Resistance Mechanisms in *Pieris* Taxa (Ericaceae) to *Stephanitis takeyai* (Hemiptera: Tingidae)

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ABSTRACT This study examines some of the potential mechanisms of resistance in selected *Pieris* (Ericaceae) taxa to the Andromeda lace bug, *Stephanitis takeyai* Drake and Maa, based on differences in resistance to lace bug feeding, and the possible role of leaf parameters such as leaf wax, toughness, nutrient composition, and stomatal characters in plant resistance. Experiments with extracts of leaf-surface lipids revealed that *Pieris* leaf wax did not have a role in resistance to lace bug feeding. Leaf wax extracts from a resistant species *P. phillyreifolia* (Hook.) DC. applied to leaves of a susceptible cultivar *P. japonica* (Thunb.) D.Don ex G.Don 'Temple Bells' did not affect feeding, oviposition, or survival of *S. takeyai*; and neither the extracts from Temple Bells induce susceptibility in *P. phillyreifolia*. Leaf penetrometer measurements indicated that significantly higher force was required to puncture *P. phillyreifolia* leaves, which also had higher fiber, lignin, and cellulose, and lower leaf moisture contents. Ultrastructural examination of leaves of *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 these were the smallest in size, whereas *P. japonica* (Thunb.) D.Don ex G.Don Temple Bells leaves had the fewest and largest stomata. Resistance in *Pieris* taxa to *S. takeyai* may be attributed to a combination of different factors including leaf toughness, moisture, and stomatal characters. The type of resistance may be described as antixenosis combined with antibiosis, because reduced adult survival and reproduction were observed on the taxa resistant to lace bug feeding.

KEY WORDS *Pieris*, *Stephanitis*, resistance, stomata, toughness

The Andromeda lace bug, *Stephanitis takeyai* Drake and Maa, is an important pest of *Pieris* D. Don spp. (Johnson and Lyon 1991), a popular ericaceous ornamental plant. Lace bugs feed by sucking cell contents, resulting in yellowish white stipples on the abaxial leaf surfaces, and leave oily, black frass spots on the adaxial surfaces. Lace bug feeding leads to reduction in photosynthetic efficiency (Buntin et al. 1996) and occasionally, plant death (Schread 1968). In ornamental plants grown for foliage and flowers, lace bug damage to even few leaves affects the esthetic value and marketability.

Pieris taxa (species, cultivars, and hybrids) show differences in susceptibility to lace bug feeding (Dunbar 1974, Labanowski and Soika 2000, Nair et al. 2012). When 60 *Pieris* taxa were compared for their susceptibility to lace bugs, the highest damage was observed on *P. japonica* (Thunb.) D.Don ex G.Don, whereas *P. phillyreifolia* (Hook.) DC. was least damaged. Among *P. japonica* cultivars, *S. takeyai* showed clear preference for 'Temple Bells' and 'Cavatine', whereas 'Variegata' and 'Prelude' were less damaged (Nair et al. 2012). The low preference for *P. floribunda* and its hybrids also are recognized (Dunbar 1974). The reasons for the preferences exhibited by *S. takeyai* are not yet known. Leaf physical and chemical parameters like toughness, pubescence, stomatal size and density, moisture content, and epicuticular wax are possible mechanisms of resistance to lace bugs and other sucking pests. Differences in leaf-surface lipid components were noted between resistant and susceptible azaleas (Balsdon et al. 1995, Wang et al. 1999) and studies with extracts of epicuticular leaf wax indicated that leaf wax serves as a primary mechanism of resistance of deciduous azalea to *S. pyrioides* (Scott) (Chappell and Robacker 2006). Leaf pubescence (Wang et al. 1998b) and stomatal characters (Kirker et al. 2008) could not be correlated with resistance to *S. pyrioides* although they varied considerably among tested azaleas. 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). Host plants are important sources of water and nutrients for phytophagous arthropods and thus can influence herbivory (Bernays and Chapman 1994). Insects can adapt well to new environments and their nutritional requirements may vary even within a species, which makes it difficult to establish whether nutritional factors confer herbivory resistance to plants (House 1961). Nevertheless, many choices made by insects during their life processes are

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influenced by nutritional needs (Slansky 1982) and therefore, determining foliar nutrient composition may provide explanation for resistance shown by plants.

Because of the varying effects of different resistance mechanisms, the general understanding is that resistance is a combination of mechanisms. This paper examines some of the above mechanisms for their potential role in resistance exhibited by *Pieris* taxa to *S. takeyai.*

Materials and Methods

Plant Material. *Pieris* taxa were obtained from the Department of Horticulture *Pieris* collection located at the University of Georgia (UGA) Horticulture Farm in Watkinsville, GA and various commercial nurseries. The plants were obtained in 11.3-liter (3 gallon) and 3.7-liter (1 gallon) pots and maintained in a screen house with regular irrigation. For the different experiments, leaves were collected from plants with at least five branches.

Lace Bugs. *Stephanitis takeyai* colonies were initiated from a population obtained from a landscape setting in Long Island, NY in April 2009. The colonies were maintained in plastic containers through the period of study at 27 ± 1°C and a photoperiod of 14:10 (L:D) h, on *Pieris* cultivars *P. japonica* 'Dodd's Crystal Cascade Falls', 'Temple Bells', and 'Scarlett O'Hara'. The host plants were rotated to avoid selection by lace bugs. For conducting the assays, 5–10-d-old adult lace bugs were aspirated into plastic tubes and then transferred to the assay dishes by using a brush.

Leaf Wax Extraction Studies. The procedure for leaf wax extraction was adopted from Chappell and Robacker (2006) and modified suitably for different assays, designated as Trials A and B.

Trial A. Forty mature leaves (fourth or fifth leaf from the bottom of a branch) from plants of a resistant (*P. phillyreifolia*) and a susceptible cultivar (*P. japonica* 'Temple Bells') (Nair et al. 2012) were collected. These were air dried for 120 h and then immersed in 100 ml of chloroform for 15 s. Chloroform was evaporated and remaining epicuticular wax resuspended in 50 ml of a 2:1 mixture of ethanol and deionized water, 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. The solution was agitated continuously to prevent the wax from settling, so that the wax was distributed as uniformly as possible over the treated surface. 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 comparisons between treated and untreated leaf surfaces. The treated leaves then were air dried and used for bioassays. Three such leaves of one cultivar placed in a petri dish with their stalks covered with sections of moist paper towel constituted one replication. Each cultivar was replicated six times. In replications 1, 2, and 3 the solvent alone was the control, whereas in replications 4, 5, and 6 the control was no treatment. Thus, two controls were prepared. This was done to test for the effect, if any, of the solvent. In all, there were three 'donors' in Trial A, (*P. phillyreifolia*, *P. japonica* 'Temple Bells', and solvent) and two 'recipients' (*P. phillyreifolia* and *P. japonica* 'Temple Bells'). Each donor–recipient combination was considered as a treatment, 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 in a growth chamber under 27 ± 1°C and a photoperiod of 14:10 (L:D) h. Observations on the number of bugs alive were taken on day 2, 7, 9, and 13. On day 13, the surviving adults were removed and the leaves were scored for percent leaf area damage by using the scoring chart developed by Klingeman et al. (2000), in which damaged leaves were chosen to represent a range of lace bug feeding injury. After scoring, the leaves were maintained and observed daily for emergence of nymphs. Both treated and untreated sides of each leaf were observed separately for leaf damage and nymph emergence. This trial was conducted twice (designated as A1 and A2).

Table 1. *S. takeyai* adult survival, leaf damage, and nymph emergence in Assay A (*Pieris* leaves treated with *Pieris* leaf wax extracts) (Averages from two repetitions, A1 and A2)

Treatments	No. of live adults	Leaf damage[a]	No. of nymphs
T6 solvent or control on *P. japonica* 'Temple Bells'	1.17a	5.49a	2.81b
T1 *P. phillyreifolia* extract on *P. japonica* 'Temple Bells'	0.67b	3.43b	5.56a
T4 *P. japonica* 'Temple Bells' extract on *P. j.* 'Temple Bells'	0.92ab	3.41b	1.63b
T3 *P. phillyreifolia* extract on *P. phillyreifolia*	0.08c	0.22c	0.00c
T5 solvent or control on *P. phillyreifolia*	0.00c	0.08c	0.00c
T2 *P. japonica* 'Temple Bells' extract on *P. phillyreifolia*	0.00c	0.00c	0.00c
F	10.01	40.58	16.84
P	<0.0001	<0.0001	<0.0001
Overall model	$F = 5.31$ df = 10,61 $P < 0.0001$	$F = 20.55$ df = 10,61 $P < 0.0001$	$F = 9.12$ df = 10,61 $P < 0.0001$

[a] Percent leaf area damaged; means in the same column bearing different letters are significantly different ($\alpha = 0.05$; LSD).

Trial B. To measure the effect of differences in extraction times and *Pieris* taxa, two sets of 40 leaves each of plants of a resistant (*P. phillyreifolia*), moderately resistant (*P. japonica* 'Variegata'), and a susceptible cultivar (*P. japonica* 'Temple Bells') (Nair et al. 2012) were air dried for 120 h. One set then was immersed in 100 ml of chloroform for 15 s and the other for 30 s. The rest of the procedure was similar to Trial A, but there were four 'donors' (*P. phillyreifolia*, *P. japonica* 'Variegata', *P. japonica* 'Temple Bells', and the solvent) and three 'recipients' (*P. phillyreifolia, P. japonica* 'Variegata', and *P. japonica* 'Temple Bells') giving 12 treatments in total. Leaves were assessed for damage by using the number of frass spots, because frass spot numbers are highly correlated with leaf damage and served as an index for the amount of *S.*

Table 2. *S. takeyai* leaf damage and nymph emergence on treated and untreated sides of *Pieris* leaves treated with *Pieris* leaf wax extracts in Trial A (Averages from two repetitions, A1 and A2)

	Leaf damage[a] (treated side)	Leaf damage[a] (untreated side)	Nymphs (treated side)	Nymphs (untreated side)
D1- *Pieris phillyreifolia*	1.33b	2.32a	1.63a	3.91a
D2- *P. japonica* 'Temple Bells'	1.51b	1.89a	0.65a	0.99b
D3- solvent	2.85a	2.72a	1.26a	1.54b
F	8.83	2.22	1.45	7.52
P	0.0004	0.1178	0.2421	0.0012
R1- *Pieris phillyreifolia*	0.07b	0.13b	0.00b	0.00b
R2- *P. japonica* 'Temple Bells'	3.73a	4.49a	2.37a	4.29a
F	128.78	182.05	24.71	42.98
P	<0.0001	<0.0001	<0.0001	<0.0001
D×R				
F	8.33	2.29	1.45	7.52
P	0.0006	0.1098	0.2421	0.0012

[a] Percent leaf area damaged; Means in the same column section bearing different letters are significantly different ($\alpha = 0.05$; LSD); D, donor (of leaf wax); R, recipient (of leaf wax).

pyrioides feeding on azaleas (Ericaceae) (Buntin et al. 1996). This trial was conducted twice (designated as B1 and B2).

Leaf Toughness. Six mature leaves (fourth or fifth leaf from the bottom of a branch) from three plants each of 11 *Pieris* taxa, showing different levels of susceptibility to *S. takeyai* (Nair et al. 2012) were collected fresh from containerized plants maintained in the screen house at the UGA Experiment Station, Griffin, GA. The leaves were detached from the plants and brought to the laboratory in plastic bags just before the toughness measurements. Toughness was measured using a force gauge (Chatillon DFX-010-NIST Digital Force Gauge, Largo, FL). To make the measurements, each leaf was placed on a platform attached to the force gauge. 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. Mature leaves (fourth or fifth leaf from the bottom of a branch) from five *Pieris* taxa showing different levels of susceptibility to *S. takeyai* (Nair et al. 2012) were collected fresh from containerized plants maintained in the screen house at the UGA Experiment Station, Griffin, GA and submitted for analysis on the same day. Moisture content, starch, water soluble carbohydrates (WSC), ethanol soluble carbohydrates (ESC), fructans, nonstructural 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. The nutrients were analyzed on as-received basis and dry matter basis. Starch was determined by using the methods of Karkalas (1985) and Holm et al. (1986). Water soluble extracts and ethanol soluble extracts were prepared according to Smith (1969) and carbohydrate in each extract was

Table 3. *S. takeyai* adult survival, leaf damage, and nymph emergence in *Pieris* leaves treated with *Pieris* leaf wax extracts prepared using two extraction times in Trial B (Averages from two repetitions, B1 and B2)

Treatments	Extraction time: 15 s			Extraction time: 30 s		
	No. of live adults	Leaf damage[a]	No. of nymphs	No. of live adults	Leaf damage[a]	No. of nymphs
T1 *P. p.* extract on *P. j.* 'Temple Bells'	0.33bc	12.78a	4.78a	0.5ab	12.45ab	7.83a
T2 *P. j.* 'Variegata' extract on *P. j.* 'Temple Bells'	1.17a	20.44a	4.33a	0.67a	12.61ab	6.39ab
T3 *P. j.* 'Temple Bells' extract on *P. j.* 'Temple Bells'	0.67b	15.70a	4.25a	0.67a	15.53a	5.64a-c
T4 *P. j.* 'Temple Bells' extract on *P. p.*	0.00c	0.06b	0.0c	0.00b	0.06c	0.00d
T5 *P. j.* 'Variegata' extract on *P. p.*	0.00c	0.22b	0.0c	0.00b	0.08c	0.00d
T6 *P. p.* extract on *P. p.*	0.00c	0.14b	0.0c	0.00b	0.11c	0.00d
T7 *P. p.* extract on *P. j.* 'Variegata'	0.00c	2.78b	0.89bc	0.00b	4.33c	1.19b-d
T8 *P. j.* 'Temple Bells' extract on *P. j.* 'Variegata'	0.00c	3.73b	0.75c	0.00b	6.36bc	3.36a-d
T9 *P. j.* 'Variegata' extract on *P. j.* 'Variegata'	0.00c	3.20b	0.72c	0.33ab	5.50bc	0.61cd
T10 solvent only on *P. j.* 'Temple Bells'	0.00c	4.80b	4.09ab	0.50ab	14.75a	5.20a-d
T11 solvent only on *P. p.*	0.00c	0.03b	0.00c	0.00b	0.22c	0.00d
T12 solvent only on *P. j.* 'Variegata'	0.00c	4.28b	2.06a-c	0.00b	5.67bc	0.94b-d
F	5.28	6.27	2.93	1.96	5.56	2.20
P	<0.0001	<0.0001	0.0039	0.0496	<0.0001	0.0266
Overall model	$F = 4.64$ df = 13,58 $P < 0.0001$	$F = 5.31$ df = 13,58 $P < 0.0001$	$F = 2.51$ df = 13,58 $P = 0.0086$	$F = 1.77$ df = 13,58 $P = 0.0705$	$F = 4.81$ df = 13,58 $P < 0.0001$	$F = 1.93$ df = 13,58 $P = 0.0450$

[a] Number of frass spots; means in the same column bearing different letters are significantly different ($\alpha = 0.05$; LSD); *P.j: Pieris japonica, P.p.: Pieris phillyreifolia.*

Table 4. *S. takeyai* leaf damage and nymph emergence on treated and untreated sides of *Pieris* leaves treated with *Pieris* leaf wax extracts prepared using two extraction times (Average values from assays B1 and B2)

	Extraction time: 15 s				Extraction time: 30 s			
	Leaf damage[a] (treated side)	Leaf damage[a] (untreated side)	Nymphs[a] (treated side)	Nymphs (untreated side)	Leaf damage[a] (treated side)	Leaf damage[a] (untreated side)	Nymphs (treated side)	Nymphs (untreated side)
D1- *Pieris phillyreifolia*	4.59a	5.87a	2.04a	1.74a	5.33a	5.93a	2.24a	3.78a
D2- *P.j.* 'Variegata'	7.67a	8.24a	1.67a	1.71a	5.65a	6.48a	2.11a	2.56a
D3- *P.j.* 'Temple Bells'	5.72a	7.26a	1.30a	2.04a	6.54a	8.09a	2.48a	3.52a
D4- solvent	3.72a	4.26a	2.39a	1.71a	5.98a	7.78a	1.71a	2.39a
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*	0.14b	0.08b	0.00b	0.00b	0.13c	0.11c	0.0b	0.0b
R2- *P.j.* 'Variegata'	3.03b	3.96b	1.21b	1.01b	5.14b	5.79b	1.17b	1.89b
R3- *P.j.* 'Temple Bells'	13.11a	15.18a	4.33a	4.39a	12.36a	15.31a	5.24a	7.29a
F	26.86	26.41	14.94	12.22	24.99	32.95	8.42	11.51
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0006	<0.0001
D×R								
F	1.34	1.21	0.28	0.29	0.11	0.21	0.22	0.31
P	0.2530	0.3153	0.9446	0.9382	0.9951	0.9733	0.9672	0.9270

[a] Number of frass spots; means in the same column section bearing different letters are significantly different ($\alpha = 0.05$; LSD); D: donor (of leaf wax), R: recipient (of leaf wax), *P.j.*: *Pieris japonica.*

colorimetrically determined through a phenol-sulfuric acid procedure on the spectrophotometer based on sucrose standard (Dubois et al. 1956). The water soluble extracts and ethanol soluble extracts represented different fractions of nonstructural carbohydrate. The difference between WSC and ESC gave an estimate of fructan content of the sample.

Hemicellulose, cellulose, and lignin were determined based on the principles of detergent fiber analyses (Van Soest 1963a, b; Van Soest and Wine 1967) that allowed the determination of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and lignin. The analyses were carried out on an Ankom$^{200/220}$ Fiber Analyzer (ANKOM Technology, Macedon, NY) by using F57 filter bags (ANKOM Technology, NY) (Ankom Technology 2006a, b). The protocols are based on the methods of the National Forage Testing Association (Undersander et al. 1993a, b). The lignin and ash contents in ADF residue were determined by using the method described by Ankom Technology (2005). Mineral concentrations in the leaves were analyzed at the Soil, Plant and Water Lab under the Agricultural and Environmental Services Laboratories, CAES Co-operative Extension Service, UGA. Phosphorus, K, Ca, Mg, Mn, Fe, Al, B, Cu, Zn, Na, Pb, Cd, Ni, Cr, and Mo were analyzed using Microwave - Acid (HNO_3) Digestion, ICP Method using CEM Mars5 microwave digestion system model 61E ICP (Thermo Jarrell-Ash, Franklin, MA). Total N and Total S were analyzed using Dry Combustion Method in LECO CNS-2000 model CNS analyzer.

Leaf Ultrastructure. Three mature leaves were chosen from each of three plants of the highly susceptible cultivar *P. japonica* 'Temple Bells', moderately resistant *P. japonica* 'Variegata', and highly resistant *P. phillyreifolia.* Ultra-thin sections of the leaves were prepared at the Histology Laboratory, Department of Pathology, UGA College of Veterinary Medicine in Athens, GA. The leaf samples were embedded 'on edge' in wax cassettes and sections were taken from the wax blocks. These sections were mounted on labeled slides that were then observed under a compound microscope (Leica DM LB; Leica Microsystems Inc., Bannockburn, IL) with a SPOT Idea camera (SPOT Imaging Solutions, Sterling Heights, MI) attached and digital images were taken under magnifications of 4, 10, and 20×. For SEM images, the leaf samples were observed at the UGA Ultrastructure Lab in Athens. These were cut into labeled bits before being examined with the SEM Variable Pressure SEM (VP-SEM) Zeiss 1450EP (Carl Zeiss NTS, Peabody, MA). Measurements of thickness of upper epidermis, upper epidermis plus 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 by using Adobe Photoshop. The numbers of stomata in 100 square micron areas also were counted. In all, 15 observations of each parameter were made from each leaf (from

Table 5. Leaf toughness measurements* in *Pieris* leaves

Pieris taxa	Direction of force	
	Upper to lower surface	Lower to upper surface
P. japonica 'Prelude'	1.72bc	1.99c
P. japonica 'Cavatine'	1.67c	1.74d
P. japonica 'Dodd's Sugar Run Falls'	1.77bc	1.7d
P. japonica 'Temple Bells'	1.65c	1.72d
P. phillyreifolia 'Little Leaf'	2.38a	3.46a
P. phillyreifolia 'Baldwin'	2.46a	3.4a
P. japonica 'Dorothy Wycoff'	1.91b	1.91c
P. taiwanensis 'Snow Drift'	1.72bc	1.65d
P. japonica 'Valley Rose'	2.27a	2.06bc
P. japonica 'Dodd's Crystal Cascade Falls'	1.46d	1.47e
P. japonica 'Variegata'	2.34a	2.2b
F	28.81	140.73
P	<0.0001	<0.0001
Overall model	$F = 24.22$ df = 12,20 $P < 0.0001$	$F = 117.89$ df = 12,20 $P < 0.0001$

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

Force in newtons required to puncture leaf.

* Averages from 18 leaves per taxon.

Table 6. Leaf nutritional parameters in *Pieris* taxa—dry matter basis

Pieris taxa	Starch (%)	Water soluble carbs (%)	Ethanol soluble carbs (%)	Fructans (%)	Non-structural carbs (%)	Acid detergent fiber (%)	Acid detergent lignin (%)	Cellulose (%)
P.j. 'Prelude'	3.02bc	10.51a	9.93a	0.69a	13.53ab	34.8b	16.31a	18.5c
P.j. 'Cavatine'	6.21a	10.58a	9.53a	1.05a	16.79a	34.81b	15.29a	19.52bc
P.j. 'Dodd's Sugar Run Falls'	5.58ab	10.7a	9.87a	0.83a	16.28a	31.16c	11.89b	19.27bc
P.j. 'Temple Bells'	4.65a-c	10.4a	9.51a	0.89a	15.04a	36.02b	16.14a	19.89b
P. p. 'Little Leaf'	2.35c	7.83b	7.68b	0.26a	10.19b	39.76a	17.02a	22.75a
F	4.11	8.34	3.59	0.71	5.57	11.29	11.21	19.74
P	0.0424	0.0059	0.0587	0.6058	0.0192	0.0023	0.0023	0.0003
Model df	6, 8	6, 8	6, 8	6, 8	6, 8	6, 8	6, 8	6, 8

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

different images) giving 45 observations per cultivar, in total.

Statistical Procedures. All the experiments were analyzed as one-way randomized complete block designs. The replications were considered as the block factor. Treatment means were analyzed separately for each study. Means of the variables were subjected to analysis of variance (ANOVA) by using the general linear model procedure (PROC GLM, 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 by using PROC CORR (SAS version 9.1) (SAS Institute 2003) to determine if any of these variables were correlated with leaf damage. Pearson's coefficient was used as the measure of correlation at a significance level of 0.05.

Results

Leaf Wax Extraction Studies. *Trial A.* The highest mean number of live bugs on day 13 after exposure was seen on control, but this was statistically similar to *P. japonica* leaves treated with wax extracts from *P. phillyreifolia* and *P. japonica* (Table 1). The greatest mean leaf damage (average of treated and untreated halves) was observed on *P. japonica* leaves treated with solvent, which was higher than those on all other treatments (Table 1). The highest mean number of nymphs (average of treated and untreated halves) was observed on *P. japonica* leaves treated with *P. phillyreifolia* extracts, which was significantly higher than those on all other treatments (Table 1). Treatments on *P. phillyreifolia* leaves showed very low or no adult survival, leaf damage, and nymph emergence.

There were no significant differences between *P. phillyreifolia* and *P. japonica* variety 'Temple Bells' as donors of leaf wax on treated halves and nontreated halves of the test leaves, except in the case of nymph emergence where the highest number of nymphs emerged when *P. phillyreifolia* was the donor. The two taxa always differed significantly when considered as recipients of leaf wax in all parameters (Table 2). R1 (*P. phillyreifolia*) always recorded significantly lower leaf damage and numbers of nymphs than R2 (*P. japonica* variety 'Temple Bells').

In all cases, replications did not differ significantly with respect to adult survival ($F = 0.6$; $P = 0.7005$), leaf damage ($F = 0.53$; $P = 0.7520$) or nymph emergence ($F = 1.4$; $P = 0.2375$). This ruled out any effect caused by the solvent.

Trial B. Pieris japonica 'Temple Bells' leaves treated with 15- and 30-s extracts of the other taxa and solvent as substrate (T1, T2, T3, and T10) showed significantly higher mean adult survival, mean numbers of frass spots, and higher nymph emergence (average of treated and untreated halves) (Table 3). Leaves of *P. phillyreifolia* and *P. japonica* 'Variegata' treated with 15-s and 30-s extracts (except for *P. japonica* 'Variegata' treated with *P. japonica* 'Temple Bells' extract) showed very low or no adult survival, low damage, and very low or no nymph emergence. The lace bugs performed best in treatments on *P. japonica* 'Temple Bells' leaves, showed moderate or low preference for

Table 7. Leaf nutritional parameters in *Pieris* taxa–as received basis

Pieris taxa	Starch (%)	Water soluble carbs (%)	Ethanol soluble carbs (%)	Fructans (%)	Non-structural carbs (%)	Acid detergent fiber (%)	Acid detergent lignin (%)	Cellulose (%)	Moisture (%)
P.j. 'Prelude'	1.28a	4.46a	4.21a	0.30a	5.74a	14.77c	6.92b	7.85d	57.58b
P.j. 'Cavatine'	2.53a	4.28a	3.86a	0.43a	6.81a	14.07cd	6.18c	7.89d	59.55a
P.j. 'Dodd's Sugar Run Falls'	2.43a	4.65a	4.28a	0.36a	7.08a	13.52d	5.153d	8.36c	56.62b
P.j. 'Temple Bells'	2.04a	4.55a	4.16a	0.40a	6.59a	15.76b	7.06b	8.70b	56.25b
P.p. 'Little Leaf'	1.21a	4.03a	3.94a	0.14a	5.23a	20.40a	8.73a	11.68a	48.69c
F	2.79	1.07	0.59	0.55	1.88	131.36	45.27	282.58	53.48
P	0.1010	0.4323	0.6819	0.7043	0.2067	<0.0001	<0.0001	<0.0001	<0.0001
Model df	6, 8	6, 8	6, 8	6, 8	6, 8	6, 8	6, 8	6, 8	6, 8

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

P. japonica 'Variegata' leaves and least preferred treatments on *P. phillyreifolia* leaves.

Extraction times did not have a significant effect on adult survival ($F = 0.31$; $P = 0.5798$), leaf damage ($F = 0.55$; $P = 0.4610$) or nymph emergence ($F = 1.48$; $P = 0.2256$). With both 15-s and 30-s extracts, there were no significant differences between *P. phillyreifolia, P. japonica* variety 'Variegata', and *P. japonica* 'Temple Bells' as donors of leaf wax and the solvent alone (Table 4). The same taxa reacted differently as recipients of leaf wax, and *P. japonica* 'Temple Bells' was the most preferred by the lace bugs. This was indicated by the higher numbers of frass spots and nymph emergence on both treated and nontreated halves of the leaves of this cultivar in all cases.

Leaf Toughness. A higher force was required to puncture leaves of *P. phillyreifolia* 'Little Leaf' and *P. phillyreifolia* 'Baldwin' in upper to lower direction and lower to upper direction (Table 5). These two taxa were statistically similar to each other, and required higher puncture force than all the other taxa. *Pieris japonica* 'Variegata' also required higher force, but lesser than that for the *P. phillyreifolia* cultivars. Force required for *P. japonica* 'Temple Bells' was similar to that for *P. japonica* 'Cavatine', *P. japonica* 'Dodd's Sugar Run Falls' and *P. taiwanensis* 'Snow Drift'; which were all 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. Greater force was required to puncture the leaves from the lower surface upward, than from the upper surface downward ($F = 8.07$; $P = 0.0063$).

Leaf Nutritional Parameters. Among the different nutritional parameters, the resistant cultivar *P. phillyreifolia* had the lowest, whereas the susceptible *P. japonica* 'Cavatine' had the highest, starch content on dry matter basis (Table 6). *Pieris phillyreifolia* was also lower in water soluble carbohydrates (WSC), ethanol soluble carbohydrates (ESC), and nonstructural carbohydrates (NSC) on dry matter basis than the other taxa. In parameters related to toughness, *P. phillyreifolia* had higher acid detergent fiber (ADF), acid detergent lignin (ADL), and cellulose on dry matter basis than in the other taxa. On 'as received' basis, WSC, ESC, fructans, NSC, and starch were not significantly different among the taxa, but *P. phillyreifolia* had the highest contents of ADF, ADL, and cellulose, and the lowest moisture (Table 7). *Pieris phillyreifolia* had the highest N content (Table 8). Phosphorus and K were the highest in the susceptible *P. japonica* 'Temple Bells' and lowest in *P. phillyreifolia.* Calcium, Mg, S, Mn, and Na contents were high, whereas Al, B, Fe, and Zn content were low, in *P. phillyreifolia. Pieris japonica* 'Temple Bells' had the highest B content. Cu content was highest in *P. japonica* 'Prelude' which was similar to that in *P. japonica* 'Cavatine' and *P. phillyreifolia.* The micronutrients Cd, Cr, Mo, Ni, and Pb were at equally low levels in all the taxa and were therefore not statistically analyzed.

Leaf Ultrastructure. Thicknesses of the epidermal layers were not significantly different among the taxa (Table 9). *Pieris phillyreifolia* had a higher number of

Table 8. Leaf mineral concentrations in *Pieris* taxa

Pieris taxa	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Al (ppm)	B (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Na (ppm)	Zn (ppm)	Cd (ppm)	Cr (ppm)	Mo (ppm)	Ni (ppm)	Pb (ppm)
P. j. 'Prelude'	1.13ab	0.14b	0.98b	1.15a	0.20b	0.13bc	70.43a	26.65b	5.93a	134.93a	348.1c	130.73cd	69.41a	<0.4	<1	<1	<2	<5
P. j. 'Cavatine'	1.07b	0.14b	1.13a	0.92b	0.17c	0.15b	48.35bc	16.63d	5.45ab	120.53b	121.67d	177.87b	34.72bc	<0.4	<1	<1	<2	<5
P. j. 'Dodd's Sugar Run Falls'	0.98b	0.14b	0.82c	1.11a	0.24a	0.21a	61.24ab	21.43c	4.93b	135.5a	164.1d	118.72d	40.02b	<0.4	<1	<1	<2	<5
P. j. 'Temple Bells'	0.98b	0.19a	1.20a	0.54c	0.14d	0.11c	39.74c	37.44a	4.18c	42.02c	912.0b	172.53bc	22.85d	<0.4	<1	<1	<2	<5
P. p. 'Little Leaf'	1.29a	0.10c	0.73d	1.11a	0.24a	0.15b	51.18bc	19.04cd	5.53ab	44.28c	1191.67a	222.87a	31.43c	<0.4	<1	<1	<2	<5
F	6.85	20.47	56.70	129.85	83.92	13.40	7.25	48.98	12.27	306.07	267.54	10.10	49.10	–	–	–	–	–
P	0.0107	0.0003	<0.0001	<0.0001	<0.0001	0.0013	0.0090	<0.0001	0.0017	<0.0001	<0.0001	0.0032	<0.0001	–	–	–	–	–
Model df	6, 8	6, 8	6, 8	6, 8	6, 8	6, 8	6, 8	6, 8	6, 8	6, 8	6, 8	6, 8	6, 8	–	–	–	–	–

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

Table 9. Analysis of variance in leaf ultrastructural parameters of *Pieris* taxa

Pieris taxa	Upper epidermis (mm)	Upper epidermis + palisade (mm)	Lower epidermis (mm)	Number of stomata in 100 sq. μ area	Length of stomatal Aperture (μ)
P. japonica 'Temple Bells'	0.027a	0.079a	0.19a	9.44b	12.20a
P. phillyreifolia 'Little Leaf'	0.029a	0.08a	0.02a	17.53a	6.07c
P. japonica 'Variegata'	0.026a	0.079a	0.02a	9.76b	9.49b
F	3.31	0.46	0.20	374.06	381.82
P	0.0412	0.6346	0.8168	<0.0001	<0.0001
Overall model	$F = 1.64$ df = 46, 88 $P = 0.0234$	$F = 0.71$ df = 46, 88 $P = 0.8999$	$F = 1.31$ df = 46, 88 $P = 0.1360$	$F = 17.01$ df = 46, 88 $P < 0.0001$	$F = 17.30$ df = 46, 88 $P < 0.0001$

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

stomata per 100 square microns, but the size of their stomatal openings was smaller. *Pieris japonica* 'Temple Bells' and 'Variegata' were similar in the numbers of stomata per 100 square microns. The stomata in 'Temple Bells' had the longest apertures, which were significantly larger than those in 'Variegata' or *P. phillyreifolia* 'Little Leaf' (Fig. 1).

Among mineral nutrients, P ($R = 0.69$; $P = 0.004$) and K ($R = 0.84$; $P = 0.0001$) were positively correlated, whereas Ca ($R = -0.82$; $P = 0.0002$) and Mg ($R = -0.84$; $P < 0.0001$) were negatively correlated with leaf damage, and whereas other nutrients were not correlated. Moisture was positively correlated ($R = 0.58$; $P = 0.022$) and leaf toughness was negatively correlated with leaf damage (upper to lower surface, $R = -0.64$; $P = 0.009$ and lower to upper surface, $R = -0.6$; $P = 0.017$). Among ultrastructural parameters, stomate size was positively correlated with leaf damage (($R = 0.84$; $P = 0.004$).

Discussion

We measured leaf wax because its role in resistance against *S. pyrioides* was established in azaleas, another ericaceous plant (Chappell and Robacker 2006) and the solvent that we used, chloroform, has been reported as the most efficient wax solvent that penetrated to the cell, but left the cell membrane intact (Nigg et al. 1981). However, our assays revealed 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 (Bodnaryk 1992). We used two methods (Buntin et al. 1996, Klingeman et al. 2000) for assessing lace bug damage, both of which gave consistent results (Nair et al. 2012).

Differences between *Pieris* taxa in physical features, such as leaf toughness, were evident in the screening experiments. *Pieris phillyreifolia* had tough, brittle leaves, whereas those of *P. japonica* cultivars were pliable (Nair et al. 2012). Force gauge measurements were consistent with these observations because *P. phillyreifolia* leaves were harder to puncture than leaves of the other cultivars. Therefore, leaf toughness could be one of the reasons for low preference for *P. phillyreifolia* by the lace bugs. Toughness in plants is attributed primarily to cellulose in the cell wall, 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 and cutin impart different mechanical properties to the plant surface, which may cause wearing of herbivore mouthparts (Hochuli 1996). These mechanical properties of plants may appear to have a greater influence on herbivory by chewing insects (Slansky 1990, Choong 1996), but sucking insects can also be influenced by plant toughness. A possible correlation between leaf toughness and resistance to the leaf hopper *Scaphytopius magdalensis* (Provancher) (Hemiptera: Cicadellidae) was noted in *Vaccinium crassifolium* Andrews (Ericaceae) (Meyer and Ballington 1990). Cuticle thickness appeared to inhibit penetration of mature leaves by the bayberry whitefly, *Parabemisia myricae* (Kuwana) (Hemiptera: Aleyrodidae) nymphs (Walker 1985). Feeding by meadow spittlebug, *Philaenus spumarius* (L.) (Hemiptera: Cercopidae) nymphs was reduced with increased lignification of stem tissues of host plants

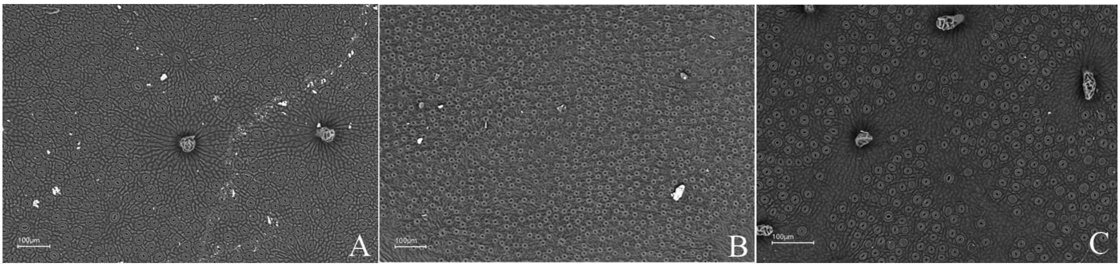

Fig. 1. Scanning electron microscope images of adaxial surfaces of leaves of *Pieris* taxa showing stomatal characters (A) *P. japonica* 'Temple Bells' (B) *P. phillyreifolia* 'Little Leaf', and (C) *P. japonica* 'Variegata'. Bars represent 100 microns.

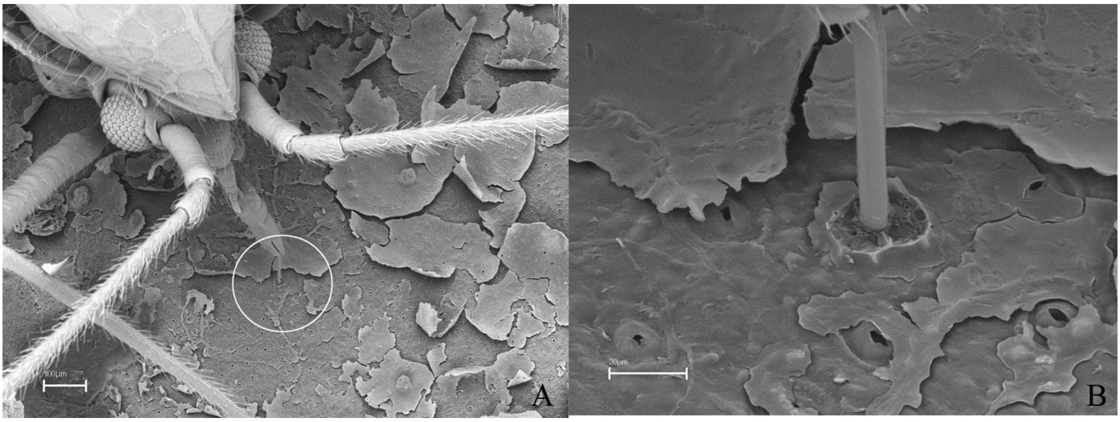

Fig. 2. Scanning electron microscope images of *Stephanitis takeyai* feeding on *P. japonica* 'Temple Bells' leaf. Bar represents 100 microns in (A) and 30 microns in (B).

(Hoffman and McEvoy 1986). However, leaf toughness did not seem to deter sessile sap feeders like scales (Hemiptera: Coccidae), that use extremely narrow stylets and enzymatic digestion to feed on tough, mature leaves (Peeters et al. 2007). Leaf toughness is commonly assessed using penetrometers, which 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).

Presence of dense pubescence on leaves affected lace bug feeding in azaleas (Braman and Pendley 1992). However, none of the *Pieris* cultivars that we examined had 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.

Pieris phillyreifolia, which was resistant to *S. takeyai,* had the lowest moisture content, whereas the highly susceptible *P. japonica* 'Cavatine' (Nair et al. 2012) had the highest moisture content. Therefore, low moisture could be one of the reasons for nonpreference of *P. phillyreifolia* by *S. takeyai.* 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). However, in measurements of azalea resistance to *S. pyrioides,* leaf water content was significantly different among taxa but was not correlated with azalea lace bug performance (Wang et al. 1998b). Moisture is 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). Low leaf moisture may explain the absence of nymphs on *P. phillyreifolia.* Moisture can also affect availability of nutrients like N (Mattson 1980), which is critical to all herbivores. Although *P. phillyreifolia* had the highest N content among the tested taxa, its low moisture may be the reason why it could not be used by the lace bugs. *Pieris phillyreifolia* leaves also had high contents of Ca, which is important in stability and function of cell wall structure (Marschner 1995), and Mn, which is essential in lignin and suberin biosynthesis (Römheld and Marschner 1991). These nutrients may therefore have a role in leaf toughness of *P. phyllireifolia.* However, *P. phyllireifolia* leaves were low in nutrients such as K, which 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 (Marschner 1995). Boron, which also has a direct function in promoting stability and rigidity of the cell wall structure (Brown et al. 2002) was low in *P. phillyreifolia,* whereas the susceptible cultivar *P. japonica* 'Temple Bells' had the highest B content. The roles, if any, played by other minerals like P, Na, Fe, Mg, S, Cu, and Zn in the resistance shown by some *Pieris* taxa could not be ascertained. Utilization of different nutrients depends on the insect's abilities to digest complex molecules (House 1961). *Pieris 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, which could also be a reason for the low preference of *P. phyllireifolia* leaves by *S. takeyai.* Lace bugs feed on cell contents, and changes in cell contents of leaves may not be entirely reflected in nutrient analysis of whole leaves. Further studies are required to determine the effects of cell or tissue contents on host plant acceptability to sap feeding herbivores.

The resistant *Pieris* cultivars did not have thicker epidermal or cuticular layers, but the differences in the number and size of stomata indicate that these parameters could have a role in host preference as exhibited by the lace bugs. We observed that *S. takeyai* feed by inserting their stylets into the leaf through the stomata (Fig. 2), as reported in *S. pyrioides. Stephanitis pyrioides* feeding injury also increased stomatal resistance in azalea leaves (Buntin et al. 1996). However, we did not observe feeding injury in the leaves of *P. phillyreifolia* that would result in increased stomatal resistance. Our observations of the stylets of *S. takeyai* led us to hypothesize that they were too thick to be inserted through the stomata of *P. phillyreifolia* leaves, which were also tough and brittle so that they were not be able to stretch open. However, the stomata in *P. japonica* 'Temple Bells' were much larger and because the leaf itself was more flexible, there were more chances for the lace bugs to feed on this cultivar by inserting their stylets and stretching open the stomata. Stomate size was seen to vary between azalea cultivars, but this was not correlated with feeding preference of *S. pyrioides* (Kirker et al. 2008). However, in our observations, stomate size was positively correlated to leaf damage.

The resistance shown by *Pieris* taxa to *S. takeyai* is most likely a combination of different mechanisms involving leaf moisture, toughness, and stomatal characters. The type of resistance may be best described as antixenosis combined with antibiosis, because we observed increased mortality and reduced adult survival and reproduction on the resistant taxa (Nair et al. 2012). Detailed examination of these parameters in all available *Pieris* taxa needs to be conducted to confirm the resistance mechanisms. Plant secondary chemicals may have a role, because there are reports of toxic substances identified from *Pieris* species, such as 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). The analysis of such plant chemicals was beyond the scope of this study and needs to be addressed in future research. Information on resistance mechanisms will be of use when screening ornamental plant germplasm for lace bug resistance, and also to plant breeders in developing hybrids desirable for long-term, sustainable landscape situations.

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