 c-g	0.0 f	1.59 l-p	2.75 c
O-15	P. j. 'Iseli Cream'	1.67 ab	3.89 d-j	7.0 d-g	2.0 a	5.92 a-j	7.25 bc
O-17	<i>P. j.</i> 'La Rocaille'	0.67 b-d	7.11 a-f	4.33 d-g	1.5 a-c	5.92 a-j	9.0 bc
O-18	<i>P. j.</i> 'Nocturne'	0.67 b-d	5.44 a-i	0.0 g	0.25 ef	2.25 ј-р	0.0 c
O-19	<i>P. j.</i> 'PI 418 531'	1.67 ab	4.33 c-j	7.0 d-g	1.0 b-e	4.67 d-n	0.0 c
O-20	P. j. 'Purity'	1.67 ab	9.33 ab	7.33 d-g	0.75 c-f	5.75 b-k	0.0 c
O-22	<i>P. j.</i> 'Red Mill'	1.33 a-c	6.22 a-h	1.67 e-g	0.25 ef	4.84 d-m	3.75 c
O-23	P. j. 'Sinfonia'	0.67 b-d	5.67 a-h	14.0 b-g	1.25 a-d	6.42 a-i	0.0 c
O-24	P. j. 'Stockman'	1.0 a-d	2.22 f-j	9.0 c-g	1.75 ab	7.83 a-e	0.0 c
O-25	<i>P. j.</i> 'T40-82A'	-	-	-	1.0 b-e	3.58 f-p	0.0 c
O-26	<i>P. j.</i> 'T44-82U'	2.0 a	5.44 a-i	5.0 d-g	1.0 b-e	6.25 a-j	0.0 c
O-27	<i>P. j.</i> 'UNH'	2.0 a	7.67 a-e	28.33 ab	1.75 ab	6.83 a-h	15.5 ab
O-29	P. j. 'Valley Valentine'x 'Kubas'	0.0 d	8.89 a-c	5.33 d-g	2.0 a	5.84 b-k	0.0 c
O-30	P. j. 'Variegata'	1.33 a-c	1.33 h-j	1.0 fg	0.0 f	1.33 m-p	0.0 c
O-31	<i>P. j.</i> 'Wada'	1.33 a-c	4.78 b-j	9.0 c-g	1.5 a-c	5.5 c-l	0.0 c
O-32	P. j. 'White Caps'	1.67 ab	4.56 b-j	17.33 a-d	0.75 c-f	6.5 a-h	3.5 c
O-33	<i>P. j.</i> 'White Cascade'	-	-	-	1.0 b-e	3.92 e-p	0.0 c
O-34	<i>P. j.</i> 'White Water'	-	-	-	1.5 a-c	9.17 a-c	8.0 bc
O-35	P. j. var. amamiana	1.67 ab	8.22 a-d	22.33 а-с	1.75 ab	7.33 a-g	5.5 c
O-36	P. j. x P. f. 'Spring Snow'	0.33 cd	8.33 a-d	11.67 c-g	1.25 a-d	4.42 d-o	0.0 c
O-37	P. j. x P. for. var. forestii 'Forest Flame'	0.33 cd	2.67 f-j	8.0 c-g	1.75 ab	7.42 a-f	9.75 bc
O-38	P. for. var. forestii	0.0 d	2.11 g-j	6.67 d-g	1.25 a-d	7.58 a-f	20.75 a
G-1	Compact Andromeda	-	-	-	1.0 b-e	4.0 e-p	0.0 c
G-2	Amami Island	-	-	-	1.0 b-e	3.34 g-p	0.0 c
G-3	Pee Wee	-	-	-	1.25 a-d	4.92 d-m	0.0 c
G-4	P. j. x P. t. 'Dodd's Bridal Veil Falls'	-	-	-	1.25 a-d	5.09 d-m	0.0 c
	F	2.3	1.66	1.77	2.9	2.57	1.34
	Р	0.0002	0.0142	0.0069	< 0.0001	< 0.0001	0.0737

Means in the same column bearing different letters are significantly different ($\alpha = 0.05$).

(B), (C) and (FF) indicate the nurseries Briggs, Cofer's and Forest Farm.

The abbreviations P.j. = Pieris japonica, P.f. = Pieris floribunda, P.t. = Pieris taiwanensis, P.for. = Pieris formosa,

HFS = Horticulture Farm Stock, O= Oregon, G = Griffin
	September 2009								
	Day 2		Da	Day 7		Day 9		Day 13	
	F	P	F	P	\boldsymbol{F}	P	F	P	
Adult survival									
Between Pieris taxa	0.83	0.6673	2.47	0.0014	1.65	0.0539	1.42	0.1267	
Between Lace bug sp.	0.02	0.8852	8.3	0.0046	24.0	< 0.0001	53.49	< 0.0001	
Overall analysis	<i>F</i> =0.79, c	lf=23,136,	F=2.47,	df=23,136,	<i>F</i> =2.41,	df=23,136,	F=3.54,	df=23,136,	
	<i>P</i> =0	.7366	<i>P</i> =0	0.0007	P=0	0.0009	<i>P</i> <	0.0001	
<u>Leaf damage</u>							F	Р	
Between Pieris taxa	-	-	-	-	-	-	2.7	0.0005	
Between Lace bug sp.	-	-	-	-	-	-	84.92	< 0.0001	
Overall analysis							<i>F</i> =6.09,	df=23,136,	
	<i>P</i> <0.0001								
				Octobe	r 2009				
Adult survival									
Between Pieris taxa	1.3	0.1957	3.72	< 0.0001	3.24	< 0.0001	2.29	0.0033	
Between Lace bug sp.	1.49	0.2239	29.24	< 0.0001	41.16	< 0.0001	50.25	< 0.0001	
Overall analysis	<i>F</i> =1.4, d	f=23,136,	<i>F</i> =4.97,	df=23,136,	<i>F</i> =5.0,	df=23,136,	<i>F</i> =4.26,	df=23,136,	
	P=0	.1198	<i>P</i> <0	0.0001	<i>P</i> <	0.0001	P <	0.0001	
<u>Leaf damage</u>									
Between Pieris taxa	-	-	-	-	-	-	3.98	< 0.0001	
Between Lace bug sp.	-	-	-	-	-	-	27.06	< 0.0001	
Overall analysis							F=4.76,	df=23,136,	
							P <	0.0001	

Table 2.3. Analysis of variance in combined assays showing differences between Pieris taxa and between lace bug species

	S. py	rioides	S. takeyai	
Pieris taxa	No. live	% leaf area	No. live	% leaf area
	bugs [£]	damaged ^{&}	bugs [£]	damaged ^{&}
<i>P. f</i> x <i>P. j</i> 'Brower's Beauty'	0.0 b	0.0 c	0.5 a-c	0.67 ef
P. j. 'Cavatine'	0.25 ab	1.67 a	1.0 ab	7.17 a
P. j 'Dodd's Crystal Cascade Falls'	0.25 ab	0.33 bc	0.25 bc	2.0 d-f
P. j. 'Dodd's Sugar Run Falls'	0.0 b	0.0 c	1.25 a	3.67 b-e
<i>P. j.</i> 'Flaming Silver'	0.0 b	0.0 c	1.0 ab	4.42 a-d
P. j. 'Mountain Fire'	0.0 b	0.0 c	0.75 a-c	1.67 d-f
<i>P. j.</i> 'Prelude'	0.0 b	0.17 bc	0.5 a-c	2.25 c-f
<i>P. j.</i> 'Scarlett O'Hara'	0.0 b	0.67 a-c	0.5 a-c	3.17 c-f
P. j. 'Shojo'	0.0 b	0.0 c	0.0 c	2.0 d-f
P. j. 'Valley Valentine'	0.0 b	0.17 bc	1.0 ab	4.17 a-d
P. t. 'Snow Drift'	0.0 b	0.0 c	1.0 ab	6.58 ab
<i>P. j.</i> 'Temple Bells'	0.0 b	0.0 c	0.25 bc	3.92 a-e
P. phyllireifolia	0.0 b	0.0 c	0.0 c	0.0 f
<i>P. j.</i> 'La Rocaille'	0.0 b	0.83 a-c	0.5 a-c	2.0 d-f
P. j. 'Purity'	0.0 b	0.34 bc	0.5 a-c	1.59 d-f
P. j. 'Variegata'	0.0 b	0.0 c	0.0 c	0.67 ef
P. j. var. amamiana	0.0 b	0.0 c	1.25 a	4.67 a-d
<i>P. j.</i> x <i>P. f.</i> 'Spring Snow'	0.0 b	1.5 a	1.25 a	4.25 a-d
P. j x P. for. var. forestii 'Forest Flame'	0.0 b	0.83 a-c	0.75 a-c	5.34 a-c
P. for. var. forestii	0.5 a	1.17 ab	0.5 a-c	3.0 c-f
F	0.89	2.21	1.76	2.75
Р	0.60	0.011	0.051	0.002

Table 2.4. Combined assay with S. pyrioides and S. takeyai for adult survival and percent leaf area damaged (September 2009)

Means in the same column followed by different letters are significantly different ($\alpha = 0.05$) [£] Significantly different at P = 0.05, [&] Significantly different at P = 0.05

The abbreviations P.j. = Pieris japonica, P.f. = Pieris floribunda, P.t. = Pieris taiwanensis, P.for. = Pieris formosa

	S. pyrioides		S. takeyai	
Pieris taxa	No. live	% leaf area	No. live	% leaf area
	bugs [£]	damaged ^{&}	bugs [£]	damaged ^{&}
<i>P. f</i> x <i>P. j</i> 'Brower's Beauty' (B)	0.5 ab	3.34 a-c	0.5 cd	2.08 d-h
P. j. 'Cavatine' (C)	0.25 ab	4.58 a	0.5 cd	4.84 a-c
P. j 'Dodd's Crystal Cascade Falls' (C)	0.0 b	1.83 b-e	0.5 cd	2.5 c-g
P. j. 'Dodd's Sugar Run Falls' (C)	0.0 b	0.5 e	0.75 b-d	2.42 c-h
<i>P. j.</i> 'Flaming Silver' (FF)	0.25 ab	1.33 b-e	0.5 cd	1.75 f-h
<i>P. j.</i> 'Mountain Fire' (B)	0.0 b	0.92 b-e	0.5 cd	1.92 e-h
<i>P. j.</i> 'Prelude' (C)	0.0 b	0.34 e	0.5 cd	3.17 c-g
<i>P. j.</i> 'Scarlett O'Hara' (B)	0.25 ab	3.5 ab	1.25 a-c	4.5 a-d
<i>P. j.</i> 'Shojo' (B)	0.0 b	0.67 de	0.0 d	1.08 gh
P. j. 'Valley Valentine' (B)	0.0 b	0.5 e	0.5 cd	0.92 gh
<i>P. t.</i> 'Snow Drift' (B)	0.0 b	0.83 c-e	1.0 a-c	3.33 c-g
<i>P. j.</i> 'Temple Bells'	0.0 b	0.34 e	1.75 a	5.92 ab
P. phyllireifolia	0.0 b	0.0 e	0.0 d	0.0 h
<i>P. j.</i> 'La Rocaille'	0.25 ab	1.5 b-e	1.0 a-c	4.25 a-e
P. j. 'Purity'	0.0 b	1.09 b-e	1.25 a-c	4.0 b-f
P. j. 'Variegata'	0.0 b	0.0 e	0.5 cd	1.75 f-h
P. j. var. amamiana	0.0 b	0.92 b-e	1.5 ab	6.0 ab
P. j. x P. f. 'Spring Snow'	0.5 ab	4.75 a	0.75 b-d	3.0 c-g
P. j x P. for. var. forestii 'Forest Flame'	0.0 b	1.5 b-e	0.5 cd	3.25 c-g
P. for. var. forestii	0.75 a	3.25 a-d	1.75 a	6.58 a
F	1.51	2.55	2.31	4.3
Р	0.116	0.003	0.008	< 0.0001

Table 2.5. Combined assay with S. pyrioides and S. takeyai for adult survival and percent leaf area damaged (October 2009)

Means in the same column bearing different letters are significantly different ($\alpha = 0.05$) ^f Significantly different at P = 0.05, [&] Significantly different at P = 0.05 The abbreviations P.j. = Pieris japonica, P.f. = Pieris floribunda, P.t. = Pieris taiwanensis, P.for. = Pieris formosa

Pieris taxa		Mean nymphs			
	Day2	Day 7	Day 9	Day 13	Day 17
<i>P. p.</i> 'Little leaf'	0.22 ± 0.11 cd	$0.22 \pm 0.11c$	$0.22 \pm 0.11 \text{ d}$	$0.22 \pm 0.11 \text{ e}$	0.0 c
P. p. 'Baldwin'	0.0 d	0.67 ± 0.38 c	$0.22 \pm 0.22 \text{ d}$	0.22 ± 0.22 e	0.0 c
<i>P. j.</i> 'Temple Bells'	8.33 ± 2.61 a	17.11 ± 3.27 a	21.89 ± 1.31 ab	31.0 ± 0.58 a	19.78 ± 9.78 a
P. j. 'Dodd's Sugar Run Falls'	7.89 ± 2.78 ab	10.11 ± 3.64 b	10.78 ± 3.91 c	18.67 ± 2.41 b	1.56 ± 1.24 c
<i>P. j.</i> 'Dorothy Wycoff'	4.33 ± 1.35 a-c	$9.89 \pm 1.72 \text{ b}$	16.78 ± 4.05 b	18.89 ± 4.89 b	11.0 ± 0.51 b
<i>P. j.</i> 'Valley Rose'	0.22 ± 0.22 cd	4.89 ± 2.11 bc	5.22 ± 2.3 cd	5.78 ± 2.59 c-e	0.56 ± 0.56 c
P. j. 'Cavatine'	8.22 ± 0.22 a	18.33 ± 3.42 a	23.67 ± 2.7 a	28.11 ± 3.23 a	10.78 ± 3.49 b
<i>P. t.</i> 'Snow Drift'	0.22 ± 0.11 cd	$0.44 \pm 0.22 \text{ c}$	$0.89 \pm 0.59 \text{ d}$	1.78 ± 1.18 de	0.0 c
P. j. 'Dodd's Crystal Cascade Falls'	1.22 ± 0.22 cd	$6.89\pm0.78~b$	8.22 ± 0.78 c	9.22 ± 1.28 cd	2.11 ± 0.73 c
<i>P. j.</i> 'Prelude'	3.67 ± 0.33 b-d	$7.0\pm0.58\ b$	10.56 ±0.87 c	12.44 ± 1.18 bc	4.78 ± 2.38 bc
F	5.49	10.69	17.36	15.51	4.89
Р	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Overall Model	F = 4.85,	F=9.05,	F=14.75,	F=13.51,	F=4.17,
	df = 11,78,	df=11,78,	df=11,78,	df=11,78,	df=13,76,
	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001

Table 2.6. Mean number of frass spots on leaves and nymphs emerged in choice trial with Pieris varieties

Means (\pm SEM) in the same column bearing different letters are significantly different ($\alpha = 0.05$; LSD). The abbreviations *P.j. = Pieris japonica*, *P.t. = Pieris taiwanensis*, *P.p. = Pieris phillyreifolia*

Pieris taxa	Day 0	Day2	Day 7	Day 9	Day 13
D = (1 + 1) + 1 + 1 + 1 + 1 + 1 + 1 + 1 + 1 +	0.22 ± 0.11	0.22 ± 0.10	0.0.0	0.11 + 0.11 -	0.0.0
P. p. Little leaf	0.22 ± 0.11 a	0.33 ± 0.19 a	0.0 C	0.11 ± 0.11 C	0.0 C
P. p. 'Baldwin'	0.44 ± 0.22 a	0.33 ± 0.19 a	$0.11 \pm 0.11 \text{ c}$	0.0 c	0.0 c
<i>P. j.</i> 'Temple Bells'	0.67 ± 0 a	1.0 ± 0.38 a	1.22 ± 0.22 a	1.67 ± 0.19 a	1.56 ± 0.41 a
<i>P. j.</i> 'Dodd's Sugar Run Falls'	0.11 ± 0.11 a	0.33 ± 0 a	0.56 ± 0.11 bc	0.33 ± 0.19 c	0.44 ± 0.41 bc
<i>P. j.</i> 'Dorothy Wycoff'	0.89 ± 0.22 a	0.78 ± 0.22 a	0.56 ± 0.29 bc	0.67 ± 0.19 bc	0.44 ± 0.29 bc
<i>P. j.</i> 'Valley Rose'	0.67 ± 0.19 a	0.22 ± 0.11 a	0.11 ± 0.11 c	0.0 c	0.0 c
<i>P. j.</i> 'Cavatine'	0.89 ± 0.29 a	0.89 ± 0.22 a	1.0 ± 0.19 ab	1.33 ± 0.38 ab	1.0 ± 0.19 ab
<i>P. t.</i> 'Snow Drift'	0.22 ± 0.11 a	0.22 ± 0.22 a	0.0 c	0.0 c	0.0 c
<i>P. j.</i> 'Dodd's Crystal Cascade Falls'	0.22 ± 0.11 a	0.44 ± 0.22 a	0.56 ± 0.11 bc	0.11 ± 0.11 c	0.0 c
<i>P. j.</i> 'Prelude'	0.44 ± 0.23 a	0.78 ± 0.22 a	0.11 ± 0.11 c	0.22 ± 0.11 c	0.0 c
F	1.6	1.2	4.59	5.85	6.41
Р	0.1288	0.3047	< 0.0001	< 0.0001	< 0.0001
Overall Model	F=1.64,	F=1.05,	F=3.86,	F=4.88,	F=5.4,
	df=11,78,	df=11,78,	df=11,78,	df=11,78,	df=11,78,
	P=0.1029	P=0.4121	P=0.0002	P<0.0001	P<0.0001

Table 2.7. Mean number of adults present on leaves in choice trial with Pieris varieties (averages from 3 replications)

Means (\pm SEM) in the same column bearing different letters are significantly different ($\alpha = 0.05$; LSD). The abbreviations *P.j. = Pieris japonica*, *P.t. = Pieris taiwanensis*, *P.p. = Pieris phillyreifolia*

	Day2	Day 7	Day 9	Day 13
<i>P. j.</i> 'Prelude'	$7.5 \pm 0.76 \text{ b}$	6.33 ± 0.8 b	4.67 ± 1.05 bc	3.5 ± 1.06 bc
<i>P. j.</i> 'Cavatine'	9.33 ± 0.49 a	8.5 ± 0.76 a	8.17 ± 0.75 a	6.0 ± 1.55 a
<i>P. j.</i> 'Dodd's Sugar Run Falls'	7.33 ± 0.49 b	6.0 ± 0.82 b	4.5 ± 0.67 c	2.83 ± 0.7 cd
<i>P. j.</i> 'Temple Bells'	8.67 ± 0.42 ab	7.67 ± 0.42 ab	6.17 ± 0.87 b	5.5 ± 1.15 ab
Pieris phillyreifolia	5.33 ± 0.56 c	2.50 ± 0.99 c	$1.83 \pm 0.91 \text{ d}$	$1.17 \pm 0.75 \text{ d}$
F	7.91	13.92	18.0	6.94
Р	0.0005	< 0.0001	< 0.0001	0.0011
Overall model	<i>F</i> =4.23,	F=8.42,	F=12.6,	F=6.64,
	df=9,20,	df=9,20,	df=9,20,	df=9,20,
	<i>P</i> =0.0035	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> =0.0002

Table 2.8. Adult survival in whole plant assay with Pieris varieties

Means (±SEM) in the same column bearing different letters are significantly different ($\alpha = 0.05$; LSD). The abbreviation *P.j. = Pieris japonica*

	Damaged leaves	Average damage	Number of
	out of 100	on 5 leaves	nymphs
<i>P. j.</i> 'Prelude'	34.33 ± 4.1 c	30.17 ± 4.07 b	30.67 ± 8.33 bc
P. j. 'Cavatine'	54.0 ± 7.65 b	74.5 ± 11.08 a	73.50 ± 14.17 a
<i>P. j.</i> 'Dodd's Sugar Run Falls'	20.67 ± 3.53 cd	16.17 ± 2.95 b	11.0 ± 2.88 cd
<i>P. j.</i> 'Temple Bells'	75.67 ± 5.01 a	78.67 ± 10.39 a	55.33 ± 17.94 ab
Pieris phillyreifolia	12.33 ± 2.42 d	9.83 ± 3.16 b	0.0 d
F	26.71	18.28	10.15
Р	< 0.0001	< 0.0001	0.0001
Overall Model	F=12.32,	F=8.45,	<i>F</i> =5.94,
	df=9,20,	df=9,20,	df=9,20,
	<i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> =0.0005

Table 2.9. Leaf damage and nymph emergence in whole plant assay with Pieris varieties

Means (±SEM) in the same column bearing different letters are significantly different ($\alpha = 0.05$; LSD). The abbreviation *P.j. = Pieris japonica*

Fig. 2.1. Scoring chart used for leaf damage assessment (Based on Klingeman et al. 2000)



Fig 2.2. Differences in adult *S. pyrioides* and *S. takeyai* survival (mean \pm SEM, N = 8) on 20 *Pieris* taxa. Data are averages of two trials conducted in September 2009 and October 2009. Bars of the same fill color bearing different letters are significantly different ($\alpha = 0.05$, LSD). The abbreviations azlb liv=No. of live *S. pyrioides* adults, anlb liv= No. of live *S. takeyai* adults



Fig 2.3. Differences in leaf damage caused by *S. pyrioides* and *S. takeyai* (mean \pm SEM, N = 8) on 20 *Pieris* taxa. Data are averages of two trials conducted in September 2009 and October 2009. Bars of the same fill color bearing different letters are significantly different ($\alpha = 0.05$, LSD). The abbreviations azlb dam=Damage by *S. pyrioides* adults, anlb dam= Damage by *S. takeyai* adults



CHAPTER 3

RESISTANCE MECHANISMS IN *PIERIS* TAXA TO *STEPHANITIS TAKEYAI*

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ABSTRACT Preliminary screening of over 60 pieris taxa for their reaction to *Stephanitis* pyrioides and S. takeyai revealed gradients in susceptibility to the two lace bug species. Wide variability in leaf shape, size, texture, color and growth habit exists among the pieris taxa, even among taxa within the same species. Based on our observations and also previous reports on the possible role of leaf parameters like toughness, moisture content, epicuticular wax, and stomatal characters in plant resistance, we examined some of the potential mechanisms of resistance in selected pieris taxa to S. takeyai. Experiments with extracts of leaf-surface lipids revealed that Pieris leaf wax does not have a role in resistance. Leaf wax extracts from the resistant species P. phillyreifolia applied on leaves of the susceptible cultivar P. japonica 'Temple Bells' did not affect feeding, oviposition or survival of S. takeyai, and neither did the reverse affect the resistance of *P. phillyreifolia*. Leaf penetrometer measurements indicated significantly higher force was required to puncture P. phillyreifolia leaves. This species also had higher fiber, lignin and cellulose content and lower leaf moisture content. Ultrastructural studies on leaves of selected Pieris taxa revealed significant differences in the number and size of stomata. P. phillyreifolia leaves had the highest number of stomata per unit area but they were the smallest in size, whereas *P. japonica* 'Temple Bells' leaves had a lower number but the largest stomata. Resistance in *Pieris* taxa to *S. takeyai* may be attributed to a combination of different factors among which leaf toughness, moisture and stomatal characters may have a significant role.

KEY WORDS: *Pieris, Stephanitis, pyrioides, takeyai,* resistance, mechanisms, leaf surface, lipids, toughness, ultrastructure, stomata

The Andromeda lace bug *Stephanitis takeyai* Drake and Maa is an important pest of *Pieris* D. Don spp. (Johnson and Lyon 1991), the popular ericaceous ornamental plant. *Pieris* is grown for its glossy evergreen foliage and attractive flowers. The brilliant spring colors on young leaves are especially notable. Among the various cultivated species of *Pieris*, Japanese pieris (*P. japonica* (Thunb.) D. Don ex G. Don) native to Japan is the preferred host of *S. takeyai* (Schread 1968). These lace bugs, like other tingids, feed by sucking cell contents resulting in disfigurement in the form of yellowish white stipples or blotches on the abaxial surfaces of the leaves as well as reduction in photosynthetic efficiency (Buntin et al. 1996). On the adaxial surface, lace bug adults and nymphs can be seen along with their shiny black frass spots. Occasionally, lace bug damage may lead to premature leaf shedding, drying up of twigs or even the whole plant (Schread 1968), but in most cases even damage to a few leaves can affect the aesthetic value of the plant and its marketability.

The extent of damage caused by *S. takeyai* to different pieris cultivars has not been studied in detail. In Poland severe damage on cultivars 'Select', 'Debutante', 'Cupido' and lesser damage was observed on cultivars 'Flaming Silver', 'Variegata' and 'Redmill' (Labanowski and Soika 2000). Apart from these few reports, very little information is available regarding the susceptibility of different *Pieris* taxa to lace bugs. The susceptibility of Japanese pieris, *P. japonica* to *S. takeyai* has been recognized (Schread 1968), as has the apparent low preference for mountain pieris, *P. floribunda* (Pursh) Benth. and Hook. f. and its hybrids (Dunbar 1974). When 60 cultivated *Pieris* taxa (species, cultivars and hybrids) were compared for their susceptibility to *S. takeyai* and the more widespread and economic tingid species, *S. pyrioides* (Scott) gradients in

susceptibility to both lace bug species were evident (Chapter 2). Among the four *Pieris* species tested the highest preference was observed for *P. japonica* as reported earlier (Schread 1968). *P. taiwanesis* Hayata and *P. formosa* (Wall.) D. Don were less damaged and *P. phillyreifolia* (Hook.) DC., was least damaged. Within *P. japonica*, 51 taxa were evaluated and among them *S. takeyai* showed clear preference for certain *P. japonica* taxa viz., 'Temple Bells' and 'Cavatine', whereas others like 'Variegata' and 'Prelude' were less damaged. The hybrids *P. floribunda* x *P. japonica* 'Brower's Beauty' were less preferred, as mentioned in an earlier report (Dunbar 1974), and so did the hybrids *P. japonica* x *P. floribunda* 'Spring Snow' and *P. japonica* x *P. formosa* var. *forestii* 'Forest Flame'. This may indicate presence of resistant genes in the less damaged species like *P. floribunda* and *P. formosa*. Knowledge of the gradients in susceptibility among the cultivated taxa can guide choice of landscape plants and minimize pesticide treatments. This information would also be helpful in breeding resistant varieties.

The reasons for the preferences exhibited by *S. takeyai* are not yet known, but the wide variability in leaf shape, size, texture, color and growth habit among the *Pieris* taxa, even within the *japonica* cultivars suggests that different mechanisms may be involved. Leaf physical and chemical parameters like toughness, pubescence, moisture content, epicuticular wax, and stomatal size and density have been studied as possible mechanisms of resistance to lace bugs and other sucking pests (Meyer and Ballington 1990; Braman and Pendley 1992; Balsdon et al. 1995; Wang et al. 1998b; Wang et al. 1999; Chappell and Robacker 2006; Kirker et al. 2008). Several aspects of resistance in the related ericaceous ornamental azalea (*Rhododendron* L. spp.) to the *S.takeyai* congeneric *S. pyrioides* have been investigated earlier (Braman and Pendley 1992;

Balsdon et al. 1995; Wang et al. 1998b; Wang et al. 1999; Chappell and Robacker 2006) but the exact mechanisms are not clear yet and likely involve a combination of different factors.

The cuticle is the plant's primary line of defense against all extraneous influences. The plant cuticle is generally composed of a mixture of epicuticular lipids. Although epicuticular lipids primarily serve to conserve moisture, they also have important ecological functions in influencing interactions between plants and their insect herbivores. The physical structure of plant surface lipids can also affect insect herbivore attachment and locomotion (Eigenbrode and Espelie 1995) and these aspects have been studied before. The relationship of leaf-surface lipid composition and response by S. *pyrioides* was evaluated in selected azalea cultivars, and it was found that there were differences in the lipid components between resistant and susceptible types. Hence, response of S. pyrioides to a particular plant may be influenced by chemical signals from these leaf-surface components (Wang et al. 1999). Most of the components were identified as *n*-alkanes and triterpenoids and these had significant correlations with S. *pyrioides* behavior on host plants as measured by oviposition, leaf area damaged, egg and nymphal development, and nymphal survivorship. Seasonal variations in their proportions were also observed. The resistant genotypes were high in ursolic acid, nhentriacontane, and *n*-nonacosane, whereas susceptible deciduous genotypes had more of α -amyrin, β -amyrin, and *n*-nonacosane. Another study revealed that the triterpenoid components α - and β -amyrin, reported to be insect feeding and/or oviposition deterrents, were present in lower levels in susceptible cultivars (Balsdon et al. 1995). Studies with extracts of epicuticular leaf wax indicated that leaf wax serves as a primary mechanism of

resistance of deciduous azalea to *S. pyrioides* (Chappell and Robacker 2006). In these experiments leaf wax extracts from resistant genotypes applied to susceptible ones resulted in resistance to both feeding and oviposition by *S. pyrioides* in the treated susceptible genotypes, and wax extracts from susceptible genotypes applied on resistant ones caused susceptibility. The effects of leaf-surface lipids have also been reported in many other plant-herbivore systems. For example, 'glossy' cruciferous vegetable varieties are seen to support lower populations of pests like cabbage aphid, *Brevicoryne brassicae* L. and diamondback moth (*Plutella xylostella* L.) (Stoner 1990).

Leaf toughness as a defense mechanism in plants was first proposed by Feeny (1970). Since then, mechanical defenses including toughness and pubescence have been widely observed and these are sometimes considered analogous to plant chemicals with multiple functions in the plant (Woodman and Fernandes 1991). Leaf toughness is a major source of protection in plants against insect herbivores and their avoidance of tough plant parts is a common observation (Howard 1988; Larsson and Ohmart 1988). In a prior study, P. phillyreifolia leaves were least preferred by S. takeyai and appeared to be the toughest among all the tested taxa. Leaf toughness has been reported previously as a probable source of resistance in blueberries (*Vaccinium* L. spp., family Ericaceae) to the leaf hopper Scaphytopius magdalensis (Provancher) (Cicadellidae: Hemiptera). A possible correlation between leaf toughness and resistance to S. magdalensis was noted in V. crassifolium Andrews, but in other species like V. ashei J.M. Reade leaf physical characteristics like thickness, toughness and glaucousness were poorly correlated with resistance (Meyer and Ballington 1990). It was also noted that the resistant V. crassifolium cultivars caused high mortality of early instar S. madgalensis nymphs, but

they supported later instars. Other studies have revealed that leaf toughness affected the development of earlier instars of insects with chewing mouthparts also (Slansky 1990). Experiments on performance of the lappet moth, *Streblote panda* Hübner

(Lasiocampidae: Lepidoptera), on blueberry cultivars (Ericaceae) showed that the only nutritional factor that was correlated with larval development was leaf toughness (Calvo and Molina 2004). In studies with other plants, herbivory losses were more correlated with toughness than with phenolics and other plant chemicals (Lowman and Box 1983, Choong 1996). Plant chemicals have been suggested as the greater limiting factor for cytoplasm consuming insects (Abe and Higashi 1991), but the role of mechanical defenses in limiting the process of the insects breaking open the cell walls to reach the cytoplasm cannot be ignored (Hochuli 1996). Similar to the cuticle, pubescence on plant surfaces is another mechanism that affects herbivore activity in addition to serving other important physiological functions like prevention of water loss. The effects of pubescence and other structural defense mechanisms seen in plants on herbivores have been studied and reviewed extensively (Hanley et al. 2007). However, in the ericaceous azaleas leaf pubescence, although varying considerably among the tested taxa, did not seem to be significantly correlated with azalea lace bug resistance (Wang et al. 1998b).

In addition to plant structures and chemicals, the concentration of water and mineral nutrients in plants is also known to influence herbivory since host plants are important sources of moisture and nutrient for phytophagous insects and other arthropods. The mineral requirements of insects is probably a lesser explored area of insect nutrition, but several reviews on insect nutrition ecology have been made over the years (Friend 1958; House 1961; Mattson 1980; Awmack and Leather 2002; Chen et al.

2010). Much of the available information is from studies with plants deficient in different minerals and observations on performance of insects fed on them. However such studies are confounded with the over-all condition of the deficient plant and so that specific deficiency effects upon the insect cannot be measured (Friend 1958). Insects can adapt well to new nutritional environments since their requirements may vary even within a species, and therefore it is difficult to establish whether nutritional factors confer any particular degree of herbivory resistance to plants (House 1961). Nevertheless, many choices made by insects during their life processes are influenced by nutritional needs (Slansky 1982) and nutrients are essential in the regulation of energy flow at all trophic levels (Mattson 1980). Hence determining the nutrient composition of leaves may provide some explanation for the responses shown by plants to herbivory.

Ultrastructural studies have enabled examination of plant surfaces for different plant structures like trichomes and stomata in greater detail. Scanning electron microscopy is widely used to describe morphological differences between hosts and nonhosts (Scott Brown and Simmonds 2006). In some cases even presence of chemical substances like wax crystals and other epicuticular components and their characters have been revealed (Balsdon et al. 1995). Compound microscopy was used in a study that examined the effects of stomatal size in azalea leaves on feeding preference of *S. pyrioides*. However it was found that although the stomates through which *S. pyrioides* feed varied in size among the selected azalea cultivars, their size or area could not be correlated to *S. pyrioides* feeding preference (Kirker et al. 2008).

Due to the varying effects of the different resistance mechanisms in different plants, the general understanding is that resistance is a combination of mechanisms

among which plant chemical composition might play the major role. Most studies that examine structural defenses in plants indicate the possibility that defensive compounds are also involved in resistance (Balsdon et al. 1995; Wang et al. 1998b; Gillman et al. 1999; Scott Brown and Simmonds 2006). In the case of *S. pyrioides* resistance in azaleas antibiosis is suggested as one of the mechanisms, indicated by lower survivorship of adults and nymphs, significantly reduced feeding, plant damage and growth rate of *S. pyrioides* (Wang et al. 1998b).

Thus, plants have different mechanisms that enable them to resist herbivory to some extent. Here we examine some of these mechanisms for their potential role in resistance exhibited by *Pieris* taxa to *S. takeyai*.

Materials and Methods

STUDIES ON EPICUTICULAR LIPIDS. This study was carried out to evaluate the effects of leaf-surface lipids on feeding, oviposition and survival of *S. takeyai* on *Pieris* leaves. **Plant material.** Leaves from selected *Pieris* taxa (species and cultivars) were obtained from the Department of Horticulture *Pieris* collection located at the UGA Horticulture Farm in Watkinsville, GA.

Lace bugs. *S. takeyai* colonies were housed in plastic containers and maintained on several cultivars of *Pieris* under conditions of $27 \pm 1^{\circ}$ C and a photoperiod of 14:10 (L:D) hours.

Leaf wax extraction. The procedure for leaf wax extraction was adopted from Chappell and Robacker (1996) and modified suitably for different assays, designated as Trials A, B and C.

Trial A. 40 leaves each of the selected *Pieris* genotypes were air dried for 120 hours. They were then immersed in 100 ml of chloroform for 15 s. Chloroform was evaporated and remaining epicuticular wax re-suspended in 50 ml 2 ethanol : 1 deionized water solution under mild heating (32 °C) and stirring. Upon cooling to room temperature $(20^{\circ}C)$, the resulting solution was applied directly to the fresh leaves by painting the leaf surface with a brush dipped in the solution. Only one half of a leaf was painted with the solution (both abaxial and adaxial surfaces on one side of the midrib), the other half being treated with solvent only or untreated. This was to facilitate pair wise comparisons between treated and untreated leaf surfaces. Thus, two controls were used, one being the solvent alone and the other without treatment at all. The treated leaves were then dried and prepared for bioassays. Three such leaves of a variety placed in a Petri dish with their stalks covered with moist tissue paper constituted one replication. Each variety was replicated six times. In replications 1, 2 and 3 the control was the solvent alone, whereas in replications 4, 5 and 6 the control was no treatment at all. This was done to test for any effect of the solvent alone. In all, there were three donors (two varieties supplying the wax, and the solvent) and two recipients (two varieties receiving the treatments) giving a total of six treatments.

Two lace bugs were released into each Petri dish. The dishes were arranged in a randomized complete block design and placed under conditions of $27 \pm 1^{\circ}$ C and a photoperiod of 14:10 (L:D) h. Observations on number of bugs alive were taken on day 2, 7, 9 and 13. After the exposure period the surviving adults were removed and the leaves were scored for percent leaf area damage using the scoring chart based on Klingeman et al. (2000). After scoring, the leaves were maintained under the same

conditions as during the exposure period and observed daily for emergence of nymphs. This trial was repeated twice (designated as A1 and A2).

Trial B. Two sets of 40 leaves each of the selected *Pieris* genotypes were air dried for 120 hours. One set was then immersed in 100 ml of chloroform for 15 s and the other for 30 s. Chloroform was evaporated and remaining epicuticular wax re-suspended in 50 ml 2 ethanol : 1 deionized water solution under mild heating (32 °C) and stirring. Upon cooling to room temperature (20°C), the resulting solution was applied directly to the fresh leaves by painting the leaf surface with a brush dipped in the solution. Only one half of a leaf was painted with the solution (both abaxial and adaxial surfaces on one side of the midrib), the other half being treated with solvent only or untreated. This was to facilitate pairwise comparisons between treated and untreated leaf surfaces. The treated leaves were then dried and prepared for bioassays. Three such leaves of a variety placed in a Petri dish with their stalks covered with moist tissue paper constituted one replication. Each variety was replicated three times. In all, there were four donors (three varieties supplying the wax, and the solvent) and three recipients (three varieties receiving the treatments) giving a total of 12 treatments.

Two lace bugs were released into each Petri dish. The dishes were arranged in a randomized complete block design and placed under conditions of $27 \pm 1^{\circ}$ C and a photoperiod of 14:10 (L:D) h. Observations on number of bugs alive were taken on day 2, 7, 9 and 13. After the exposure period the surviving adults were removed and the leaves were assessed for leaf damage using the number of frass spots as an indication of feeding (Buntin et al. 1996). After damage assessment, the leaves were maintained under

the same conditions as during the exposure period and observed daily for emergence of nymphs. This trial was repeated twice (designated as B1 and B2).

Trial C. Two sets of 40 leaves each of the selected *Pieris* genotypes were freshly collected. One set was then immersed in 100 ml of chloroform for 30 s and the other for 5 minutes. Chloroform was evaporated and remaining epicuticular wax re-suspended in 50 ml 2 ethanol : 1 deionized water solution under mild heating (32 °C) and stirring. Upon cooling to room temperature (20°C), the resulting solution was applied directly to the fresh leaves being tested by dipping them in the solution.

Four leaves of each variety were used in a replication. Two leaves were entirely dipped in the wax solution for 30 s and then removed and air dried. The other two leaves were left untreated. This was to facilitate pair wise comparisons between treated and untreated leaves. The four leaves of a variety arranged in a Petri dish with their stalks covered with moist tissue paper constituted one replication. The two treated leaves were kept on one side of the tissue paper and the two untreated leaves on the other side. Each variety was replicated three times. In all, there were three donors (three varieties supplying the wax) and three recipients (three varieties receiving the treatments) giving a total of 9 treatments.

Four lace bugs were released into each Petri dish. The dishes were arranged in a randomized complete block design and placed under conditions of $27 \pm 1^{\circ}$ C and a photoperiod of 14:10 (L:D) h. Observations on number of bugs alive were taken at day 2, 7, 9 and 13. After the exposure period the surviving adults were removed and the leaves were assessed for leaf damage using the number of frass spots as an indication of feeding (Buntin et al. 1996).

LEAF TOUGHNESS. Leaves from 11 *Pieris* varieties were collected fresh from containerized plants maintained in the screenhouse at the UGA Experiment Station Griffin, GA. The plants were obtained from different plant nurseries near Griffin as well as outside Georgia. The leaves were detached from the plants and brought to the laboratory in plastic bags just prior to the toughness measurements. Toughness was measured using a force gauge (Chatillon DFX-010-NIST Digital Force Gauge).

Six mature leaves (fourth or fifth leaf from the bottom of a branch) were selected from each variety. To make the measurements, each leaf was placed on a special platform provided with the force gauge for the purpose and the gauge was operated in the prescribed manner and observations recorded. Six punctures were made on each leaf, three from the upper (abaxial) to lower (adaxial) surface and three from the lower to upper surface. The force required to puncture each leaf was recorded in Newtons. LEAF NUTRITIONAL PARAMETERS. Leaves from five selected Pieris varieties were collected fresh from containerized plants maintained in the screenhouse at the UGA Experiment Station Griffin, GA and submitted for analysis on the same day. Leaf nutrients including moisture content, starch, water soluble carbohydrates (WSC), ethanol soluble carbohydrates (ESC), fructans, non-structural carbohydrates (NSC), acid detergent fiber (ADF), acid detergent lignin (ADL) and cellulose were analyzed at the Feed and Environmental Water Lab under the Agricultural and Environmental Services Laboratories, UGA CAES Cooperative Extension Service, Athens, GA. ESC represents simple sugars mainly, glucose, fructose, and sucrose and WSC represents simple sugars and fructans. The nutrients were analyzed on as-received basis as well as dry matter basis. Starch was determined following the methods described by Karkalas

(1985) and Holm et al. (1986). The procedure solubilized starch via enzymatic extraction and determined glucose in the extracts. Percent starch per sample was calculated based on the concentration of glucose. Water soluble extracts and ethanol soluble extracts were prepared according to Smith (1969) then carbohydrate in each extract was colorimetrically determined through a phenol-sulfuric acid procedure on the spectrophotometer based on sucrose standard as described by (Dubois et al. 1956). The water soluble extracts and ethanol soluble extracts represented different fractions of nonstructural carbohydrate. The WSC included simple sugars plus fructans, whereas the ESC included simple sugars with negligible fructans (Harris 2003). The difference between WSC and ESC gave an estimate of fructan content of the feed sample.

The procedures followed to determine the hemicellulose, cellulose, and lignin were based on the principles of detergent fiber analyses developed by Van Soest (1963a; 1963b) and Van Soest and Wine (1967) which allow the determination of:

- (a) Neutral Detergent Fiber (NDF), which is the residue remaining after digesting the sample in a neutral detergent solution (30.0 g sodium dodecyl sulfate; 18.61g EDTA disodium salt, dihydrate; 6.81 g sodium borate; 4.56 g sodium phosphate dibasic, anhydrous; and 10.0 ml triethylene glycol, dissolved in 1 L distilled water with necessary agitation and heating to aid solution followed by pH of the solution adjusted in the range from 6.9 to 7.1). The fiber residues are predominantly hemicelluloses, cellulose, and lignin;
- (b) Acid Detergent Fiber (ADF), which is the residue remaining after digesting the sample with an acid detergent solution (20g cetyl trimethylammonium bromide

added to 1 L standardized $1.0N H_2SO_4$). The fiber residues are predominantly cellulose and lignin; and

(c) Lignin, which is the combustible carbon compounds in the residue after cellulose fraction in ADF is dissolved in 24 N H₂SO₄.

The analyses of NDF and ADF were carried out on an Ankom^{200/220} Fiber Analyzer (ANKOM Technology, NY) using F57 filter bags (ANKOM Technology, NY), constructed from chemically inert and heat resistant filter media, capable of being heat sealed closed and able to retain 25 µm particles while permitting rapid solution penetration (Ankom Technology 2006a; 2006b). The protocols are based on the basic principles of the methods 5.1 and 4.1 of National Forage Testing Association

(Undersander et al. 1993a; 1993b). The lignin and ash contents in ADF residue were determined following the method described by Ankom Technology (2005). Finally, the contents of hemicellulose and cellulose, and were estimated from NDF, ADF, lignin and ash follows:

%Hemicellulose = %NDF - %ADF

%Cellulose = %ADF - (%Lignin + %Ash).

Mineral concentrations in the leaves were analyzed at the Soil, Plant and Water Lab under the Agricultural and Environmental Services Laboratories, CAES Cooperative Extension Service, UGA. P, K, Ca, Mg, Mn, Fe, Al, B, Cu, Zn, Na, Pb, Cd, Ni, Cr, and Mo were analyzed using Microwave - Acid (HNO₃) Digestion, ICP Method using CEM Mars5 microwave digestion system, Thermo Jarrell-Ash model 61E ICP. Total N and Total S were analyzed using Dry Combustion Method in LECO CNS-2000 model CNS analyzer.

LEAF ULTRASTRUCTURE. Leaf samples were collected from containerized plants maintained in the screenhouse at the UGA Experiment Station Griffin, GA and submitted for analysis on the same day. Ultra thin sections of the leaves were prepared at the Histology Laboratory, Department of Pathology, UGA College of Veterinary Medicine in Athens, GA. For preparing leaf sections, three mature leaves each were chosen from three plants of three selected Pieris varieties viz., P. japonica 'Temple Bells', P. japonica 'Variegata' and P. phillyreifolia. The leaf samples were processed and then embedded 'on edge' in wax cassettes and the sections were taken from these wax blocks. These sections were received mounted on labeled slides which were later observed under a compound microscope [Leica DM LB (Leica Microsystems Inc. Bannockburn, USA) with a SPOT Idea camera (SPOT Imaging Solutions, Sterling Heights, MI, USA) attached] and digital images taken under magnifications of 4X, 10X and 20X. For SEM images, the leaf samples were observed at the UGA Ultrastructure Lab in Athens. Three mature leaves each were chosen from three plants of three selected Pieris varieties viz., P. japonica 'Temple Bells', P. japonica 'Variegata' and P. phillyreifolia. These were cut into labeled bits before viewing as is with the SEM [Variable Pressure SEM (VP-SEM)] Zeiss 1450EP (Carl Zeiss NTS, Peabody, MA, USA)].

Measurements of thickness of upper epidermis, upper epidermis + palisade layer, lower epidermis and size of stomatal opening were made by comparing the digital images of the samples with images of scales with the corresponding magnification using Adobe Photoshop®. The numbers of stomata in 100 square micron areas were also counted. For this, a line of the same length as the 100 micron scale bar was first drawn, also in Adobe Photoshop®. Using this line as a side, a square was constructed and superimposed

over the image of the leaf surface showing stomata. The square was moved to different locations on the image and the number of stomata within this square in each location was counted. In all, 15 observations of each parameter were made from each leaf (from different images) giving a total of 45 observations per variety.

STATISTICAL PROCEDURES. The experiments were analyzed as randomized complete block designs. The replications were considered as the block factor. Treatment means were analyzed separately for each study. Meansof the variables were subjected to analysis of variance (ANOVA) using the general linear model procedure (SAS Institute 2003). Means were separated with Fisher protected least significant difference (LSD) test. Leaf toughness, nutrient and mineral contents and ultrastructural parameters were used to perform a correlation analysis using PROC CORR (SAS Version 9.1) to determine if any of these variables were associated with leaf damage. Pearson's coefficient was used as the measure of correlation at a significance level of 0.05.

Results

Trial A. In part A1 (Table 3.1), all the treatments on *P. japonica* 'Temple Bells' leaves showed significantly higher mean numbers of live bugs and leaf damage compared to treatments on *P. phillyreifolia* leaves. The highest mean number of live bugs on day 13 after exposure (1.33) (F = 5.77, df = 5, P = 0.0011) was seen on T6 (Solvent only on *P. japonica* 'Temple Bells') but this was not significantly different from that on T1 (*P. phillyreifolia* extract on *P. japonica* 'Temple Bells') and T4 (*P. japonica* 'Temple Bells') extract on *P. japonica* 'Temple Bells'). None of the treatments on *P. phillyreifolia* leaves showed adult survival and leaf damage. Maximum mean leaf damage (percent leaf area damaged scored on day 13) (F=35.17, df=5, P<0.0001) on day 13 (5.33) was seen on T6

(Solvent only on *P. japonica* 'Temple Bells') and this was significantly higher than that on T1 (*P. phillyreifolia* extract on *P. japonica* 'Temple Bells') (3.47) and T4 (*P. japonica* 'Temple Bells' extract on *P. japonica* 'Temple Bells') (2.47). All treatments on *P. phillyreifolia* leaves had significantly low leaf damage compared to those on *P. japonica* 'Temple Bells' leaves. Nymph emergence was recorded only on *P. japonica* 'Temple Bells' leaves. Highest mean number of nymphs (F=12.1, df=5, P<0.0001) was observed on T1 (*P. phillyreifolia* extract on *P. japonica* 'Temple Bells'). It was interesting to note that T1 (*P. phillyreifolia* extract on *P. japonica* 'Temple Bells') had the highest number of nymphs while none of the treatments on *P. phillyreifolia* leaves (i.e., T2, T3 and T5) supported nymph emergence..

Similar trends were seen when this assay was repeated (Part A2, Table 3.1). *P. japonica* 'Temple Bells' leaves showed significantly higher mean numbers of live bugs and mean leaf damage whereas *P. phillyreifolia* leaves had very low or no adult survival and leaf damage. The highest mean number of live bugs on day 13 after exposure (1.0) (F=5.27, df=5, P=0.0019) was seen on T6 (Solvent only on *P. japonica* 'Temple Bells'). Maximum mean leaf damage on day 13 (5.64) (F=17.12, df=5, P<0.0001) was also seen on T6 (Solvent only on *P. japonica* 'Temple Bells'). Nymph emergence was again recorded only on *P. japonica* 'Temple Bells' leaves and T1 (*P. phillyreifolia* extract on *P. japonica* 'Temple Bells') had the highest mean number of nymphs (5.42) (F=6.14, df=5, P=0.0008). Table 3.1 presents the average values for live bugs, leaf damage and nymphs emerged over all three leaves in a replication.

Table 3.2 takes into account the differences between the treated and non-treated halves of each leaf. We recorded the leaf damage and nymph emergence values from the

treated and untreated sides of each leaf and then analyzed for the difference between them. Part A1 shows the results of the first assay. On treated halves as well as nontreated halves of the test leaves highest mean damage (2.92 and 2.53 respectively) was seen when the donor was D3 (Solvent), but these were not significantly different from the other two donors D1 (*P. phillyreifolia*) and D2 (*P. japonica* var. 'Temple Bells') in most cases. Highest mean number of nymphs emerged where the donor was D1 (*P. phillyreifolia*) (1.92 and 3.78 on treated and non-treated halves respectively). Except in the case of nymphs emerged from untreated half (column Nym-nt), there are no significant differences between the two *Pieris* taxa viz., *P. phillyreifolia* and *P. japonica* var. 'Temple Bells' as donors of leaf wax. However, the same two plants differed significantly when considered as recipients of leaf wax in all the parameters. R1 (*P. phillyreifolia*) always recorded significantly lower leaf damage and number of nymphs than R2 (*P. japonica* var. 'Temple Bells'). Similar results were obtained when this assay was repeated (Table 3.2, Part A2).

In all cases, replications did not differ significantly, which meant that both the controls used, solvent alone and absolute control (no solvent) were at par with each other. This also ruled out any effect caused by the solvent alone (data not shown). *Trial B.* With the 15 second extracts, significantly highest mean adult survival on day 13 (1.17 out of 2) (F=5.26, df=11, P<0.0001) was noted on T2 (P. *japonica* 'Variegata' extract on P. *japonica* 'Temple Bells'), followed by T3 (P. *japonica* 'Temple Bells' extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *phillyreifolia* extract on P. *japonica* 'Temple Bells') and T1 (P. *ph*

frass spots on day 13) (F=6.49, df=11, P<0.0001) was also noted on T2 (20.44) which was significantly higher than all other treatments except T3 (15.7). T3 was not statistically different from T1 (12.78). All the other treatments showed statistically similar and low damage. Significantly higher nymph emergence (F=3.01, df=11, P=0.0030) was observed on all the treatments on P. japonica 'Temple Bells' leaves (T1, T2, T3 and T10- Solvent only on *P. japonica* 'Temple Bells') as well as T12 (Solvent only on P. japonica 'Variegata') which were all on par. The other treatments showed very low or no nymph emergence. With the 30 second extracts also (Table 3.3), highest adult survival on day 13 (F=1.98, df=11, P=0.0465) was observed in the treatments on P. *japonica* 'Temple Bells' leaves which were all statistically similar. Treatments on P. *japonica* 'Variegata' and *P. phillyreifolia* leaves did not show significant adult survival. Significantly higher leaf damage on day 13 (F=5.61, df=11, P<0.0001) and nymph emergence (F=2.24, df=11, P=0.0233) were observed in the treatments on P. japonica 'Temple Bells' leaves, whereas the observations in the other treatments were negligible or none. In general, treatments on *P. phillyreifolia* leaves were least preferred by the lace bugs, whereas they performed best in treatments on *P. japonica* 'Temple Bells' leaves and showed moderate or low preference for *P. japonica* 'Variegata' leaves. Table 3.4 shows average values of leaf damage and nymph emergence on treated and non-treated halves of the test leaves, from two assays (B1 and B2). With both 15 second as well as 30 second extracts, there were no significant differences between the three varieties viz., D1 (P. phillyreifolia), D2 (P. japonica var. 'Variegata'), D3 (P. japonica var. 'Temple Bells') as donors of leaf wax and D4 which referred to the solvent alone. The same varieties reacted differently as recipients of leaf wax, and P. japonica var. 'Temple Bells'

was the most preferred by the lace bugs. This was indicated by the significantly higher leaf damage and nymph emergence on both treated and non-treated halves of the leaves of this variety in all cases.

Table 3.5 shows the analysis of variance in the assays B1 and B2. Pieris taxa were always significantly different. The two extraction times did not differ significantly with respect to the adult survival, leaf damage or nymph emergence in all cases except one (leaf damage in assay B1).

Trial C. Table 3.6 shows that the treatments on P. phillyreifolia leaves showed significantly lower mean numbers of live bugs on day 13 with 30 second (F=1.79, df=8, P=0.1526) as well as 5 minute extraction time (F=1.63, df=8, P=0.1932) than those on P. *japonica* 'Temple Bells' and *P. japonica* 'Variegata' leaves, although the differences were not significant with both extraction times. However mean leaf damage (number of frass spots on day 13) was significantly higher on *P. japonica* 'Temple Bells' leaves. With 30 second extracts, the highest mean leaf damage (F=9.93, df=8, P<0.0001) (34.92) was observed on T3 (P. japonica 'Temple Bells' extract on P. japonica 'Temple Bells') and this was on par with T2 (P. japonica 'Variegata' extract on P. japonica 'Temple Bells') (27.92). T1 (*P. phillyreifolia* extract on *P. japonica* 'Temple Bells') showed 15.33 frass spots, which was higher than that on all the other remaining treatments, but not statistically different. With the 5 minute extracts, highest mean leaf damage (F=15.83, df=8, P<0.0001) was again on T3 (P. japonica 'Temple Bells' extract on P. *japonica* 'Temple Bells'), and this was on par with T1 (*P. phillyreifolia* extract on *P*. japonica 'Temple Bells') and T2 (P. japonica 'Variegata' extract on P. japonica 'Temple Bells'). Treatments on *P. japonica* 'Variegata' leaves were at par with each other and

significantly higher than treatments on *P. phillyreifolia* leaves which were also at par with each other. Both these varieties showed lower damage than *P. japonica* 'Temple Bells'. Table 3.7 compares the treated and non-treated halves of the leaves in assay C, and it was clear that the recipient of the leaf wax extract (substrate) had a more significant effect on leaf damage and nymph emergence as compared to the donor. The lower part of the tables shows that the recipients differed significantly in all the parameters. This indicates that immaterial of the wax extract or solvent applied on a leaf, it is the substrate that determined whether the lace bugs fed or oviposited on it or not. The use of fresh leaves to extract wax does not appear to have changed the effect of the wax. These observations are similar to what would have been observed in the case of untreated leaves and hence indicate that the leaf wax did not affect the lace bugs. From the three assays, the order of lace bug preference of the three varieties could be identified as *P. japonica* 'Temple Bells' > *P. japonica* 'Variegata' > *P. phillyreifolia*.

LEAF TOUGHNESS. Significant differences were observed in the force required to puncture leaves of the tested *Pieris* taxa (Table 3.8). Greater force was required to puncture the leaves from the lower surface upward, than from the upper surface downward in most cases. Maximum force was required to puncture leaves of *P. phillyreifolia* 'Little Leaf' and *P. phillyreifolia* 'Baldwin' in the lower to upper direction (*F*=112.98, df=10, *P*<0.0001). These two taxa were statistically similar and significantly higher than all the other taxa with respect to puncture force. *P. japonica* 'Variegata' also required higher force, but significantly lesser than that for the *P. phillyreifolia* varieties. *P. japonica* 'Temple Bells' required slightly higher force to puncture and it was not statistically different from that for *P. japonica* 'Cavatine', *P. japonica* 'Dodd's Sugar

Run Falls' and *P. taiwanensis* 'Snow Drift'; but they were all significantly lower than that for the *P. phillyreifolia* leaves. Leaves of *P. phillyreifolia* 'Little Leaf' and *P. phillyreifolia* 'Baldwin' required significantly higher force to puncture from upper to lower direction also (*F*=28.07, df=10, *P*<0.0001), and this was on par with *P. japonica* 'Variegata' and *P. japonica* 'Valley Rose'. *P. japonica* 'Dodd's Sugar Run Falls', *P. japonica* 'Prelude', *P. taiwanensis* 'Snow Drift', *P. japonica* 'Cavatine' and *P. japonica* 'Temple Bells' required significantly less force to puncture and they were all significantly lower than that for the *P. phillyreifolia* leaves. The least force was required to puncture *P. japonica* 'Dodd's Crystal Cascade Falls' leaves in both directions.

LEAF NUTRITIONAL PARAMETERS. Among the different nutritional parameters (Table 3.9) *P. phillyreifolia* 'Little Leaf' had significantly lower starch (2.35%) on dry matter basis than the other taxa but it was on par with *P. japonica* 'Prelude' (3.02%). *P. japonica* 'Cavatine' had the highest starch content (6.21%) which was on par with that of *P. japonica* 'Dodd's Sugar Run Falls' (5.58%) and *P. japonica* 'Temple Bells' (4.65%). *P. phillyreifolia* 'Little Leaf' was also significantly lower in water soluble carbohydrates (WSC) (7.83%), ethanol soluble carbohydrates (ESC) (7.68%) and non-structural carbohydrates (NSC) (10.19%) on dry matter basis than the other taxa, which were statistically similar to each other in these parameters. *P. phillyreifolia* 'Little Leaf' also had the lowest fructans (0.26%) but the differences were not significant. On the other hand in parameters related to toughness, *P. phillyreifolia* 'Little Leaf' was significantly higher than all the other taxa. It had the highest acid detergent fiber (ADF) (39.76%) and acid detergent lignin (ADL) (17.02%) on dry matter basis which were both significantly higher than 31.16% ADF and 11.89% ADL in *P. japonica* 'Dodd's Sugar Run Falls'

which were the lowest values. *P. japonica* 'Cavatine' and *P. japonica* 'Temple Bells' were slightly higher in ADF and ADL than *P. japonica* 'Dodd's Sugar Run Falls', but they were still significantly lower than *P. phillyreifolia* 'Little Leaf'. Cellulose on dry matter basis was also significantly higher in *P. phillyreifolia* 'Little Leaf' (22.75%) for which the lowest value was 18.5% in *P. japonica* 'Prelude', and this was not significantly different from the other three taxa. Among the nutrients on 'as received' basis (Table 3.10) WSC, ESC, fructans, NSC and starch were not significantly different among the taxa. ADF, ADL and cellulose were significantly different, with *P. phillyreifolia* 'Little Leaf' again showing highest contents of all three (20.4%, 8.73% and 11.6% respectively). Moisture was significantly low in *P. phillyreifolia* 'Little Leaf' (48.69%) as opposed to *P. japonica* 'Cavatine' which had the highest moisture (59.55%). The other three taxa were similar with respect to moisture content.

All mineral concentrations were significantly different among the *Pieris* taxa (Table 3.11). Among the major mineral nutrients N, P and K, *P. phillyreifolia* 'Little Leaf' had highest N content (1.29%) and this was not significantly different from that in *P. japonica* 'Prelude' (1.13%), while the other three taxa were on par. P and K were highest in *P. japonica* 'Temple Bells' (0.19% and 1.2% respectively) and lowest in *P. phillyreifolia* 'Little Leaf' (0.1% and 0.73% respectively). Ca, Mg and S contents were high in *P. phillyreifolia* 'Little Leaf' and they were on par with *P. japonica* 'Prelude' and *P. japonica* 'Dodd's Sugar Run Falls'. *P. japonica* 'Dodd's Sugar Run Falls'. Al content in *P. phillyreifolia* 'Little Leaf' was low and on par with that in *P. japonica* 'Temple Bells' and *P. japonica* 'Cavatine', whereas in the case of B content, *P.*

phillyreifolia 'Little Leaf' was again low and on par with Cavatine, but significantly different from that in *P. japonica* 'Temple Bells' which had the highest B content. Cu content was highest in *P. japonica* 'Prelude' which was on par with *P. japonica* 'Cavatine' and *P. phillyreifolia* 'Little Leaf'. Mn and Na contents were both significantly high in *P. phillyreifolia* 'Little Leaf' than all the other taxa. Fe and Zn content were highest in *P. japonica* 'Prelude' and low in *P. phillyreifolia* 'Little Leaf'. The micronutrients Cd, Cr, Mo, Ni and Pb were at equally low levels in all the taxa and hence were not statistically analyzed.

LEAF ULTRASTRUCTURE. Tables 3.12, 3.13 and 3.14 give measurements of ultrastructural parameters of the selected *Pieris* taxa and Table 3.15 presents the analysis of variance of these parameters. There were no significant differences among the taxa with respect to thickness of the epidermal layers (Plate 3.1), but there were significant differences in the stomatal number and size (Plate 3.2). *Pieris phillyreifolia* 'Little Leaf' had a significantly higher number of stomata per unit area of 100 square microns (17.53), but their size of the stomatal opening was significantly lower (6.07 microns). The numbers of stomata per 100 square microns area was not significantly different between *P. japonica* 'Temple Bells' (9.44) and *P. japonica* 'Variegata' (9.76). However, the stomata in *P. japonica* 'Temple Bells' had the longest apertures (12.2 microns), which were significantly larger than those in *P. japonica* 'Variegata' (9.49 microns) or *P. phillyreifolia* 'Little Leaf' (6.07 microns). We also made some measurements of the thickness of the stylet of *S. takeyai* and found that they averaged between 10 and 11 microns.

Correlation analysis. Some of the nutritional parameters and leaf toughness showed correlation with leaf damage at 0.05 significance level (Table 3.16). Among the nutrients, moisture alone showed significant positive correlation with leaf damage (R=0.58, P=0.022). Starch, WSC, fructans, NSC and ESC (dry) showed trends of positive correlation and ADF, ADL and cellulose showed trends of negative correlation, but these were not significant. Leaf toughness measured by the puncture force in both directions – upper to lower as well as lower to upper surface – showed significant negative correlation with leaf damage. Among the mineral nutrients Ca (R= - 0.83, P = 0.0002) and Mg (R = - 0.84, P<0.0001) and Al (R=-0.52, P=0.048) showed significant negative correlation with leaf damage, whereas P (R= 0.69, P=0.004) and K (R=0.84, P=0.0001) showed significant positive correlation. The other mineral nutrients were not significant positive correlation. The other mineral nutrients were not significant positive correlation.

Discussion

In the above described studies, we have made preliminary assessment of some mechanisms for their potential role in resistance exhibited by *Pieris* taxa to *S. takeyai*. We first chose to evaluate leaf wax because its effect on the related species *S. pyrioides* was established in azaleas, which also belong to the same plant family (Ericaceae) as pieris. In studies with the wax extracts, it was found that extracts from resistant cultivars imparted resistance to susceptible genotypes and vice versa, which indicated that leaf wax serves as a primary mechanism of resistance in deciduous azalea to *S. pyrioides* (Chappell and Robacker 2006). The procedure that we used for extracting the leaf surface lipids from *Pieris* taxa were similar to those used by Chappell and Robacker (2006) with suitable modifications. Chloroform has also been reported as the most
efficient waxsolvent that penetrated to the cell, but left the cell membrane intact (Nigg et al. 1981). However our first set of assays with the chloroform-extracted leaf surface materials from the selected pieris leaves did not indicate any effect on the lace bugs. The recipient of the extract had a more significant effect on leaf damage and nymph emergence as compared the donor and these results were similar to those on untreated leaves that we have observed in our screening experiments. One of the possible reasons for our observations in the previous trial could be the method used for wax extraction. According to the procedure followed, the dried leaves were immersed in chloroform for 15 seconds and then the chloroform was evaporated off. This duration was probably not sufficient to extract a substantial amount of leaf wax. Another speculation is that we used two different species of *Pieris* viz. *P. japonica* and *P. phillyreifolia* which may have two totally different mechanisms in operation at the plant-insect interface. Comparison of two varieties belonging to the same species of Pieris e.g., P. japonica var. 'Temple Bells' and *P. japonica* var. 'Variegata' would probably give different results. Therefore, we modified our experiment to accommodate these points and repeated the study twice (referred to as B1 and B2). However the second set of assays also revealed that the wax extract or solvent painted on a leaf did not matter but it was the substrate that determined the preference of the lace bugs. In our third set of assays (Assay C) we tried fresh leaves instead of dry ones, since brief extractions (10-60 s) of fresh foliage at room temperature are also recommended for surface lipids extraction (Eigenbrode and Espelie 1995). The results of these assays with the chloroform-extracted material from the leaf surfaces confirmed our observations that *Pieris* leaf wax was not a primary mechanism of defense against S. takeyai.

The ineffectiveness of epicuticular wax in deterring herbivores has been observed earlier. For example, pests like flea beetles (*Phyllotreta* Alticini spp.) caused more damage on glossy *B. oleracea* L. than on normal cultivars (Bodnaryk 1992) whereas glossy cultivars did not support other pests like aphids and diamondback moth (Stoner 1990). Thus, in addition to the physical structure of the cuticle, factors like chemical composition, polarity and post-ingestive effects of cuticular components may also influence herbivore responses to the plant (Eigenbrode and Espelie 1995).

We had noted differences in the physical features of the pieris leaves during our screening experiments. P. phillyreifolia leaves were always tough and brittle to the touch as compared to leaves of the other species like P. japonica or P. taiwanensis which were more flexible. Evaluation of toughness using the force gauge was based on these observations, and this helped to quantify our observations that *P. phillyreifolia* leaves were harder to puncture, and this could be one of the reasons for their avoidance by the lace bugs. Leaf toughness is a widespread mechanism of resistance in plants to herbivory, and it has been widely observed and studied, also within the plant family Ericaceae (Meyer and Ballington 1990). Increased toughness in leaves and other plant parts is seen to increase the difficulty in obtaining as well as digesting and absorbing food by the young larvae, which affected the size, weight, performance and survival of the later instars (Slansky 1990), which may be the reason for avoidance of tough plant parts by herbivores. Like toughness, presence of dense pubescence on leaves not only hinders feeding by adults and larvae but also causes behavioral changes by inducing frequent movement to more suitable sites. However, all varieties of pieris that we examined lacked pubescence in the form of hairs or trichomes. Microscopic examination revealed

occasional peg-like structures on the adaxial leaf surface, but their role in defense against herbivory could not be ascertained.

Toughness in plants is primarily attributed to cellulose in the cell wall (cellulose microfibers set in a hemmicellulose or lignin matrix) which is indigestible to most phytophagous insects (Martin 1991; Lucas et al. 2000). In addition to affecting digestibility, cellulose and other components of plant cell walls like lignin, cutin and minerals like silica also impart different mechanical properties to the plant surface. This is also known to cause wearing of herbivore mouthparts (Hochuli 1996). The term 'fiber' does not imply a chemical like cellulose but the cell wall itself, and the composition of fiber varies greatly (Mattson and Scriber 1987). The composite cell wall can be up to ten times tougher its individual components (Lucas et al. 2000). Therefore individual components of the cell wall (like cellulose) have been the focus of studies with digestive enzymes regarding their indigestibility to insects (Martin 1991). There are various methods for evaluating toughness of plant parts and laboratory measurements of toughness have been made in several broad-leaved plants (Lucas and Pereira 1990). Penetrometers are commonly used although they do not directly measure toughness, but the force or energy required to puncture or penetrate a material (Lucas and Pereira 1990; Lucas et al. 1991). This is difficult in field conditions, but approximations may be obtained by using corrections for leaf lamina thickness (Choong et al. 1992).

Analysis of leaf samples for nutrient contents revealed that the *Pieris* taxa were significantly different with respect to many of the variables. This information was not available earlier. It is well known that phytophagous insects and other arthropods depend on their host plants for moisture and nutrients. Among the nutrients, moisture was

significantly low in *P. phillyreifolia*, which showed good resistance to *S. takeyai* whereas P. japonica 'Cavatine' which was highly susceptible had the highest moisture content. Therefore, low moisture could be one of the reasons for the resistance shown by P. phillyreifolia to S. takeyai. Water is considered as a macronutrient by some researchers and often ignored by others, but its importance is universally recognized. Foliage feeding insects generally require a high moisture intake and low leaf water may be more limiting for them than low protein content (Barbehenn et al. 1999). In some plants even the presence of urticant hairs did not deter defoliation in young leaves with higher moisture content (Cano-Santana and Oyama 1992). Water content in plants has marked effects on their utilization as food by insects (Scriber 1977; Reese and Beck 1978) and changes in moisture content of leaves can alter the preferences of insects feeding on them (Mattson 1980; Scriber and Slansky 1981). Water stress in plants is generally thought to predispose them to insect damage. A study on inducing leaf water deficits in crucifers found that the induced stress led to increased defoliation by chewing and leaf-mining insects, but not by sap-feeders (Louda and Collinge 1992). Conversely, many herbivores are negatively affected by prolonged water stress. This may be because even though stress increases nitrogen levels, the herbivore's ability to utilize it is reduced by the loss in turgor pressure (Landsberg and Wylie 1983; Huberty and Denno 2004). Decreased dietary water affects nutrient digestion and absorption. Further, the absorbed nutrients may be diverted to produce metabolic water instead of body mass. Energy costs to excrete excess nutrients may also be increased (Slansky and Scriber 1985). Overall, woody plants usually have significantly lower moisture levels and associated structural and chemical features like thicker cell walls and possibly higher levels of allelochemics

such as tannins, which reduce their attractiveness to insect herbivores (Feeny 1976). However not all studies were able to correlate plant palatability with leaf moisture content, nitrogen or energy (Howard 1987). In evaluations of azalea resistance to *S. pyrioides*, leaf water content was significantly different among taxa but it was not correlated with azalea lace bug performance (Wang et al. 1998b). Moisture is also an important cue used by insects to determine the suitability of a host for oviposition and to support their developing young (Jaenike 1978; Craig and Ohgushi 2002). This may also explain the absence of nymph emergence in *P. phillyreifolia*. Female insects are observed to choose oviposition sites near preferred feeding sites and sites that are less prone to desiccation. On water-stressed plants, females moved their oviposition sites from the leaf lamina to the petioles (Seagraves et al. 2011).

Nutrients in insect diets are similar to those required by plants and vertebrates, and are commonly grouped as macronutrients (protein, carbohydrates, lipids, water) and micronutrients (vitamins, minerals, trace elements) (Barbehenn et al. 1999). Utilization of different nutrients depends on the insect's abilities to digest complex forms to simpler absorptive forms and on the degree of absorption (House 1961). For example, phytophagous insects vary in their ability to utilize starch (Friend 1958). Among the *Pieris* taxa analyzed, *P. phillyreifolia* was significantly low in starch and carbohydrates, whereas it was highest in fiber, lignin and cellulose. These factors point to a low nutritive value for herbivores.

The quantitative mineral nutrient requirements for insects are largely unknown (Barbehenn et al. 1999). Essential elements have been identified for certain insects like *Drosophila melanogaster* Meigen, *Blatella germanica* L. and *Tenebrio molitor* L. (House

1961). Among the mineral nutrients, the primary nutrients are nitrogen, potassium and phosphorus. Nitrogen (N) plays a pivotal role in physiological processes in all organisms and hence it is a critical and limiting factor growth. The importance of N is mainly as a major building block of proteins, enzymes and amino acids. Protein quality is a determining factor in host plant suitability for insects and can significantly affect herbivore performance (Felton 1996). N and water content are usually positively correlated and therefore it is difficult to separately identify the effects of each (Mattson 1980). However low nitrogen content is reported to prolong feeding and growing periods and these predispose the insects to biotic and abiotic mortality factors. Therefore the indirect effects of low N are negative for all herbivores (Mattson 1980). In some cases herbivores preferred foliage with higher N and phosphorus (P), even though it was covered with dense trichomes (Cano-Santana and Oyama 1992). Low N (often accompanied by decreased water content and increased leaf toughness) discouraged feeding in several herbivores (Kraft and Denno 1982). However although P. phillyreifolia had the highest N content among the tested taxa, its low moisture may be the reason why it could not be utilized by the lace bugs.

Potassium (K) promotes the development of thick outer walls in epidermal cells and thus has an essential role in tissue hardening and stomatal opening patterns, which can influence feeding activity of sap sucking insects. Phosphorus (P) is part of many vital organic molecules like DNA and RNA, but its effect on herbivore performance is variable (Marschner 1995). An experiment comparing the response of alfalfa varieties that were resistant or susceptible to aphids, to various levels of nutrients found that phosphorus deficiency increased aphid resistance in some varieties whereas potassium

deficiency decreased resistance and nitrogen had no effect on resistance (McMurty 1962). Likewise in the case of *P. phillyreifolia* which had the lowest P and K and *P. japonica* 'Temple Bells' which had the highest of both, the effects of these nutrients seemed to be variable. The high Ca content of *P. phillyreifolia* is supported by the role of Ca in the toughness of plants. Ca is reported to be important for the stability and function of plant membranes and cell wall structure (Marschner 1995) and thereby may influence herbivory. Ca accumulating in the tissues of certain plants in the form of calcium oxalate crystals is reported to protect conifer bark against wood boring insects (Hudgins et al. 2003). These star shaped crystals (raphides) have been recorded in several plant families, seen grouped as bundles within the plant cells (Franceschi and Nakata 2005) and protect foliage from invertebrate defoliators (Korth et al. 2006). P. phillyreifolia was also high in Mn, which seems to be explained well because Mn is essential in lignin and suberin biosynthesis (Römheld and Marschner 1991) and therefore may have a role in toughness. B also has a direct function in promoting stability and rigidity of the cell wall structure and therefore supports the shape and strength of the plant cell (Marschner 1995; Brown et al. 2002). B is also involved in the metabolism of phenolics or lignin and in the integrity of the plasma membrane (Blevins and Lukaszewski 1998; Brown et al. 2002; Dordas and Brown 2005). However *P. phillyreifolia* was significantly low in B, whereas the susceptible variety *P. japonica* 'Temple Bells' had the highest B content. Among the other minerals, Na and Fe are also considered essential nutrients because many basic biological functions depend on them (Barbehenn et al. 1999). Mg, S and Cu which function as cofactors for specific enzymes have been found to be essential for several insects. Zinc is essential in protein and starch synthesis, and therefore a low zinc

concentration induces accumulation of amino acids and reducing sugars in plant tissue (Marschner 1995; Römheld and Marschner 1991), which in turn may affect plant suitability to insects. However the roles if any, played by these minor nutrients and trace elements in the resistance shown by some *Pieris* taxa remains to be ascertained. Correlation analysis of the nutritional and structural parameters of the *Pieris* taxa with leaf damage showed that very few of the correlations were significant, but most of them were as expected. For example the nutrients like starch and carbhohydrates were positively correlated to leaf damage, while fiber, lignin and cellulose were negatively correlated.

Examination of the ultrastructure of the leaves provided valuable insights into the actual differences between the selected *Pieris* taxa. Measurements of the epidermal layers in thin sections of the leaves showed that the resistant varieties did not have thicker epidermal or cuticular layers. However the differences in the number and size of stomata indicate that these parameters could definitely have a role in feeding by the lace bugs. It has been proved earlier that lace bugs feed by inserting their stylets into the leaf through the stomata (Buntin et al. 1996) and we have also observed this (Plate 3.3). Our measurements of the bug's stylets suggest that they are too thick to be inserted through the stomata of *P. phillyreifolia* leaves which are also tough so that they may not be able to stretch open. On the other hand the stomata in *P. japonica* 'Temple Bells' are much larger and since the leaf itself is more flexible, there are more chances for the lace bugs to feed on this variety by inserting their stylets as well as stretching open the stomata. It has been reported earlier that azalea cultivars also vary in stomata size but were not correlated with feeding preference of *S. pyrioides* (Kirker et al. 2008).

The resistance shown by *Pieris* taxa to *S. takeyai* is most likely a combination of different mechanisms among which leaf moisture, toughness and stomatal characters appear to have significant influence. These factors suggest antixenosis to be the type of resistance exhibited combined with antibiosis because we observed increased mortality as well as reduced adult survival and reproduction on the resistant taxa. Further detailed examination of these parameters in all available *Pieris* taxa needs to be taken up to confirm these findings. The role of plant secondary chemicals cannot be disputed, especially because there have been previous reports of toxic substances identified from *Pieris* species like asebotoxin III, a diterpenoid from the leaves of *P. japonica* 'Asebi' (Takeya et al. 1981) and grayanoids from *P. formosa* which possess antifeedant and insecticidal properties (Ding et al. 1998, Wang et al. 1998a). Also, the chemical composition of leaf-surface lipids needs to be examined further to determine why the extracts did not affect *S. takeyai*. However the analysis of such plant chemicals was beyond the scope of this study and needs to be taken up in future research.

Our studies detailed above provide new information on some of the possible mechanisms of resistance exhibited by *Pieris* to the lace bug *S. takeyai*. A lot more work has been done on related plants like azaleas and their pests like *S. pyrioides* and similar results would be expected, but the responses were different in many cases. Although we have not been able to pinpoint the primary mechanism of resistance, our studies can provide directions for future work on these lines. Information from such studies will be of use when screening germplasm of ornamental plants for lace bug resistance and also to plant breeders in developing hybrids desirable for long term landscape situations.

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		Al			A2	
Treatments	No. of	Leaf	No. of	No. of	Leaf	No. of
	live	damage*	nymphs	live bugs	damage*	nymphs
	bugs					
T1 P. p. extract on P. j. 'Temple Bells'	1.0 a	3.47 b	5.7 a	0.33 b	3.39 b	5.42 a
T2 P. j. 'Temple Bells' extract on P. p.	0.0 b	0.0 c	0.0 c	0.0 b	0.0 c	0.0 b
T3 P. p. extract on P. phillyreifolia	0.0 b	0.17 c	0.0 c	0.17 b	0.28 c	0.0 b
T4 P. j. 'Temple Bells' extract on P. j. 'Temple Bells'	0.83 a	2.47 b	0.81 c	1.0 a	4.34 ab	2.47 b
T5 Solvent only on <i>P. p.</i>	0.0 b	0.11 c	0.0 c	0.0 b	0.06 c	0.0 b
T6 Solvent only on P. j. 'Temple Bells'	1.33 a	5.33 a	3.2 b	1.0 a	5.64 a	2.42 b
F	5.77	35.17	12.1	5.27	17.12	6.14
Р	0.0011	< 0.0001	< 0.0001	0.0019	< 0.0001	0.0008

Table 3.1. Adult survival, leaf damage and nymph emergence in pieris leaves treated with pieris leaf wax extracts A1

		A	1			Α	.2	
	Dam-t	Dam-nt	Nym-t	Nym-nt	Dam-t	Dam-nt	Nym-t	Nym-nt
D1- Pieris phillyreifolia	1.28 b	2.36 a	1.92 a	3.78 a	1.39 a	2.28 a	1.36 a	4.06 a
D2- <i>P</i> . <i>j</i> . 'Temple Bells'	0.89 b	1.58 a	0.31 a	0.5 b	2.14 a	2.28 a	1.0 a	1.47 b
D3- Solvent	2.92 a	2.53 a	1.14 a	2.06 ab	2.78 a	2.92 a	1.39 a	1.03 c
F	12.17	1.96	3.2	5.12	2.71	0.81	0.09	3.44
P	0.0002	0.1617	0.0579	0.0137	0.0857	0.4581	0.9142	0.048
R1- Pieris phillyreifolia	0.11 b	0.07 b	0.0 b	0.0 b	0.04 b	0.19 b	0.0 b	0.0 b
R2- <i>P</i> . <i>j</i> . 'Temple Bells'	3.28 a	4.24 a	2.24 a	4.22 a	4.17 a	4.74 a	2.5 a	4.37 a
F	78.97	100.35	18.57	25.47	71.73	80.39	8.96	18.43
P	<.0001	<.0001	0.0002	<.0001	< 0.0001	< 0.0001	0.0061	0.0002
D*R								
$oldsymbol{F}$	9.92	1.74	3.2	5.12	3.18	1.25	0.09	3.44
<i>P</i>	0.0007	0.1965	0.0579	0.0137	0.0586	0.3046	0.9142	0.048

Table 3.2. Leaf damage and nymph emergence on treated and untreated leaf surfaces of pieris leaves treated with pieris leaf wax extracts

Means in the same column section bearing different letters are significantly different ($\alpha = 0.05$; LSD).

The abbreviations Dam-t = Leaf damage on treated side of leaf, Dam-nt = Leaf damage on untreated side of leaf, Nym-t = Nymphs emerged on treated side of leaf, Nym-nt = Nymphs emerged on untreated side of leaf, D = donor (of leaf wax), R = recipient (of leaf wax)

	Extraction times:		15 seconds			30 seconds	
Tre	atments	No. of	Leaf	No. of	No. of	Leaf	No. of
		live bugs	damage*	nymphs	live bugs	damage*	nymphs
T1	P. p.extract on P. j. 'Temple Bells'	0.33 bc	12.78 b	4.78 a	0.5 ab	12.45 ab	7.83 a
T2	P. j. 'Variegata' extract on P. j. 'Temple Bells'	1.17 a	20.44 a	4.33 a	0.67 a	12.61 ab	6.39 ab
Т3	P. j. 'Temple Bells' extract on P. j. 'Temple Bells'	0.67 b	15.7 ab	4.25 a	0.67 a	15.53 a	5.64 a-c
T4	P. j. 'Temple Bells' extract on P. p.	0.0 c	0.06 c	0.0 c	0.0 b	0.06 c	0.0 d
T5	P. j. 'Variegata' extract on P. p.	0.0 c	0.22 c	0.0 c	0.0 b	0.08 c	0.0 d
T6	P. p.extract on P. p.	0.0 c	0.14 c	0.0 c	0.0 b	0.11 c	0.0 d
T7	P. p.extract on P. j. 'Variegata'	0.0 c	2.78 c	0.89 bc	0.0 b	4.33 c	1.19 b-d
T8	P. j. 'Temple Bells' extract on P. j. 'Variegata'	0.0 c	3.73 c	0.75 c	0.0 b	6.36 bc	3.36 a-d
Т9	P. j. 'Variegata' extract on P. j. 'Variegata'	0.0 c	3.2 c	0.72 c	0.33 ab	5.5 bc	0.61 cd
T10	Solvent only on <i>P. j.</i> 'Temple Bells'	0.0 c	4.8 c	4.09 ab	0.5 ab	14.75 a	5.2 a-d
T11	Solvent only on <i>P</i> . <i>p</i> .	0.0 c	0.03 c	0.0 c	0.0 b	0.22 c	0.0 d
T12	Solvent only on P. j. 'Variegata'	0.0 c	4.28 c	2.06 a-c	0.0 b	5.67 bc	0.94 b-d
F		5.26	6.49	3.01	1.98	5.61	2.24
Р		<.0001	<.0001	0.0030	0.0465	< 0.0001	0.0233

Table 3.3. Adult survival, leaf damage and nymph emergence in pieris leaves treated with pieris leaf wax extracts prepared using two extraction times (Averages from assays B1&B2)

* Number of frass spots

		15 sec	conds			30 seco	nds	
	Dam-t	Dam-nt	Nym-t	Nym-nt	Dam-t	Dam-nt	Nym-t	Nym-nt
D1- Pieris phillyreifolia	4.59 a	5.87 a	2.04 a	1.74 a	5.33 a	5.93 a	2.24 a	3.78 a
D2- <i>P.j.</i> 'Variegata'	7.67 a	8.24 a	1.67 a	1.71 a	5.65 a	6.48 a	2.11 a	2.56 a
D3- <i>P.j.</i> 'Temple Bells'	5.72 a	7.26 a	1.3 a	2.04 a	6.54 a	8.09 a	2.48 a	3.52 a
D4- Solvent	3.72 a	4.26 a	2.39 a	1.71 a	5.98 a	7.78 a	1.71 a	2.39 a
F	1.26	0.97	0.50	0.05	0.13	0.45	0.09	0.29
P	0.2967	0.4152	0.6855	0.9871	0.9411	0.7201	0.9660	0.8343
R1- Pieris phillyreifolia	0.14 b	0.08 b	0.0 b	0.0 b	0.13 c	0.11 c	0.0 b	0.0 b
R2- P.j. 'Variegata'	3.03 b	3.96 b	1.21 b	1.01 b	5.14 b	5.79 b	1.17 b	1.89 b
R3- <i>P.j.</i> 'Temple Bells'	13.11 a	15.18 a	4.33 a	4.39 a	12.36 a	15.31 a	5.24 a	7.29 a
F	26.86	26.41	14.94	12.22	24.99	32.95	8.42	11.51
P	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0006	<.0001
D*R								
F	1.34	1.21	0.28	0.29	0.11	0.21	0.22	0.31
Р	0.2530	0.3153	0.9446	0.9382	0.9951	0.9733	0.9672	0.9270

Table 3.4. Leaf damage and nymph emergence on treated and untreated leaf surfaces of pieris leaves treated with pieris leaf wax extracts prepared using two extraction times (Average values from assays B1&B2)

Means in the same column section bearing different letters are significantly different ($\alpha = 0.05$; LSD).

The abbreviations Dam-t = Leaf damage on treated side of leaf, Dam-nt = Leaf damage on untreated side of leaf, Nym-t = Nymphs emerged on treated side of leaf, Nym-nt = Nymphs emerged on untreated side of leaf, D = donor (of leaf wax), R = recipient (of leaf wax), P.j. = Pieris japonica

]	B1]	B2			
	F	Р	F	Р			
Adult survival							
Pieris taxa	3.96	0.0005	7.24	< 0.0001			
Extrn time	1.96	0.1685	0.07	0.7927			
Pieris taxa* Extrn	1.69	0.1058	0.37	0.9594			
time							
Model	<i>P</i> =0	0.0002					
	df=	25,46	df=	25,46			
	F=	= 2.6	F=	3.39			
Leaf Damage							
Pieris taxa	10.19	< 0.0001	12.88	< 0.0001			
Extrn time	13.22	0.0007*	0.25	0.6187			
Pieris taxa* Extrn	2.2	0.0309	1.14	0.3574			
time							
Model	P<0	0.0001	<i>P</i> =0	P=0.0024			
	df=	25,46	df=	25,46			
	F=	6.16	$F^{=}$	= 2.6			
Nymph emergence							
Pieris taxa	2.46	0.0164	5.12	< 0.0001			
Extrn time	1.55	0.2188	0.91	0.3459			
Pieris taxa* Extrn	2.43	0.0177	0.35	0.9693			
time							
Model	<i>P</i> =0	0.0072	<i>P</i> =0	0.0024			
	df=	25,46	df=	25,46			
	$F^{=}$	= 2.3	$F^{=}$	= 2.6			

Table 3.5. Analysis of variance in wax assay B showing treatment effects on adult survival and leaf damage.

	30 second	extraction	5 minute e	xtraction
Treatments	No. of live bugs	Leaf damage*	No. of live bugs	Leaf damage*
P. p.extract on P. j. 'Temple Bells'	1.33 a	15.33 b	1.67 a	22.92 ab
P. j. 'Variegata' extract on P. j. 'Temple Bells'	1.67 a	27.92 a	1.33 a	16.83 b
<i>P. j.</i> 'Temple Bells' extract on <i>P. j.</i> 'Temple Bells'	2.0 a	34.92 a	1.67 a	23.08 a
P. j. 'Temple Bells' extract on P. p.	0.0 a	0.0 c	0.33 a	0.58 d
P. j. 'Variegata' extract on P. p.	0.0 a	0.17 c	0.0 a	0.67 d
P. p.extract on P. p.	0.0 a	0.08 c	0.0 a	0.67 d
P. p. extract on P. j. 'Variegata'	0.33 a	7.75 bc	1.0 a	7.92 c
P. j. 'Temple Bells' extract on P. j. 'Variegata'	1.33 a	11.75 bc	0.67 a	7.42 c
P. j. 'Variegata' extract on P. j. 'Variegata'	0.33 a	7.33 bc	0.67 a	9.08 c
F	1.79	9.93	1.63	15.83
Р	0.1526	< 0.0001	0.1932	< 0.0001

Table 3.6. Adult survival and leaf damage in pieris leaves treated with pieris leaf wax extracts prepared using two extraction times (Averages from assay C)

* Number of frass spots

		30 second	extraction	5 minute	extraction
		Dam-t	Dam-nt	Dam-t	Dam-nt
D1- Pieris phillyreifolia		12.45 a	11.33 a	10.78 a	10.22 a
D2- <i>P.j.</i> 'Variegata'		8.22 a	7.0 a	8.11 a	9.61 a
D3- <i>P.j.</i> 'Temple Bells'		12.89 a	18.28 a	8.11 a	12.61 a
	F	1.11	2.87	2.01	0.81
	P	0.3524	0.0858	0.1666	0.4613
R1- Pieris phillyreifolia		0.11 b	0.06 c	0.33 c	0.94 c
R2- P.j. 'Variegata'		7.22 b	10.67 b	7.72 b	8.56 b
R3- <i>P.j.</i> 'Temple Bells'		26.22 a	25.89 a	18.94 a	22.94 a
	F	30.6	14.97	74.41	40.35
	P	< 0.0001	0.0002	< 0.0001	< 0.0001
D^*R					
	F	0.62	2.12	2.43	1.55
	P	0.6562	0.1252	0.0905	0.2367

Table 3.7. Leaf damage on treated and untreated leaf surfaces of pieris leaves treated with pieris leaf wax extracts prepared using two extraction times (Average values from assay C)

Means in the same column bearing different letters are significantly different ($\alpha = 0.05$; LSD).

The abbreviations Dam-t = Leaf damage on treated side of leaf, Dam-nt = Leaf damage on untreated side of leaf, D = donor (of leaf wax), R = recipient (of leaf wax), PP= *Pieris phillyreifolia*, VA= *Pieris japonica* 'Variegata', TB= *Pieris japonica* 'Temple Bells'

Pieris taxa	Direction	n of force
	Upper to lower surface	Lower to upper surface
P. japonica 'Prelude'	1.72 bc	1.99 c
P. japonica 'Cavatine'	1.67 c	1.74 de
P. japonica 'Dodd's Sugar Run Falls'	1.77 bc	1.7 e
P. japonica 'Temple Bells'	1.65 c	1.72 de
P. phillyreifolia 'Little Leaf'	2.38 a	3.46 a
P. phillyreifolia 'Baldwin'	2.46 a	3.4 a
P. japonica 'Dorothy Wycoff'	1.91 b	1.91 cd
P. taiwanensis 'Snow Drift'	1.72 bc	1.65 ef
P. japonica 'Valley Rose'	2.27 a	2.06 bc
P. japonica 'Dodd's Crystal Cascade Falls'	1.46 d	1.47 f
P. japonica 'Variegata'	2.34 a	2.2 b
F	28.07	112.98
Р	<.0001	<.0001

Table 3.8. Leaf toughness measurements in *Pieris* leaves (Force in newtons required to puncture leaf)

Means in the same column bearing different letters are significantly different ($\alpha = 0.05$; LSD).

Таха	Starch	Water	Ethanol	Fructans	Non-	Acid	Acid	Cellulose
		soluble	soluble		structural	detergent	detergent	
		carbs	carbs		carbs	fiber	lignin	
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
<i>P.j.</i> 'Prelude'	3.02 bc	10.51 a	9.93 a	0.69 a	13.53 b	34.8 b	16.31 ab	18.5 c
<i>P.j.</i> 'Cavatine'	6.21 a	10.58 a	9.53 a	1.05 a	16.79 a	34.81 b	15.29 b	19.52 bc
P.j. 'Dodd's Sugar Run Falls'	5.58 a	10.7 a	9.87 a	0.83 a	16.28 ab	31.16 c	11.89 c	19.27 bc
<i>P.j.</i> 'Temple Bells'	4.65 ab	10.4 a	9.51 a	0.89 a	15.04 ab	36.02 b	16.14 b	19.89 b
P. p. 'Little Leaf'	2.35 c	7.83 b	7.68 b	0.26 a	10.19 c	39.76 a	17.02 a	22.75 a
F	5.11	7.43	4.1	0.8	6.63	14.09	13.93	23.55
<u>P</u>	0.0166	0.0048	0.032	0.553	0.0071	0.0004	0.0004	< 0.0001

Table 3.9. Leaf nutritional parameters in Pieris taxa - dry matter basis

Taxa	Starch	Water soluble	Ethanol soluble	Fructans	Non- structural	Acid detergent	Acid detergent	Cellulose	Moisture
		carbs	carbs		carbs	fiber	lignin		
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
<i>P.j.</i> 'Prelude'	1.28 b	4.46 a	4.21 a	0.3 a	5.74 a	14.77 c	6.92 b	7.85 d	57.58 b
<i>P.j.</i> 'Cavatine'	2.53 a	4.28 a	3.86 a	0.43 a	6.81 a	14.07 cd	6.18 c	7.89 d	59.55 a
<i>P.j.</i> 'Dodd's Sugar Run Falls'	2.43 a	4.65 a	4.28 a	0.36 a	7.08 a	13.52 d	5.153 d	8.36 c	56.62 b
<i>P.j.</i> 'Temple Bells'	2.04 ab	4.55 a	4.16 a	0.4 a	6.59 a	15.76 b	7.06 b	8.7 b	56.25 b
<i>P.p.</i> 'Little Leaf'	1.21 b	4.03 a	3.94 a	0.14 a	5.23 a	20.4 a	8.73 a	11.68 a	48.69 c
F	3.49	1.07	0.69	0.61	2.27	148.23	56.0	228.38	60.53
Р	0.0496	0.4206	0.6156	0.6627	0.1333	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 3.10. Leaf nutritional parameters in Pieris taxa – as received basis

Таха	Ν	Р	K	Ca	Mg	S	Al	В	Cu
	(%)	(%)	(%)	(%)	(%)	(%)	(ppm)	(ppm)	(ppm)
<i>P. j.</i> 'Prelude'	1.13 ab	0.14 b	0.98 b	1.15 a	0.2 b	0.13 bc	70.43 a	26.65 b	5.93 a
P. j. 'Cavatine'	1.07 b	0.14 b	1.13 a	0.92 b	0.17 c	0.15 b	48.35 bc	16.63 d	5.45 ab
<i>P. j.</i> 'Dodd's Sugar Run Falls'	0.98 b	0.14 b	0.82 c	1.11 a	0.24 a	0.21 a	61.24 ab	21.43 c	4.93 b
<i>P. j.</i> 'Temple Bells'	0.98 b	0.19 a	1.2 a	0.54 c	0.14 d	0.11 c	39.74 c	37.44 a	4.18 c
P. p. 'Little Leaf'	1.29 a	0.1 c	0.73 c	1.11 a	0.24 a	0.15 b	51.18 bc	19.04 cd	5.53 ab
F	5.9	21.17	46.91	120.37	68.65	15.25	6.12	45.78	9.1
Р	0.0105	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0003	0.0094	< 0.0001	0.0023

Table 3.11. Leaf mineral concentrations in Pieris taxa

Taxa	Fe	Mn	Na	Zn	Cd	Cr	Мо	Ni	Pb
	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)	(ppm)
<i>P. j.</i> 'Prelude'	134.93 a	348.1 c	130.73 c	69.41 a	< 0.4	<1	<1	<2	<5
<i>P. j.</i> 'Cavatine'	120.53 b	121.67 d	177.87 b	34.72 bc	< 0.4	<1	<1	<2	<5
<i>P. j.</i> 'Dodd's Sugar Run Falls'	135.5 a	164.1 d	118.72 c	40.02 b	< 0.4	<1	<1	<2	<5
<i>P. j.</i> 'Temple Bells'	42.02 c	912.0 b	172.53 b	22.85 d	< 0.4	<1	<1	<2	<5
P. p. 'Little Leaf'	44.28 c	1191.67 a	222.87 a	31.43 c	< 0.4	<1	<1	<2	<5
F	272.03	227.93	11.72	43.64					
Р	< 0.0001	< 0.0001	0.0009	< 0.0001					

Table 3.11. Leaf mineral concentrations in *Pieris* taxa (contd.)

Observation	Upper	Upper	Lower	Number of	Length of
#	epidermis	epidermis	Epidermis	Stomata	stomatal
	(mm)	+palisade	(mm)	in 100 sq. u	aperture
	()	(mm)	()	area	(µ)
1	0.025	0.085	0.02	9	14.0
2	0.03	0.07	0.015	10	12.5
3	0.02	0.08	0.02	8	13.0
4	0.025	0.085	0.02	9	11.0
5	0.02	0.075	0.015	11	12.0
6	0.03	0.08	0.025	10	15.5
7	0.025	0.085	0.015	9	13.0
8	0.025	0.08	0.02	8	14.5
9	0.02	0.08	0.025	9	13.0
10	0.03	0.07	0.03	12	15.0
11	0.035	0.075	0.015	8	12.5
12	0.02	0.07	0.02	10	12.0
13	0.02	0.075	0.02	9	14.5
14	0.025	0.075	0.015	11	13.0
15	0.025	0.08	0.015	12	12.5
16	0.02	0.08	0.02	10	12.5
17	0.035	0.085	0.02	9	12.0
18	0.035	0.09	0.015	11	11.0
19	0.03	0.09	0.02	12	11.5
20	0.035	0.08	0.025	10	13.0
21	0.025	0.075	0.02	9	14.5
22	0.03	0.07	0.015	8	13.0
23	0.03	0.075	0.02	9	12.0
24	0.025	0.08	0.025	10	10.0
25	0.035	0.08	0.025	8	12.0
26	0.02	0.075	0.015	7	10.5
27	0.03	0.085	0.015	10	11.5
28	0.025	0.08	0.02	9	10.5
29	0.025	0.07	0.02	8	12.0
30	0.025	0.075	0.02	9	12.5
31	0.03	0.07	0.015	8	11.0
32	0.02	0.08	0.025	9	11.5
33	0.03	0.075	0.025	10	10.5
34	0.025	0.075	0.025	12	12.0
35	0.02	0.085	0.02	8	14.0
36	0.025	0.08	0.015	10	12.0
37	0.03	0.07	0.015	11	12.5
38	0.035	0.075	0.025	9	13.0
39	0.03	0.08	0.02	8	11.0
40	0.025	0.08	0.025	12	10.5
41	0.035	0.085	0.02	/	9.5
42	0.03	0.085	0.02	9	10.5
45	0.025	0.09	0.025	ð 10	11.0
44 15	0.03	0.075	0.015	10	12.3
4J Moon	0.023	0.0794	0.013	0 4444	12 2000
Std day	0.027	0.0780	0.0198	7.4444 1 2577	12.2000
5iu. ucv.	0.0049	0.0037	0.0041	1.3377	1.3731

Table 3.12. Ultrastructural parameters of leaves of Pieris japonica 'Temple Bells'

Observation	Upper	Upper	Lower	Number of	Length of
#	epidermis	epidermis	Epidermis	Stomata	stomatal
	(mm)	+palisade	(mm)	in 100 sq. µ	aperture
		(mm)		area	(μ)
1	0.03	0.07	0.025	19	6.0
2	0.03	0.08	0.015	21	5.0
3	0.035	0.085	0.02	20	5.5
4	0.025	0.07	0.015	18	6.5
5	0.02	0.075	0.015	18	7.0
6	0.03	0.075	0.02	19	6.0
7	0.03	0.08	0.025	20	6.5
8	0.02	0.085	0.015	21	5.5
9	0.02	0.07	0.02	20	5.0
10	0.035	0.07	0.02	21	5.0
11	0.03	0.075	0.02	18	6.0
12	0.025	0.085	0.015	19	5.5
13	0.025	0.08	0.02	17	6.0
14	0.025	0.08	0.025	18	7.0
15	0.03	0.075	0.025	17	5.5
16	0.035	0.085	0.025	15	6.5
17	0.03	0.09	0.02	18	5.5
18	0.035	0.085	0.02	19	5.0
19	0.025	0.075	0.015	17	6.0
20	0.025	0.08	0.03	15	6.5
21	0.035	0.075	0.025	16	7.0
22	0.035	0.075	0.015	17	7.5
23	0.03	0.08	0.02	15	6.0
24	0.035	0.085	0.02	16	5.0
25	0.04	0.085	0.015	18	5.5
26	0.025	0.09	0.02	19	4.5
27	0.035	0.09	0.025	18	5.0
28	0.03	0.085	0.03	20	5.5
29	0.03	0.085	0.025	17	6.5
30	0.025	0.08	0.02	18	6.0
31	0.02	0.085	0.015	15	7.0
32	0.025	0.08	0.02	16	6.5
33	0.035	0.075	0.025	15	7.0
34	0.03	0.075	0.02	14	8.0
35	0.035	0.08	0.015	18	6.5
36	0.03	0.07	0.015	17	7.0
37	0.025	0.08	0.02	20	6.5
38	0.035	0.085	0.025	15	5.5
39	0.02	0.075	0.02	16	5.5
40	0.025	0.08	0.02	14	5.5
41	0.025	0.08	0.015	16	6.0
42	0.03	0.075	0.015	17	6.5
43	0.035	0.09	0.02	18	6.0
44	0.02	0.085	0.015	19	7.5
45	0.035	0.075	0.02	15	6.0
Mean	0.029	0.0798	0.0200	17.5333	6.0667
Std. dev.	0.0054	0.0058	0.0043	1.9377	0.7877

Table 3.13. Ultrastructural parameters of leaves of Pieris phillyreifolia 'Little Leaf'

Observation	Upper	Upper	Lower	Number of	Length of
#	epidermis	epidermis	Epidermis	Stomata	stomatal
	(mm)	+palisade	(mm)	in 100 sq. µ	aperture
		(mm)		area	(μ)
1	0.02	0.08	0.02	10	9.0
2	0.03	0.075	0.015	9	9.5
3	0.035	0.07	0.025	10	10.0
4	0.02	0.08	0.02	12	9.5
5	0.02	0.08	0.03	9	11.0
6	0.025	0.075	0.035	10	9.0
7	0.02	0.08	0.02	8	8.5
8	0.03	0.07	0.015	10	9.0
9	0.03	0.07	0.02	11	11.5
10	0.035	0.08	0.015	8	10.0
11	0.03	0.08	0.015	9	9.0
12	0.025	0.085	0.015	10	9.5
13	0.02	0.085	0.015	10	8.5
14	0.02	0.085	0.03	12	9.0
15	0.035	0.085	0.025	8	9.0
16	0.035	0.075	0.02	12	9.5
17	0.03	0.07	0.015	8	10.0
18	0.03	0.08	0.02	10	10.5
19	0.025	0.075	0.025	9	9.0
20	0.03	0.075	0.025	11	9.5
21	0.025	0.07	0.02	10	8.5
22	0.02	0.08	0.015	11	9.5
23	0.025	0.085	0.025	9	9.5
24	0.03	0.075	0.02	10	9.0
25	0.025	0.08	0.02	10	9.0
26	0.02	0.07	0.02	9	10.0
27	0.03	0.075	0.015	9	10.5
28	0.035	0.075	0.02	10	10.0
29	0.025	0.08	0.025	8	9.5
30	0.03	0.075	0.015	10	10.0
31	0.025	0.08	0.02	9	11.0
32	0.03	0.085	0.025	10	9.5
33	0.025	0.075	0.02	8	8.5
34	0.025	0.08	0.015	12	9.0
35	0.025	0.08	0.02	11	9.5
36	0.025	0.09	0.02	9	9.0
37	0.03	0.09	0.025	10	9.0
38	0.025	0.085	0.015	8	8.5
39	0.02	0.09	0.015	9	11.0
40	0.025	0.075	0.02	10	9.5
41	0.025	0.075	0.02	12	10.0
42	0.035	0.08	0.025	11	9.0
43	0.02	0.085	0.025	9	9.0
44	0.03	0.09	0.015	10	9.5
45	0.025	0.09	0.02	9	9.0
Mean	0.0267	0.0793	0.0203	9.7556	9.4889
Std. dev.	0.0049	0.0060	0.0048	1.1900	0.7268

Table 3.14. Ultrastructural parameters of leaves of Pieris japonica 'Variegata'

Таха	Upper	Upper	Lower	Number of	Length of
	epidermis	epidermis	Epidermis	Stomata	stomatal
	(mm)	+palisade	(mm)	in 100 sq. µ	aperture
		(mm)		area	(μ)
P. japonica 'Temple Bells'	0.027 a	0.079 a	0.19 a	9.44 b	12.2 a
P. phillyreifolia 'Little Leaf'	0.029 a	0.08 a	0.02 a	17.53 a	6.07 c
P. japonica 'Variegata'	0.026 a	0.079 a	0.02 a	9.76 b	9.49 b
F	2.78	0.50	0.18	404.25	419.55
P	0.0655	0.6052	0.8347	< 0.0001	< 0.0001

Table 3.15. Analysis of variance in ultrastructural parameters of Pieris taxa

Means in the same column bearing different letters are significantly different ($\alpha = 0.05$; LSD).

Parameter	R	Р
Moisture	0.58	0.022
Starchdry	0.29	0.279
WSCdry	0.42	0.117
Fructansdry	0.34	0.214
NSCdry	0.38	0.158
ESCdry	0.30	0.270
ADFdry	-0.01	0.977
ADLdry	0.21	0.441
Starch	0.22	0.423
WSC	0.11	0.706
Fructans	0.30	0.271
NSC	0.21	0.463
Cellulosedry	-0.29	0.291
ESC	-0.11	0.690
ADF	-0.35	0.201
ADL	-0.15	0.582
Cellulose	-0.48	0.072
Tough U-L	-0.64	0.009
Tough L-U	-0.60	0.017

Table 3.16. Correlation analysis of leaf parameters with leaf damage in *Pieris* taxa

Parameter	R	Р
Са	-0.82	0.0002
Mg	-0.84	< 0.0001
Al	-0.52	0.048
Р	0.69	0.004
Κ	0.84	0.0001
В	0.45	0.093
Na	0.02	0.950
Cu	-0.49	0.060
Fe	-0.18	0.518
Mn	-0.09	0.764
Zn	-0.33	0.233
S	-0.48	0.073
Ν	-0.48	0.074

Table 3.17. Correlation analysis of leaf mineral content with leaf damage in *Pieris* taxa

Plate 3.1. Cross sections of leaves of *Pieris* taxa showing various layers (20X magnification) (A) *Pieris japonica* 'Temple Bells' (B) *P. phillyreifolia* 'Little Leaf' and (C) *P. japonica* 'Variegata'


Plate 3.2 Scanning electron microscope images of adaxial surfaces of leaves of *Pieris* taxa showing stomatal characters (A) *Pieris japonica* 'Temple Bells' (B) *P. phillyreifolia* 'Little Leaf' and (C) *P. japonica* 'Variegata'







CHAPTER 4

HOST PLANT UTILIZATION WITHIN FAMILY ERICACEAE

BY THE ANDROMEDA LACE BUG STEPHANITIS TAKEYAI

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Nair, S. and S. K. Braman

ABSTRACT The preferred and major reproductive host of the Andromeda lace bug Stephanitis takeyai Drake and Maa is the Japanese Andromeda Pieris japonica. S. takeyai is known to exhibit non-obligate seasonal host alternation between its two main hosts, both of which belong to the family Ericaceae. In the United States, the presence of S. takeyai has been reported from several other hosts which are members of different plant families and not closely related. This study was undertaken to evaluate host plant utilization by S. takeyai within the family Ericaceae and also confirm some of the earlier reports. We tested the acceptability of ten ornamental, landscape and fruit plants belonging to the family Ericaceae viz., Rhododendron calendulaceum (flame azalea), Rhododendron 'Hampton Beauty', Rhododendron 'Autumn Empress', Vaccinium arboreum (sparkleberry), Vaccinium virgatum (rabbiteye blueberry), Calluna vulgaris (heather), Kalmia latifolia (mountain laurel) along with the Pieris species P. floribunda (mountain pieris), *Pieris phillyreifolia* (climbing fetterbush) and *P. japonica* 'Temple Bells'(Japanese pieris) to S. takeyai. In no-choice tests adult survival did not vary significantly among the taxa. Maximum leaf damage was recorded on *P. japonica* and *R.* calendulaceum, while slight but non-significant damage was noted on V. arboreum and *Rhododendron* 'Hampton Beauty'. Nymph emergence occurred on *P. japonica* (highest), R. calendulaceum and Rhododendron 'Hampton Beauty'. In multi-choice tests adult presence on leaves did not vary significantly except on day 9. Maximum leaf damage was recorded on *P. japonica*. Several plants like *Rhododendron* and *Vaccinium* spp., which may not be favorable hosts, could still serve as reservoirs for the pest.

KEY WORDS: *Pieris, Rhododendron, Vaccinium, Stephanitis takeyai*, host range, Ericaceae

The Andromeda lace bug, *Stephanitis takeyai* Drake and Maa (1955), is an important pest of the ericaceous ornamental plant *Pieris* D. Don spp. (Johnson and Lyon 1991). *S. takeyai* adults and nymphs feed by piercing the leaf surface and drawing out cell contents, which results in symptoms like yellowish white stipples and blotches on the upper leaf surfaces, while on the lower surfaces oily black frass spots can be seen along with the lace bug colonies. Lace bug feeding also reduces photosynthetic efficiency of the leaves (Buntin et al. 1996). In ornamental plants like pieris which are valued for their foliage as well as flowers even slight damage to leaves can seriously affect the market value. Sometimes lace bug damage can reach severe levels causing premature leaf shedding, drying up of twigs or even the whole plant (Schread 1968).

Polyphagy is considered to be an ancestral character in the genus *Stephanitis*, and monophagous species are supposed to have developed later (Tsukada 1994c). This finding adds to the importance of more studies on *S. takeyai* and its relation to other members of the genus. *S. takeyai* has been reported to be polyphagous in Japan as well as the various countries where it has spread and established, attacking host plants of different unrelated families (Tsukada 1994b). However relatively few studies have been done on *S. takeyai*. The Japanese Andromeda, *Pieris japonica* (Thunb.) D. Don ex G. Don, is the preferred and major reproductive host of *S. takeyai* (Schread 1968) from which the pest derives its common name. In its country of origin Japan, *S. takeyai* is known to exhibit non-obligate seasonal host alternation between *P. japonica* and its other major host, the deciduous shrub *Lyonia elliptica* (Siebold & Zucc.) Hand.-Mazz., both of which belong to the family Ericaceae. The bugs feed on *P. japonica* during the winter and on *L. elliptica* during the summer. If *L. elliptica* is scarce, *S. takeyai* may continue to

feed on *P. japonica* (Tsukada 1994b). *S. takeyai* has also been reported to attack the persimmon tree (*Diospyros kaki* Thunb., Family Ebenaceae, the camphor tree (*Cinnamomum camphora* (L.) J. Presl, Family Lauraceae), the Chinese onise (*Illicium religiosum* Siebold & Zucc., Family Illiciaceae) and other useful plants in Japan (Takeya 1963). The occurrence of *S. takeyai* in Japanese pine stands, on *Pinus densiflora* Siebold and Zucc. and *P. thunbergii* Parl. was noted by Watanabe (1983). This occurrence led to the speculation that the lace bug might become a pest of forestry (MacLeod 1998).

The first report of *S. takeyai* in North America was in 1950 on *P. japonica* plants in Connecticut when it was speculated to become a serious pest of *Pieris* and other ornamental Ericaceae (Bailey 1950). Later, the presence of *S. takeyai* has been recorded in the United States from several other hosts like *Andromeda* sp., *Aperula* sp., *Cinnamomum* sp., *Lindera* sp., *Lyonia* sp., *Pieris* sp. and *Salix* sp. which are members of different unrelated plant families (Drake and Ruhoff 1965). Bailey (1974) recorded the occurrence of *S. takeyai* on the rhododendron, *R. calendulaceum* (Michx.) Torr., when the branches of *P. japonica* and *R. calendulaceum* were contiguous. Wheeler (1977) reported spicebush (*Lindera benzoin*) and sassafras (*Sassafras albidum*) both belonging to family Lauraceae, as hosts of *S. takeyai*. In Poland, plants within the Hippocastanaceae, Magnoliaceae, Rosaceae, Saxifragaceae and Styracaceae are reported to be hosts for *S. takeyai* (Soika and Labanowski 1999).

These reports suggest the polyphagous nature of S. *takeyai* which can be a cause for concern in the context of increasing commercial trade in plant material worldwide. Several plants, which may not be favorable hosts, could still serve as reservoirs for the pest. Hence a proper understanding of the host plant utilization of different plants by *S*.

takeyai would be essential in choosing plants for gardens and landscapes as well as formulating management strategies. We undertook this study to evaluate host plant utilization by *S. takeyai* within the family Ericaceae and also confirm some of the earlier reports of host suitability.

Pieris spp., the major reproductive hosts of *S. takeyai*, are popular broad-leaved evergreen shrubs commonly planted in landscapes and gardens for their attractive foliage and flowers (ARS, USDA 2011a). Japanese pieris (*P. japonica*) native to Japan, is the most common *Pieris* species (Heriteau 2006) and also the most preferred by *S. takeyai* (Schread 1968). Mountain pieris (*P. floribunda* (Pursh) Benth. and Hook. f.) is native to North America, indigenous to the Appalachian mountains of the U.S. extending from Virginia southward into Georgia (Starrett et al. 1996). *P. phillyreifolia* (Hook.) DC. (Climbing fetterbush) is also native to North America and is common in the Southeastern states of Alabama, Florida, Georgia, Mississippi and South Carolina. *P. floribunda* is considered resistant to the pest (Dunbar 1974) and *P. phillyreifolia* was observed to be resistant in our studies (Chapters 2 and 3).

The genus *Rhododendron* L. comprises a large group of over 1,000 species of woody ornamentals mostly known for their showy flowers (ARS, USDA 2011c). Azaleas (*Rhododendron* sp.), native to North America, Europe as well as Asia (Scariot et al. 2007), comprise two of the eight subgenera of the genus (Chamberlain and Rae 1990; Chamberlain et al. 1996) and are among the most widely cultivated ornamental and landscape plants (Raupp and Noland 1984). They are considered key plants in landscapes of the southeastern United States (Raupp et al. 1985). Breeding has resulted in thousands of azalea cultivars with diverse plant and floral characters (Galle 1987;

Salley and Greer 1992). We chose the cultivars *Rhododendron* 'Hampton Beauty' and Rhododendron 'Autumn Empress' based on availability. Azaleas are also severely attacked by different pests among which the azalea lace bug S. pyrioides is predominant (Neal and Douglas 1988). S. takeyai however is not generally listed as a problem to azalea cultivation. Azaleas are widely distributed, but their diversity is threatened by urbanization and related developments in the landscape (Pellett et al. 1991). The flame azalea R. calendulaceum is a species occurring naturally in the mountainous and adjoining regions of the eastern United States (Lee 1965) as a common understory shrub (Gleason and Cronquist 1963). It is characterized by early spring flowering and produces inflorescences with attractive reddish-orange to golden yellow flowers. R. *calendulaceum* has been reported to be susceptible to *S. pyrioides* (Braman and Pendley 1992) and to support populations of *Heterothrips azaleae* Hood (Braman and Beshear 1994) but there are no reports about susceptibility to *S.takeyai*. Native ornamental and landscape plants like *R. calendulaceum* are currently being sought by homeowners with renewed interest which has also increased the availability of planting material (Ault 2003). This change may result in changes in the pest scenario as well, since the native plants may show susceptibility to introduced pests.

Vaccinium L. is a genus comprising over 150 species (ARS 2011) of shrubs producing edible fruit, some of which are of commercial importance like the blueberry *V*. *virgatum* Aiton (rabbiteye blueberry). *V. arboreum* Marshall (sparkleberry) is a widespread and abundant diploid blueberry species native in the southeastern United States (Vander Kloet, 1988). It is important in breeding as a source of vigor and increased drought tolerance, tolerance to higher soil pH and low organic matter (Lyrene

1997, 2011). Several pests are recorded on blueberries (Turner and Liburd 2007) and the thrips *Frankliniella vaccinii* Morgan, *Catinathrips vaccinicola* Nakahara and *Haplothrips rectipennis* Hood have been recorded on *V. arboreum* (Braman et al. 1996). Lace bugs have not been reported on these two species of *Vaccinium*. However *Stephanitis oberti* Kol. has been reported on lingonberry (*V. vitis-idaea* L.) (Paal 2006).

The genus *Calluna* is monotypic, with the low-growing perennial shrub *C*. *vulgaris* (L.) Hull being the only species recorded in this genus (ARS, USDA 2011d). It is native to Europe, Africa as well as temperate Asia but is naturalized in Australasia and North America. *C. vulgaris* is an effective colonist and its successful dominance of heathlands can be attributed to several characteristics, mainly the ability to survive under conventional forms of use and management. An important use for *C. vulgaris* is grazing, especially when the landscape is covered by snow, and sheep and cattle can graze on the tips of its shoots (Gimingham 1989). Major pests of heather include the heather beetle *Lochmaea suturalis* (Thomson) (Chrysomelidae, Coleoptera) (Ladekarl et al. 2001) and various lepidopteran defoliators (Haysom and Coulson 1998). Lace bugs have not been reported on *Calluna* sp.

Kalmia latifolia L. (mountain laurel) comprises an important evergreen component in the deciduous forests of the southern Appalachians (Monk et al. 1985). The sap of *Kalmia* is a source of cytotoxins (Mancini and Edwards 1979) and antifeedant diterpenes (Elnaggar et al. 1980). *K. latifolia* is listed as a host for *S. pyrioides* (Drake and Ruhoff 1965; Stonedahl et al. 1992) and also for *S. rhododendri* Horváth (Bailey 1959), but not for *S. takeyai*. We tested the acceptability of these ten plant species belonging to the family Ericaceae using no-choice as well as choice Petri dish assays.

Materials and Methods

PLANT MATERIALS. The selected ericaceous ornamentals were obtained from plant nurseries near Griffin, GA and elsewhere. Leaves were obtained from plants maintained in three gallon (11.356 liter) and one gallon (3.785 liter) pots in a screen house at the Experiment Station, Griffin, GA. The plants were provided with regular irrigation. Pesticides were not used in the screen house.

LACE BUGS. *S. takeyai* colonies were initiated from a population obtained from Cornell in April 2009. The colonies were housed in plastic containers and maintained on several cultivars of *Pieris* under conditions of $27 \pm 1^{\circ}$ C and a photoperiod of 14:10 (L:D) h. For the assays, known numbers of adults were first collected in plastic tubes using an aspirator and then transferred into the testing Petri dishes using a brush.

NO- CHOICE PETRI DISH ASSAYS. For our no-choice assays, three leaves of a variety placed in a Petri dish with their stalks covered with moist tissue paper constituted one replication. Each variety was replicated three times. Three adult lace bugs were released into each Petri dish. The dishes were arranged in a randomized complete block design and placed under conditions of $27 \pm 1^{\circ}$ C and a photoperiod of 14:10 (L:D) h. Observations on number of bugs alive were taken at 48 hour intervals. After the exposure period the surviving adults were removed and the leaves were assessed for leaf damage using the number of frass spots as an indication of feeding (Buntin et al. 1996). After damage assessment, the leaves were maintained under the same conditions as

during the exposure period and observed daily for emergence of nymphs. This assay was performed twice in December 2010.

MULTI -CHOICE ASSAY. For these assays, leaves were collected from the ten selected ericaceous plants (Table 4.1) as and when required for the assays. Three leaves of each variety were placed as a group with their bases covered with moist tissue paper. Ten such groups of leaves placed in a circular pattern inside a large 30 cm Petri dish constituted one replication and there were three such replications. The leaf groups were arranged randomly within the circular pattern in each replication. Twenty adult lace bugs were released into each Petri dish. All the Petri dishes were placed inside a growth chamber under conditions of $27 \pm 1^{\circ}$ C and a photoperiod of 14:10 (L:D) h. Observations on number of bugs present on each of the leaves was recorded one hour after releasing the bugs, and then again at the start of each observation on the 2^{nd} , 7^{th} , 9^{th} and 13^{th} day. After the 13th day the surviving adults were removed and the leaves were assessed for leaf damage using the number of frass spots as an indication of feeding. After damage assessment, the leaves were placed back in their positions and maintained under the same conditions as during the exposure period and observed daily for emergence of nymphs. Nymphs were counted and removed as and when they were observed.

STATISTICAL PROCEDURES. The experiments used a randomized complete block design. The replications were considered as the block factor. Treatment means were analyzed separately for each kind of trial. Meansof the variables (adult survival, leaf damage and nymph emergence in no-choice assays; adult presence on leaves, leaf damage and nymph emergence in choice assays) were subjected to analysis of variance (ANOVA) using the

general linear model procedure (SAS Institute 2003). Means were separated with Fisher protected least significant difference (LSD) test.

Results

NO- CHOICE PETRI DISH ASSAYS. The taxa showed differences in adult survival on day 7 and 9 after exposure in the first assay (Table 4.1). Highest adult survival was on *P. japonica* 'Temple Bells' on both days. On days 2 and 13 there were no significant differences in adult survival among the taxa. Leaf damage was significantly different among the taxa on all four days of observation. Highest number of frass spots indicative of feeding was seen on *R. calendulaceum* and *P. japonica* 'Temple Bells' on all four days of observation. Highest number of all four days of observation. The progressive number of spots on *R. calendulaceum* on day 2, 7, 9 and 13 were 19.0, 39.89, 47.89 and 51.0 respectively and for *P. japonica* 'Temple Bells' these values were 17.67, 40.33, 47.67 and 52.78 respectively. These two taxa were significantly more damaged than all the other taxa among which slight but non-significant damage was seen on *V. arboreum* and *Rhododendron* 'Hampton Beauty'.

In the repeat assay (Table 4.2) highest adult survival was again on *P. japonica* 'Temple Bells' and this was significantly higher than the other taxa on days 9 and 13. On days 2 and 7 there were no significant differences in adult survival among the taxa. Highest leaf damage indicated by frass spots was seen on *P. japonica* 'Temple Bells' and *R. calendulaceum* on all days of observation. *P. japonica* 'Temple Bells' exhibited 15.67, 35.44, 52.22 and 60.22 spots, respectively on day 2, 7, 9 and 13 whereas *R. calendulaceum* had 14.56, 29.0, 37.89 and 44.22 spots, respectively on these days. The other taxa showed significantly lower amounts of damage. Multiple choice trials indicated the preference of the lace bugs for *P. japonica* 'Temple Bells' (Table 4.3) which received significantly higher leaf damage on all four days of observation (11.67, 21.56, 43.33 and 89.56 spots respectively on day 2, 7, 9 and 13). *R. calendulaceum, V. virgatum* and *V. arboreum* showed slight but non-significant damage which was on par with the other less damaged taxa. Adult presence on leaves did not vary significantly among the taxa (Table 4.4) except on day 9, where the most adults were found on *P. japonica* 'Temple Bells'. Nymph emergence was observed only on *P. japonica* 'Temple Bells' in most cases in both no-choice as well as choice trials (Table 4.5). However *R. calendulaceum*, and *Rhododendron* 'Hampton Beauty' supported nymphs in one replication each and the nymphs on *R. calendulaceum* survived till adulthood.

Discussion

We tested the acceptability of ten ornamental and landscape plants belonging to the family Ericaceae viz., *Rhododendron calendulaceum* (flame azalea), *Vaccinium arboreum* (sparkleberry), *Pieris floribunda* (mountain pieris), *Calluna vulgaris* (heather), *Vaccinium virgatum* (rabbiteye blueberry), *Pieris japonica* 'Temple Bells'(Japanese pieris), *Pieris phillyreifolia* (climbing fetterbush), *Rhododendron* 'Hampton Beauty', *Rhododendron* 'Autumn Empress' and *Kalmia latifolia* (mountain laurel). In our assays we used methodology that has been previously used in studies that examined susceptibility of host plants to lace bugs (Wang *et al.* 1998). We conducted no-choice, as well as choice assays. No-choice tests are valuable tools to assess feeding, survival and acceptance for oviposition by insects in various potential host plants to which the test insect has not been exposed before. These tests are mostly conducted before multi-choice tests or field testing so that plants that are not attacked can be classified as non-hosts (Van Driesche and Murray 2004). Multi-choice assays better represent the natural situation because in nature, the insects may be exposed to several plants simultaneously. A no-choice situation is mostly unnatural and the feeding or oviposition behavior elicited in a herbivore may be forced, in order to prevent starvation or death (Van Driesche and Murray 2004). Therefore both kinds of tests are essential to arrive at accurate conclusions regarding host range.

The USDA ARS GRIN Online Database lists 228 genera in the family Ericaceae and over 3,300 taxa are known (Brummitt 1992; ARS, USDA 2011b). These include several species that occupy predominance in the nursery industry. Over 1,000 species including synonyms are recorded in the genus *Rhododendron* alone (Davidian 1995; ARS, USDA 2011c) and there are numerous varieties under each (Salley and Greer 1992). These species and varieties show adaptations to different agro-climatic zones throughout the world which cannot be strictly demarcated and this increases the risk of spread of pests like *S. takeyai*.

The ten plant species that we chose for our screening assays represent the diversity in the family Ericaceae. Many of these plants are widely cultivated ornamental and landscape plants and some are commercially important for their fruit, while others have importance in breeding. Of these *Pieris* sp. are the natural hosts of *S. takeyai*. However *P. floribunda* and *P. phillyreifolia* were not acceptable to *S. takeyai* for feeding or oviposition and P. *japonica* "Temple Bells' was the most preferred host. We have observed similar reactions in our earlier screening assays with *Pieris* taxa as well (Chapter 2). The high preference for *P. japonica* among the different *Pieris* species

(Schread 1968) as well as the resistance of *P. floribunda* and low preference of *P. floribunda* x *japonica* hybrids to *S. takeyai* (Dunbar 1974) have been reported earlier.

The reaction of S. takeyai to R. calendulaceum was particularly notable because in the no-choice assays it recorded the highest leaf damage comparable to the most preferred taxon P. japonica 'Temple Bells' as well as nymph emergence in one replication. However in the multi-choice assays it recorded very low leaf damage. S. takeyai has been recorded earlier on R. calendulaceum, when its branches were contiguous with those of *P. japonica* (Bailey 1974) but this report does not describe the nature of damage or its pest status on R. calendulaceum. Our observations confirm this report and show that S. takeyai can feed as well as reproduce on R. calendulaceum in conditions when its preferred host is absent. In the presence of its preferred host it may not attack *R. calendulaceum*. The presence of *S. takeyai* on other azaleas has also been reported earlier (Bailey 1951; Wheeler 1977; Neal 1988). Hybrid evergreen azaleas were found to be suitable feeding and breeding hosts for S. takeyai, and the possibility that the pest could develop into a late season threat to azalea production has also been suggested (Neal 1988). In our assays we recorded slight, non-significant damage to both azaleas tested viz., Rhododendron 'Hampton Beauty' and Rhododendron 'Autumn Empress' in no-choice tests and no damage in multi-choice tests. We also recorded nymph emergence in *Rhododendron* 'Hampton Beauty' in one replicate of the no-choice test. Our assays also recorded slight leaf damage on both *Vaccinium* species showing that S. takeyai can potentially survive on Rhododendron and Vaccinium species in conditions where its preferred host is absent.

C. vulgaris and *K. latifolia* recorded very low or no damage in both no-choice and multi-choice assays. Both these plants are known to possess various allelopathic effects (Ballester et al. 1982; Mallik 1987) on other plants, as well as various tannins and other antifeedant principles (Elnaggar et al. 1980; Gonzalez-Hernandez et al. 2003) which may have a role in their avoidance by insects.

Our results document the susceptibility of selected ericaceous plants to *S. takeyai*. This information has not been reported earlier. Information on the host reactions under laboratory conditions will be helpful in selecting hosts for organizing pest surveys to monitor the populations of new and minor pests like *S. takeyai*. The results from our preliminary laboratory studies may be different from those observed in natural conditions owing to presence of other plants or influence of other abiotic factors. However they will be useful in predicting potential hosts of *S. takeyai* and also other *Stephanitis* lace bugs. Further testing of more ericaceous host plants as well as plants of other related and unrelated families mentioned in the literature as potential hosts needs to be taken up, to determine the actual host range of this pest.

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Taxa	Adult survival (Number of adults)			Leaf damage*				
	Day2	Day7	Day9	Day13	Day2	Day7	Day9	Day13
Flame Azalea	2.33 a	1.33 ab	0.67 b	0.67 a	19.0 a	39.89 a	47.89 a	51.0 a
Sparkleberry	2.0 a	0.0 c	0.0 b	0.0 a	0.78 b	4.11 b	5.67 b	7.89 b
Pieris floribunda	1.0 a	0.0 c	0.0 b	0.0 a	0.11 b	0.33 b	0.33 b	0.33 b
Heather	1.33 a	0.0 c	0.0 b	0.0 a	0.0 b	0.22 b	0.22 b	0.22 b
Kalmia latifolia	1.33 a	0.0 c	0.0 b	0.0 a	0.0 b	0.0 b	0.0 b	0.0 b
<i>P.j.</i> 'Temple Bells'	2.67 a	2.0 a	1.67 a	1.0 a	17.67 a	40.33 a	47.67 a	52.78 a
<i>P.p.</i> 'Little Leaf'	1.67 a	0.0 c	0.0 b	0.0 a	0.0 b	0.0 b	0.0 b	0.0 b
Encore Azalea	0.67 a	0.0 c	0.0 b	0.0 a	0.0 b	0.44 b	0.44 b	0.44 b
'Hampton Beauty' Azalea	1.67 a	0.67 bc	0.33 b	0.67 a	0.0 b	5.67 b	7.11 b	8.11 b
Blueberry	1.0 a	0.0 c	0.0 b	0.0 a	0.0 b	0.78 b	0.78 b	0.78 b
F	2.10	6.12	4.45	1.59	7.5	13.65	13.94	14.38
Р	0.09	0.0006	0.0034	0.1916	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 4.1. No-choice assay 1 for adult survival and leaf damage by S. takeyai on ericaceous hosts

*number of frass spots Means in the same column bearing different letters are significantly different ($\alpha = 0.05$). The abbreviations *P.j.* = *Pieris japonica*, *P.p.* = *Pieris phillyreifolia*

Taxa	Adult survival				Leaf damage*			
	Day2	Day7	Day9	Day13	Day2	Day7	Day9	Day13
Flame Azalea	2.0 a	2.0 a	1.33 a	0.67 ab	14.56 a	29.0 a	37.89 a	44.22 b
Sparkleberry	1.67 a	0.67 a	0.33 b	0.0 b	1.33 b	1.33 b	1.33 b	1.33 c
Pieris floribunda	1.33 a	0.67 a	0.33 b	0.33 b	0.11 b	0.11 b	0.11 b	0.11 c
Heather	1.67 a	0.67 a	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 c
Kalmia latifolia	1.33 a	0.67 a	0.0 b	0.0 b	0.11 b	0.11 b	0.11 b	0.11 c
<i>P.j.</i> 'Temple Bells'	3.0 a	2.33 a	1.33 a	1.33 a	15.67 a	35.44 a	52.22 a	60.22 a
<i>P.p.</i> 'Little Leaf'	1.67 a	1.0 a	0.0 b	0.0 b	0.22 b	0.22 b	0.22 b	0.22 c
Encore Azalea	1.67 a	1.0 a	0.33 b	0.33 b	1.11 b	1.11 b	1.11 b	1.22 c
'Hampton Beauty' Azalea	1.67 a	1.0 a	0.33 b	0.33 b	0.11 b	0.56 b	0.89 b	1.0 c
Blueberry	1.33 a	1.0 a	0.33 b	0.0 b	0.0 b	1.22 b	1.22 b	1.22 c
F	0.98	1.89	3.72	3.63	6.2	9.77	11.6	16.19
Р	0.48	0.1205	0.0085	0.0095	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 4.2. No-choice assay 2 for adult survival and leaf damage by S. takeyai on ericaceous hosts

*number of frass spots

Means in the same column bearing different letters are significantly different ($\alpha = 0.05$). The abbreviations *P.j. = Pieris japonica*, *P.p. = Pieris phillyreifolia*

Таха	Day2	Day7	Day9	Day13
Flame Azalea	1.0 b	1.22 b	2.56 b	3.89 b
Sparkleberry	0.0 b	0.56 b	2.0 b	4.89 b
Pieris floribunda	0.0 b	0.0 b	0.0 b	0.0 b
Heather	0.0 b	0.0 b	0.0 b	0.0 b
Kalmia latifolia	0.0 b	0.0 b	0.0 b	0.0 b
<i>P.j.</i> 'Temple Bells'	11.67 a	21.56 a	43.33 a	89.56 a
<i>P.p.</i> 'Little Leaf'	0.0 b	0.0 b	0.0 b	0.0 b
Encore Azalea	0.0 b	0.0 b	0.0 b	0.0 b
'Hampton Beauty' Azalea	0.0 b	0.0 b	0.0 b	0.0 b
Blueberry	2.0 b	4.56 b	8.78 b	13.33 b
F	5.14	9.03	8.22	10.16
Р	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 4.3. Mean leaf damage* and *S. takeyai* nymphs emerged in choice trial on ericaceous hosts (averages from 3 replications)

*number of frass spots

Means in the same column bearing different letters are significantly different ($\alpha = 0.05$). The abbreviations *P.j.* = *Pieris japonica*, *P.p.* = *Pieris phillyreifolia*

Таха	Day0	Day2	Day7	Day9	Day13
Flame Azalea	0.44 a	0.22 a	0.44 a	0.22 b	0.56 a
Sparkleberry	0.11 a	0.22 a	0.33 a	0.22 b	0.33 a
Pieris floribunda	0.67 a	0.33 a	0.22 a	0.44 b	0.44 a
Heather	0.33 a	0.56 a	0.56 a	0.11 b	0.22 a
Kalmia latifolia	0.56 a	0.22 a	0.11 a	0.11 b	0.33 a
<i>P.j.</i> 'Temple Bells'	0.44 a	0.56 a	0.56 a	1.11 a	0.67 a
<i>P.p.</i> 'Little Leaf'	0.56 a	0.33 a	0.44 a	0.33 b	0.22 a
Encore Azalea	0.11 a	0.22 a	0.33 a	0.0 b	0.22 a
'Hampton Beauty' Azalea	0.0 a	0.56 a	0.33 a	0.44 b	0.56 a
Blueberry	0.11 a	0.11 a	0.22 a	0.22 b	0.11 a
F	1.43	0.77	0.67	2.72	0.91
Р	0.1879	0.6476	0.7308	0.0082	0.5228

Table 4.4. Mean number of *S. takeyai* adults present on leaves in choice trial with ericaceous hosts (averages from 3 replications)

Means in the same column bearing different letters are significantly different ($\alpha = 0.05$). The abbreviations *P.j.* = *Pieris japonica*, *P.p.* = *Pieris phillyreifolia*

Taxa	Trial 1	Trial 1 Trial 2	
	(No-choice)	(No-choice)	trial
Flame Azalea	0.56 b	0.0 b	0.0 b
Sparkleberry	0.0 b	0.0 b	0.0 b
Pieris floribunda	0.0 b	0.0 b	0.0 b
Heather	0.0 b	0.0 b	0.0 b
Kalmia latifolia	0.0 b	0.0 b	0.0 b
<i>P.j.</i> 'Temple Bells'	11.67 a	20.56 a	19.33 a
<i>P.p.</i> 'Little Leaf'	0.0 b	0.0 b	0.0 b
Encore Azalea	0.0 b	0.0 b	0.0 b
'Hampton Beauty' Azalea	0.56 b	0.0 b	0.0 b
Blueberry	0.0 b	0.0 b	0.0 b
F	7.98	10.54	6.3
Р	< 0.0001	< 0.0001	< 0.0001

Table 4.5. Mean number of S. takeyai nymphs emerged in no-choice and choice trials with ericaceous hosts

Means in the same column bearing different letters are significantly different ($\alpha = 0.05$). The abbreviations *P.j.* = *Pieris japonica*, *P.p.* = *Pieris phillyreifolia*

CHAPTER 5

INTEGRATION OF CHEMICALS AVAILABLE TO THE HOME OWNER WITH NATURAL ENEMY

CHRYSOPERLA CARNEA FOR MANAGEMENT OF AZALEA LACE BUG

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ABSTRACT The azalea lace bug *Stephanitis pyrioides* (Scott) is the major cosmopolitan tingid species attacking the widely popular ornamental azalea. Their importance has grown with the increasing popularity of azaleas and their management has been extensively studied. Presently, lace bug management strategies focus on the use of chemical insecticides to control the lace bug populations. However due to the rising demand for less hazardous methods several other materials and use of natural enemies has been tested, with limited success. We evaluated the effectiveness of five selected insecticides available to the homeowner, supplemented with a natural enemy Chrysoperla carnea (Stephens) (green lacewing) in suppressing the azalea lace bug. Each material and a water check were applied individually as well as in combination with green lacewing larvae, giving a total of 12 treatments. We found that treatments using traditional insecticides (acephate and imidacloprid) were significantly more effective in controlling the lace bugs than alternative means and that C. carnea did not contribute significantly to the control. The integration of natural enemies with chemical insecticides for lace bug suppression needs to be studied further.

KEY WORDS: Azalea, Stephanitis pyrioides, Chrysoperla carnea, management

Azaleas (*Rhododendron* spp.) are one of the most popular and widely cultivated ornamentals worldwide. They originated in North America, Europe and Asia and have been spread to different regions of the world by transport of plant material (Scariot et al. 2007). The azalea lace bug Stephanitis pyrioides (Scott) is the major cosmopolitan tingid species attacking azaleas and causes severe economic damage to landscape and cultivated azaleas in most countries where they are grown (Shrewsbury and Smith-Fiola 2000; Klingeman III et al. 2001). The importance of the azalea lace bug problem has increased along with the increasing use and cultivation of azaleas (Neal and Schaefer 2000). The lace bug is considered to be native to Japan, and was first introduced to the United States in New Jersey (Weiss 1916) from where it spread to Pennsylvania and Washington D.C. (Weiss 1918; McAtee 1923). Since then it has continued its spread to different states of the country (Torres-Miller 1989; Nielsen 1997) as well as other parts of the world. Adults and nymphs of S. pyrioides feed by piercing and drawing out cell contents on the underside of the leaves resulting in characteristic symptoms like chlorotic stippling or bleached appearance of leaves in severe cases. Oily black tar-like frass spots and exuviae can be seen on the underside of leaves along with the adults and nymphs.

Lace bugs are often considered hard to manage due to their many adaptations. The adults and nymphs are usually found on the undersurface of leaves and may evade reach by conventional pest control equipment and chemicals. Eggs are inserted into the leaf tissue and the tar-like frass deposited by the female over the eggs protects them from desiccation as well as from extraneous materials reaching the eggs (Livingstone and Yacoob 1987; Nalepa and Baker 1994).

Relatively few specific parasites and predators are reported on tingids (Wheeler Jr et al. 1975; Gordh and Dunbar 1977; Livingstone et al. 1982; Livingstone and Yacoob 1983; Henry et al. 1986; Livingstone and Yacoob 1986; Sathiamma et al. 1998; Sathiamma et al. 1999). The nymphs especially are usually free of parasitism or predation (Neal Jr and Schaefer 2000) and the secretions seen to exude from bristles covering their bodies may have a role in deterring predators (Neal Jr 1988). However, some natural enemies have been reported to attack *S. pyrioides* viz., the mymarid egg parasitoid *Anagrus takeyanus* Gordh, recovered from eggs of *S. pyrioides* (Braman et al. 1992; Balsdon et al. 1993; Balsdon et al. 1996); the Japanese mirid *Stethoconus japonicus* Schumacher, an aggressive obligate predator of *S. pyrioides* (Henry et al. 1986); the mirid *Rhinocapsus vanduzeei* Uhler, the green lacewing *Chrysoperla carnea* (Stephens) and *C. rufilabris* (Burmeister) (Braman and Beshear 1994; Shrewsbury and Smith-Fiola 2000; Stewart et al. 2002) and various spiders (Shrewsbury et al. 2004).

Lace bug management recommendations always begin with regular and thorough inspection of plants for presence of overwintering lace bug adults, eggs, and newly hatched nymphs. The bugs can be washed off with a jet of running water and use of chemicals can be avoided in case of mild infestations but heavy infestations often warrant chemical control (Sparks et al. 2002). Earlier studies used dimethoate and phorate for controlling azalea lace bugs (Johnson 1960; Schread 1968). Presently the recommended chemicals for lace bug control include carbaryl, acephate, dimethoate, malathion and bifenthrin. Systemic insecticides like imidacloprid have been reported to be effective in controlling infestations (Hommes and Westhoff 2004). Nymphs are especially vulnerable to pesticides and early spraying of plants prevents further

generations from developing (Neal Jr and Schaefer 2000). This also reduces the amount of insecticides used and thereby, plant protection costs. Non-chemical methods like use of botanicals (Wedge et al. 2009; Tabanca et al. 2010) and insecticidal soaps (M-Pede®) or horticultural oils (Sparks et al. 2002) are also reported to be effective in controlling lace bug populations. M-Pede® (insecticidal soap) provided short-term control of avocado lace bug (Hoddle et al. 2005).

However current recommendations for lace bug management focus on the use of chemical insecticides. Many concerns have been raised about the environmental effects of these chemicals. Chemical formulations designated for use on ornamental plants are higher in persistence and toxicity and are therefore not suited for plants used for consumption. With increasing public awareness about these issues, there is a great demand for alternative pest management strategies which are cost effective and efficient at the same time. Especially from the home owners' point of view, safer, yet effective management measures are always sought after.

Integration of chemical control with other safer methods has been examined. In one study with insecticides, parasitism by the mymarid wasp *A. takeyanus* was not seen to be affected by any of the chemicals, among which acephate proved to be the most cost-effective and provided long-term suppression (Balsdon et al. 1993). This indicates a possibility of integrating this or other natural enemies with chemical control for effective lace bug suppression. Integrating augmentative release of *C. carnea* larvae into azalea lace bug management programs was also found to be feasible (Shrewsbury and Smith-Fiola 2000).

The present study aimed at evaluating the effectiveness of chemicals available to the home owner, supplemented with a commercially available natural enemy (*C. carnea*) in suppressing the azalea lace bug. *C. carnea* fulfills most of the essential requirements for an effective biological control agent (Daanel and Yokota 1997). They are voracious predators often found in association with *S. pyrioides* in landscape situations and they have been demonstrated to prey on *S. pyrioides*. They are also easily available from commercial suppliers (Olkowski et al. 1992; Hunter 1994).

Materials and Methods

PLANTS. 120 azalea plants of the susceptible variety 'Girard's Rose' were selected in a location at the UGA Experiment Station, Griffin, GA. The plants were planted in between pecan trees. They were provided with irrigation and weed control was performed in the interspaces. Pesticides were not used on the plants. At the time of start of the experiment (June 2010), they were healthy and free of lace bug damage symptoms. We selected this location because the pecan trees would give sufficient shade suitable for the green lacewing larvae.

LACE BUGS. *S. pyrioides* colonies were established and periodically replenished using adult azalea lace bugs collected from natural populations found near Griffin, GA. The colonies were housed in 1.0-m³ screen cages in the entomology insect rearing facility at Griffin, GA. These colonies were reared on several cultivars of evergreen azaleas under conditions of $27 \pm 1^{\circ}$ C and a photoperiod of 14:10 (L:D) h.

To initiate a lace bug population on our test plants, male and female adult lace bugs were first collected in separate plastic tubes using aspirators. A branch with sufficient green foliage was selected on each of the test plants and enclosed in a sleeve

cage (BugDorm). Ten male and ten female adult lace bugs were then transferred into the sleeve cages using a brush or by tapping the tube and left to multiply. The releases were done on 8th, 9th and 10th June 2010. Nymphs were noticed three weeks later. A precount of nymphs was recorded from each sleeve cage on 6th July 2010. To facilitate easy application of treatments, they were color coded by different colored ribbons tied to the selected branches. The next day (7th July 2010) the spray materials were applied to the branches in the sleeve cages using a meter jet gun with a CO₂ sprayer. Two chemical insecticides (acephate and imidacloprid), an insecticidal soap (M-Pede®), horticultural oil (Suffoil), a biopesticide (Tick Ex) and a water check were chosen as the treatment materials. These were each applied individually as well as in combination with green lacewing larvae, giving a total of 12 treatments. The treatments and rates of formulation used for 400 ml of spray fluid were as follows: T1-Acephate (119.83 mg), T2-Imidacloprid (26.65 mg), T3-Soap: M-Pede® (6 ml), T4-Oil: Suffoil (5.99 ml), T5-Biopesticide: Tick Ex (0.908 ml), T6-Water, T7-Acephate (119.83 mg) + 10 green lacewing (GLW) larvae, T8-Imidacloprid (26.65 mg) + 10 GLW larvae, T9-Soap: M-Pede® (6 ml) + 10 GLW larvae, T10-Oil: Suffoil (5.99 ml) + 10 GLW larvae, T11-Biopesticide: Tick Ex (0.908 ml) + 10 GLW larvae, T12-Water + 10 GLW larvae. The treatments were laid out on the azaleas in a randomized complete block design with ten replications.

On the following day (8 July 2010) post treatment counts were recorded. Two days later, on 10 July 2010 *C. carnea* larvae were released in the sleeve cages according to the treatment schedule. The *C. carnea* larvae were purchased from (BioServe) and were received in cut pieces of corrugated cardboard with silkscreen glued to either

side. Each cell of the corrugated cardboard contained a single larva and they were moved to diet cups with a moist filter paper disc and maintained at 15°C until release into the sleeve cages on the azalea plants. Final post counts of both nymphs and adults were taken on 13 July 2010, six days after spraying. For the final counts the treated branches along with their sleeve cages were clipped off the plants and brought to the lab where the counting was done.

STATISTICAL PROCEDURES. The experiments used a randomized complete block design. The replications were considered as the block factor. Meansof the variables (pretreatment counts of nymphs, post-treatment count 1 of nymphs, post-treatment count 2 of nymphs and post-treatment count 2 of adults) were subjected to analysis of variance (ANOVA) using the general linear model procedure (SAS Institute 2003). Means were separated with Fisher protected least significant difference (LSD) test. Data points greater than three times the standard deviation were considered outliers and removed. Since there was variation in the original data, log transformation and square-root transformation was applied. Both untransformed and transformed data were analyzed and presented.

Results

In the original data (untransformed, and keeping all observations including the outliers) there was significant difference in the number of adults recorded in post-count 2, after the treatments were imposed (Table 5.1). The other counts (pre count, post count 1-nymphs and post count 2-nymphs) did not differ significantly between the treatments. The highest numbers of adults were noted in treatment 12 (water + GLW) which was on par with treatment 6 (Water). These were significantly higher than all other treatments.

The lowest numbers of adults were seen in treatments 1 (Acephate) and 7 (Acephate + GLW). After removing outliers (Table 5.2), we saw that treatment 2 (Imidacloprid) and treatment 7 (Acephate + GLW) had the lowest number of adult lace bugs (0) and these were significantly lower that treatments 4 (Oil) and 12 (water + GLW).

Contrasts performed on the untransformed data with outliers (Table 5.4) showed that the treatments with water check (T6 and T12) were significantly different from other treatments (P = 0.0021), and also the treatments that used chemicals (T1-Acephate, T2-Imidacloprid, T7- Acephate + GLW and T8- Imidacloprid + GLW) were significantly different from the treatments that did not use chemicals (P = 0.0026). The treatments using GLW on the whole were not significantly different from those that did not use GLW (P = 0.6614). Individual treatments with and without GLW (for example T1 Vs. T7) were not significantly different either. The same contrasts performed on the data after removing outliers yielded similar results (Table 5.5).

Applying log transformation to the original data again resulted in significant differences in lace bug adults in post count 2 after spraying (Table 5.3). Treatments 2 (Imidacloprid) and 7 (Acephate + GLW) recorded significantly lower number of lace bug adults, but they were on par with all the other treatments except treatment 4 (oil), 6 (water) and 12 (water + GLW). Square root transformation also showed similar results.

Application of contrasts to the log transformed data showed similar results as with the untransformed data (Tables 5.6-5.9). Applying contrasts to the analysis helped to show that the green lacewings did not contribute significantly to the control, and also that treatments using chemicals (acephate and imidacloprid) were significantly better in controlling the lace bugs than those not using chemicals.

Discussion

In the present study, we evaluated the effectiveness of chemicals available to the home owner, supplemented with a natural enemy (*C. carnea*) in suppressing the azalea lace bug. All spray materials used in our experiments were available to homeowners at local stores except *Metarhizium anisopliae* strain F52 (029056) Biopesticide. We found that the chemical insecticides acephate and imidacloprid offered the best control of lace bugs and the effect of *C. carnea* in suppressing the lace bug population was not significant. The other alternative materials like soap, oil and the fungal product (Tick ex) were not as effective as the chemicals. Comparing the cost of the homeowner accessible materials in Athens, GA we found that imidacloprid was the most cost-effective.

Both acephate (Balsdon et al. 1993; Sparks et al. 2002) and imidacloprid (Gill et al. 1999; Sparks and Hudson 1999; Hommes and Westhoff 2004) have been successfully used to control lace bug populations. Acephate proved to be more cost-effective and persistent in suppression of azalea lace bugs compared to dimethoate, bendiocarb, cyfluthrin, abamectin, azadirachtin, insecticidal soap and horticultural oil. In this study imidacloprid was an experimental compound (Balsdon et al. 1993). Imidacloprid was the first neonicotinoid insecticide to be used in nursery and landscape pest control. Due to its broad spectrum activity against several kinds of insect pests, it is one of the most popular insecticides today (Szczepaniec and Raupp 2007). Soil and trunk injection with imidacloprid were shown to be effective for controlling sucking insects that feed on ornamental trees, as an alternative to conventional spraying applications (Gill et al. 1999). Imidacloprid also showed residual toxicity when applied on leaves of cotoneasters, especially to nymphs of hawthorn lace bug *Corythuca cydoniae* (Fitch).
This reduced the need for repeated applications and thus saved resources for pest management (Szczepaniec and Raupp 2007).

Insecticidal soaps and horticultural oils are less hazardous and of shorter persistence than chemical insecticides and therefore are considered for integration into many pest management programs (Miller 1989; Davidson et al. 1990). Even though the control offered is not as effective as in chemical insecticides, the safety aspect prompts homeowners and landscapers to consider using these materials (Balsdon et al. 1993). Biopesticides like *M. anisopliae* are being used widely for control of spittlebugs (Cercopidae) in South American sugarcane and pastures (Faria and Wraight 2007) though its use on lace bugs is limited. Another fungus *Sporothrix insectorum* has been reported to control the rubber lace bug *Leptopharsa heveae* (Li et al. 2010).

The natural enemy *C. carnea* did not contribute significantly to lace bug suppression in our experiment. Although *C. carnea* are resistant to a wide range of insecticides, owing partially to enhanced metabolism of these chemicals, metabolism depends on the pesticide and decline of resistance has been reported. Pesticides used in tree fruit control have been found to be toxic to populations of *C. carnea* (Pree et al. 1989). Very low numbers of *C. carnea* larvae were recovered during the second post count and these were not significantly different.

The feasibility of integrating natural enemies with chemical control for effective lace bug suppression needs to be examined further. Augmentative releases of *C. carnea* as well as other natural enemies may help to increase the effectiveness of the biological control component in azalea lace bug management.

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Treatment	Pre count	Post count 1	Post count 2	Post count 2
	(nymphs)	(nymphs)	(nymphs)	(adults)
Acephate	14.1 a	8.6 a	1.8 a	0.5 b
Imidacloprid	14.9 a	5.9 a	4.8 a	1.7 b
Soap	17.3 a	4.6 a	3.2 a	1.5 b
Oil	12.3 a	15.9 a	4.4 a	9.4 a
Tick Ex	16.4 a	6.7 a	1.3 a	1.5 b
Water	10.9 a	4.4 a	5.9 a	4.8 ab
Acephate + 10 GLW	15.3 a	10.8 a	0.4 a	0.0 b
Imidacloprid + 10 GLW	15 a	8.3 a	4.2 a	1.5 b
Soap $+$ 10 GLW	10.8 a	6.7 a	4.4 a	1.6 b
Oil + 10 GLW	15.8 a	12.7 a	4.3 a	8.0 a
Fungus + 10 GLW	9.7 a	9.6 a	1.7 a	0.9 b
Water + 10 GLW	11.1 a	11.7 a	6.1 a	10.5 a
F	0.37	0.86	0.61	3.34
Р	0.9649	0.5834	0.8142	0.0006

Table 5.1. Effect of treatments for azalea lace bug control : Mean separation in untransformed data (keeping outliers)

Means in the same column bearing different letters are significantly different ($\alpha = 0.05$). The abbreviation GLW = Green Lacewing

Treatment	Pre count	Post count 1	Post count 2	Post count 2
	(nymphs)	(nymphs)	(nymphs)	(adults)
Acephate	14.1 a	8.6 a	1.8 bc	0.5 bc
Imidacloprid	16.56 a	6.56 a	0.22 c	0.0 c
Soap	17.3 a	4.6 a	3.2 abc	1.5 bc
Oil	7.71 a	11.0 a	5.14 a	6.43 a
Tick Ex	16.4 a	6.7 a	1.3 bc	1.5 bc
Water	9.44 a	4.67 a	3.11 abc	3.44 ab
Acephate + 10 GLW	14.0 a	6.11 a	0.44 c	0.0 c
Imidacloprid + 10 GLW	15.0 a	8.3 a	4.2 ab	1.5 bc
Soap + 10 GLW	10.38 a	3.0 a	0.13 c	0.87 bc
Oil + 10 GLW	7.71 a	6.57 a	1.29 bc	2.0 bc
Fungus + 10 GLW	9.7 a	9.6 a	1.7 bc	0.9 bc
Water + 10 GLW	10.38 a	11.5 a	2.5 abc	5.62 a
F	0.78	0.65	1.84	2.88
Р	0.6625	0.7852	0.0575	0.0026

Table 5.2. Effect of treatments for azalea lace bug control : Mean separation in untransformed data (after removing outliers)

Means in the same column bearing different letters are significantly different ($\alpha = 0.05$). The abbreviation GLW = Green Lacewing

	Log transformed			Square root		
	with	Log tran	sformed	transformed	Square root	transformed
	outliers	without	outliers	with	without	outliers
				outliers		
Treatment	Post count	Post count	Post count	Post count	Post count	Post count
	2 (adults)	2 (nymphs)	2 (adults)	2 (adults)	2 (nymphs)	2 (adults)
Acep	0.28 d	0.79 abc	0.28 c	0.37 d	1.01 ab	0.37 c
Imida	0.29 d	0.12 c	0.0 c	0.41 d	0.16 b	0.0 c
Soap	0.65 bcd	1.13 a	0.65 bc	0.87 bcd	1.44 a	0.87 bc
Oil	1.67 a	1.23 a	1.37 a	2.38 a	1.64 a	1.86 a
TicEx	0.4 cd	0.59 abc	0.4 c	0.56 cd	0.77 ab	0.56 c
Water	1.34 ab	1.16 a	1.17 ab	1.78 ab	1.48 a	1.52 ab
Acep+ GLW	0.0 d	0.23 bc	0.0 c	0.0 d	0.3 b	0.0 c
Imida + GLW	0.58 bcd	0.99 ab	0.58 bc	0.74 bcd	1.33 a	0.74 bc
Soap + GLW	0.61 bcd	0.09 c	0.39 c	0.81 bcd	0.13 b	0.53 c
Oil + GLW	1.14 bcd	0.55 abc	0.64 bc	1.71 abc	0.71 ab	0.84 bc
TicEx + GLW	0.49 bcd	0.66 abc	0.49 bc	0.66 bcd	0.82 ab	0.66 bc
Water + GLW	1.87 a	0.8 abc	1.48 a	2.68 a	1.04 ab	1.98 a
F	4.04	2.05	3.48	3.90	2.01	3.46
Р	<.0001	0.0321	0.0004	<.0001	0.0355	0.0004

Table 5.3. Mean separation in log transformed and square root transformed data

Means in the same column bearing different letters are significantly different ($\alpha = 0.05$). The abbreviation GLW = Green Lacewing

	Pre count	Post count 1	Post count 2	Post count 2
	(nymphs)	(nymphs)	(nymphs)	(adults)
Treatment P values	0.9649	0.5834	0.8142	0.0006
		Contrast P	values	
With GLW Vs Without GLW	0.5736	0.295	0.9708	0.6614
T1 Vs T7	0.84	0.6797	0.6761	0.8626
T2 Vs T8	0.9866	0.6524	0.8578	0.9448
T3 Vs T9	0.2755	0.6935	0.7202	0.9724
T4 Vs T10	0.5563	0.5483	0.9762	0.6281
T5 Vs T11	0.2611	0.5864	0.9049	0.8355
T6 Vs T12	0.9732	0.1725	0.9524	0.0507
Water vs others	0.3328	0.7499	0.1101	0.0021
Chem vs non-chem, control	0.4878	0.7823	0.4437	0.0026

Table 5.4. Contrasts performed on untransformed data (keeping outliers)

	Pre count (nymphs)	Post count 1 (nymphs)	Post count 2 (nymphs)	Post count 2 (adults)		
Treatment P values	0.6625	0.7852	0.0575	0.0026		
	Contrast P values					
With GLW Vs Without GLW	0.3014	0.7879	0.257	0.5449		
T1 Vs T7	0.9853	0.5648	0.3861	0.7551		
T2 Vs T8	0.7749	0.6864	0.0122	0.3504		
T3 Vs T9	0.2192	0.7198	0.0587	0.7057		
T4 Vs T10	1.000	0.3791	0.0358	0.0192		
T5 Vs T11	0.2074	0.4908	0.7924	0.7006		
T6 Vs T12	0.8715	0.1369	0.7114	0.2002		
Water vs others	0.3442	0.6945	0.3391	0.0015		
Chem vs non-chem, control	0.1173	0.922	0.3627	0.0017		

Table 5.5. Contrasts performed on untransformed data after removing outliers

	Pre count (nymphs)	Post count 1 (nymphs)	Post count 2 (nymphs)	Post count 2 (adults)
Treatment P values	0.9239	0.8858	0.2236	<.0001
		Contrast	P values	
With GLW Vs Without GLW	0.3377	0.9787	0.3165	0.9568
T1 Vs T7	0.5708	0.4639	0.1974	0.5057
T2 Vs T8	0.7203	0.2608	0.278	0.4897
T3 Vs T9	0.514	0.7868	0.1367	0.9224
T4 Vs T10	0.8478	0.4812	0.5709	0.2018
T5 Vs T11	0.0713	0.7283	0.8838	0.8205
T6 Vs T12	0.893	0.3214	0.737	0.2095
Water vs others	0.8958	0.468	0.026	<.0001
Chem vs non-chem, control	0.4889	0.8349	0.0946	<.0001

Table 5.6. Contrasts performed on log transformed data, keeping outliers

	Pre count (nymphs)	Post count 1 (nymphs)	Post count 2 (nymphs)	Post count 2 (adults)
Treatment P values	0.6227	0.8093	0.0321	0.0004
Contrasts		Contrast	<i>P</i> values	
With GLW Vs Without GLW	0.1804	0.6017	0.0771	0.7514
T1 Vs T7	0.3922	0.1893	0.1353	0.4233
T2 Vs T8	0.9224	0.3559	0.023	0.0976
T3 Vs T9	0.6385	0.5782	0.0085	0.4839
T4 Vs T10	0.6347	0.3644	0.1215	0.0725
T5 Vs T11	0.0552	0.706	0.856	0.779
T6 Vs T12	0.6704	0.3162	0.376	0.4042
Water vs others	0.9034	0.6464	0.1191	<.0001
Chem vs non-chem, control	0.2392	0.674	0.1496	0.0001

Table 5.7. Contrasts performed on log transformed data, after removing outliers

	Pre count (nymphs)	Post count 1 (nymphs)	Post count 2 (nymphs)	Post count 2 (adults)
Treatment P values	0.9492	0.883	0.4285	<.0001
		Contras	t P values	
With GLW Vs Without GLW	0.4105	0.7381	0.4224	0.8791
T1 Vs T7	0.7927	0.7094	0.2728	0.5406
T2 Vs T8	0.803	0.3727	0.4467	0.5936
T3 Vs T9	0.3962	0.9434	0.3108	0.9226
T4 Vs T10	0.719	0.4747	0.6643	0.2784
T5 Vs T11	0.1031	0.9725	0.9369	0.8755
T6 Vs T12	0.9013	0.2641	0.7957	0.1413
Water vs others	0.654	0.539	0.0344	<.0001
Chem vs non-chem, control	0.4614	0.9882	0.1408	0.0001

Table 5.8. Contrasts performed on square root transformed data, keeping outliers

	Pre count (nymphs)	Post count 1 (nymphs)	Post count 2 (nymphs)	Post count 2 (adults)
Treatment P values	0.6358	0.8523	0.0355	0.0004
		Contra	st P values	
With GLW Vs Without GLW	0.2104	0.7425	0.088	0.7189
T1 Vs T7	0.5881	0.2631	0.1535	0.4206
T2 Vs T8	0.8682	0.4551	0.019	0.1131
T3 Vs T9	0.4445	0.6058	0.0113	0.482
T4 Vs T10	0.7676	0.336	0.1075	0.0625
T5 Vs T11	0.0781	0.9686	0.9119	0.8321
T6 Vs T12	0.7274	0.246	0.4044	0.3443
Water vs others	0.6545	0.8105	0.1325	<.0001
Chem vs non-chem, control	0.1683	0.7659	0.1688	0.0001

Table 5.9. Contrasts performed on square root transformed data, after removing outliers

CHAPTER 6

SUMMARY

The genus *Stephanitis* comprises over 60 species of lace bugs that include pests of tropical and temperate fruit and ornamental plants worldwide. The Andromeda lace bug Stephanitis takeyai is one of the most important pests of Pieris sp. a popular ericaceous ornamental shrub. *Pieris* are grown for their evergreen foliage that takes on magnificent spring coloration as well as for their attractive flowers. Relatively less work has been done on S. takeyai especially aspects of pest management, the azalea lace bug (S. *pyrioides*) being the more studied species. Our studies examined the range of susceptibility to S. takeyai among cultivated Pieris taxa. We first conducted screening using no-choice Petri dish assays to evaluate *Pieris* taxa for their susceptibility to the two species of lace bugs S. takeyai and S. pyrioides. The azalea lace bug, S. pyrioides was included in the study being the more economic and widespread tingid species which is also known to infest other ericaceous hosts. In our no-choice assays, over 60 Pieris taxa, including species, cultivars and hybrids, were evaluated for their susceptibility to the two species of lace bugs based on leaf damage, adult survival on the leaves and emergence of nymphs. We used four *Pieris* species among which the highest preference was for *P*. japonica. P. taiwanesis and P. formosa were less preferred and P. phillyreifolia, was least preferred among the four species tested. The maximum number of taxa evaluated represented the species P. japonica (51). Among them S. takeyai again showed preference for certain *P. japonica* taxa viz., 'Temple Bells' and 'Cavatine', whereas

others like 'Variegata' and 'Prelude' were less preferred. Overall, *P. phillyreifolia* and *P. japonica* 'Variegata' were consistently resistant to both species of lace bugs while *P. japonica* 'Cavatine' was consistently susceptible to both. *P. japonica* 'Temple Bells' and was notable in being highly susceptible to *S. takeyai*, but resistant to *S. pyrioides*. Oviposition was noted only with *S. takeyai* on some *Pieris* taxa, whereas *S. pyrioides* did not oviposit on any of the *Pieris* taxa. Comparing the responses of the two species of lace bugs, it was clear that *Pieris* were not favored hosts for *S. pyrioides*, whereas although reported on other hosts, the preference of *S. takeyai* for *P. japonica* was clearly indicated by better adult survival, higher leaf damage and most importantly, nymph emergence on different *Pieris* taxa) were also conducted using *S. takeyai* alone, which confirmed the resistance of *P. phillyreifolia* and susceptibility of *P. japonica* 'Temple Bells'.

Along with the gradients in susceptibility to the two lace bug species revealed in our screening assays, we also observed wide variability in leaf shape, size, texture, color and growth habit among the *Pieris* taxa, even within the same species. Based on our observations and also previous reports on the possible role of leaf parameters like toughness, moisture content, epicuticular wax, and stomatal characters in plant resistance, we examined some of the potential mechanisms of resistance in selected *Pieris* taxa to *S. takeyai*. Our experiments with extracts of *Pieris* leaf-surface lipids revealed that *Pieris* leaf wax does not have a role in resistance. Leaf wax extracts from the resistant species *P. phillyreifolia* applied on leaves of the susceptible cultivar *P. japonica* 'Temple Bells' did not affect feeding, oviposition or survival of *S. takeyai*, and

neither did the reverse affect the resistance of *P. phillyreifolia*. Changes in leaf wax extraction procedure, like increasing the extraction time, did not affect the results. Among other leaf parameters tested, leaf toughness measurements revealed that significantly higher force was required to puncture P. phillyreifolia leaves which were resistant to the pest. This plant species also had higher fiber, lignin and cellulose content and lower leaf moisture content as compared to the susceptible cultivar P. japonica 'Temple Bells'. Mineral nutrient analysis of the leaves showed significantly higher Ca and Mn content in *P. phillyreifolia* leaves. Both these minerals are associated with toughness of cell walls. However the levels of other minerals could not be related to the response to lace bugs. Further to leaf parameter analyses, leaf ultrastructure of selected Pieris taxa were conducted and these revealed significant differences in the number and size of stomata. *P. phillyreifolia* leaves had the highest number of stomata per unit area but they were the smallest in size, whereas *P. japonica* 'Temple Bells' leaves had a lower number but the largest stomata. From our observations, we found that resistance in Pieris taxa to S. takeyai appears to be a combination of different factors among which leaf toughness, moisture content and stomatal characters may have a significant role. Although we could not arrive at the primary mechanism of resistance, information from these studies will be useful in screening ornamental plant germplasm for lace bug resistance and also in breeding resistant plants.

Although *P. japonica* is the preferred and reproductive host of *S. takeyai*, it is known to exhibit seasonal, non-obligate host alternation between its two main hosts: *P. japonica* and *Lyonia elliptica*, both ericaceous ornamentals, in its country of origin Japan. Polyphagy of *Stephanitis* lace bugs has been reported by some workers, and *S. takeyai*

has been reported from several other hosts of different related and unrelated plant families. We undertook a study to evaluate host plant utilization by S. takeyai within the family Ericaceae and also confirm some of the earlier reports. We tested the acceptability of ten ornamental, landscape and fruit plants belonging to the family Ericaceae viz., Rhododendron calendulaceum (flame azalea), Rhododendron 'Hampton Beauty', Rhododendron 'Autumn Empress', Vaccinium arboreum (sparkleberry), Vaccinium virgatum (rabbiteye blueberry), Calluna vulgaris (heather), Kalmia latifolia (mountain laurel) along with the *Pieris* species *P. floribunda* (mountain pieris), *Pieris* phillyreifolia (climbing fetterbush) and P. japonica 'Temple Bells' (Japanese pieris) to S. takeyai. In no-choice tests adult survival varied significantly among the taxa. Maximum leaf damage was recorded on P. japonica and R. calendulaceum, while slight damage was noted on V. arboreum and Rhododendron 'Hampton Beauty'. Nymph emergence was recorded on P. japonica (highest), R. calendulaceum and Rhododendron 'Hampton Beauty'. In multi-choice tests maximum leaf damage was recorded on *P. japonica* whereas R. calendulaceum suffered only slight damage. This showed that reactions of the lace bugs to the plants may vary with the situation and hosts that may be attacked under no-choice situation may be ignored in presence of the favored host. However several plants, which may not be favorable hosts, could still serve as reservoirs for the pest. Such information could be useful in predicting potential hosts of S. takeyai and also other Stephanitis lace bugs.

Existing lace bug management recommendations emphasize the use of chemical insecticides to control the lace bug populations especially considering the aesthetic value. Public demand for less hazardous methods has led to the testing of several other materials

as well as use of natural enemies. However the alternative methods have met with limited success. The azalea lace bug *S. pyrioides* (Scott) is the most important pest of azaleas. Their importance has grown with the increasing popularity of azaleas and their management has been extensively studied. We evaluated the effectiveness of five insecticides available to the homeowner viz., acephate, imidacloprid, insecticidal soap, horticultural oil and *Metarhizium anisopliae*, supplemented with a natural enemy *Chrysoperla carnea* (green lacewing) in suppressing the azalea lace bug. Each material and a water check were applied individually as well as in combination with green lacewing larvae, giving a total of 12 treatments. We found that treatments using chemical insecticides (acephate and imidacloprid) were significantly better in controlling the lace bugs than those not using chemicals and that *C. carnea* did not contribute significantly to the control. The integration of natural enemies with chemical insecticides for lace bug suppression needs to be studied further.

Our studies have resulted in new information on gradients in susceptibility of cultivated *Pieris* taxa to the two species of lace bugs, *S. pyrioides* and *S. takeyai*, their host range and potential mechanisms of resistance in *Pieris* taxa. *S. takeyai* is a relatively new pest in the southeastern United States and is capable of causing significant damage to several *Pieris* cultivars. Since relatively less information is available regarding its potential host range among Ericaceae and other related families, it would be worthwhile to conduct further studies along these lines as well as new integrated management strategies for both species of lace bugs.