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The Effects of Stomatal Size on Feeding Preference of Azalea Lace Bug, *Stephanitis pyrioides* (Hemiptera: Tingidae), on Selected Cultivars of Evergreen Azalea

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Abstract. **Azalea lace bug (ALB), *Stephanitis pyrioides* (Scott), is an important economic pest of azaleas in the southeastern United States. In this study, 33 commercially available cultivars of evergreen azalea, *Rhododendron* spp., were evaluated for *S. pyrioides* feeding preference in both choice and no-choice feeding bioassays. Mean stomatal length and area, which were hypothesized to affect ALB feeding preference, were also measured for each of 33 cultivars and results were correlated with indices of ALB feeding (mean feces) and fecundity (mean eggs). An azalea cultivar, Fourth of July, was least preferred by ALB in both no-choice and choice tests, whereas 'Watchet' was most preferred. Cultivars Fourth of July and Delaware Valley White had the smallest mean stomatal areas despite their disparate susceptibilities to ALB feeding. Although stomates through which ALB insert their proboscides vary in size among azalea cultivars, they confer no obvious resistance to ALB feeding preference. Therefore, the mechanism for lace bug resistance in azalea remains elusive.**

The azalea lace bug (ALB), *Stephanitis pyrioides* (Scott), is an economically important hemipteran pest on azalea and a serious problem to azalea production nurseries in the southeastern United States. Eggs overwinter in midribs of leaves and nymphs hatch in early spring. Adults, appearing black and spiny with netted wings (Schultz and Shetlar, 1994), feed on the underside of leaves through the upper palisade parenchyma of stomates causing significant leaf chlorosis. Chlorosis appears as yellow patches or stippling on the upper leaf surface, which can reduce photosynthesis and gas exchange in response to feeding as a result of stomatal restriction (Buntin et al., 1996).

Plants can protect themselves against insect attack using both physical and chemical mechanisms. Physical modifications to the leaf structure can be an effective barrier against insect pests through the adaptation of leaf surfaces and hairs. Plants can also sequester secondary metabolites in their vascular tissue that provide a chemical defense against insect feeding (Panda and Kush, 1995). We have observed that *Rhododendron oldhamii* Maxim. 'Fourth of July', a cultivar with small stomates, has little ALB feeding. Based on this observation and the fact that lace bugs feed through stomata on the underside of leaves (Ishihara and Kawai, 1981; Mathen et al., 1988), we hypothesize that smaller stomatal size confers to azalea some physical resistance to ALB feeding by restricting access to the feeding site. All Encore Autumn™ cultivars of azaleas have 'Fourth of July' as one of their parents. Therefore, we tested Encore™ cultivars as well as other common azalea cultivars from various lineages for resistance to ALB. In this study, we chose to examine the effects of stomatal area and length on ALB feeding preference on 33 commercial cultivars of azalea to determine if these leaf characteristics influence ALB preference. Choice and no-choice ALB feeding bioassays were performed in the laboratory to determine possible varietal feeding preference on azaleas by *S. pyrioides*.

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Materials and Methods

Stephanitis pyrioides

ALBs were obtained from azaleas planted at the Southern Horticultural Laboratory and from bushes planted around the city of Poplarville (Pearl River County, MS). The ALBs used in the laboratory trials were from natural populations. Voucher specimens of *S. pyrioides* are deposited in the National Museum of Natural History, Washington, DC.

Azaleas

Liners of evergreen azalea cultivars (Table 1) used in this study were obtained from Flowerwood Nursery (Loxley, AL) and Van der Giessen Nursery (Semmes, AL) and transplanted to 2.8-L containers on 19 Feb. 2004. Sixteen plants of each cultivar were potted in pine bark media amended with 0.9 $kg \cdot m^{-3}$ micronutrients mix (Micromax; The Scotts Co., Marysville, OH) and 8.3 $kg \cdot m^{-3}$ 15N–9P–12K slow-release fertilizer (Osmocote; The Scotts Co.). Cultivar abbreviations used throughout the body of this text are explained in Table 1.

Laboratory tests

Both choice and no-choice tests were conducted in the laboratory from 33 azalea cultivars. On 9 to 12 July 2004, six blocks containing one of each cultivar were placed on a container pad in a completely randomized block design in 0.25 m × 0.5-m spacing. Azaleas were overhead irrigated daily.

In both laboratory tests, cuttings were taken from each plant and held in plastic bags in an ice chest for transport to the laboratory. The stem of each cutting was placed through a hole in the lid of a 29.6-mL, water-filled cup (Jet Plastica Industries, Inc., Hatfield, PA). To equalize the area of photosynthetically active tissue, all but one leaf was removed from stems of larger-leaf cultivars, and two to five leaves were left on the other cultivars. Only mature leaves were used in the test because the newly expanded leaves would differ in their morphological characteristics.

No-choice test. This test was replicated twice; the first replicate began on 23 Aug. 2004 and the second replicate on 1 Nov. 2004. Cuttings from each plant were prepared as described previously. Five ALBs (four females and one male) were placed on each cutting. In the no-choice test, the feeding chambers used in the no-choice test consisted of two 29.6-mL cups held together with a piece of stretched parafilm (parafilm "M"; Pechiney Plastic Packaging, Chicago, IL). The inverted cup on top was to prevent ALBs from escaping, and the bottom cup supplied water to the cuttings. Cups were placed in an incubator (I-30BLL; Percival Scientific, Perry, IA) set at day/night temperatures of 27 °C for 14 h and 22 °C for 10 h and photoperiod of 16:8 light:dark.

For 4 d, cups were checked daily and any dead lace bugs were recorded. The test was ended after 4 d and the number of lace bug days and female lace bug days were calculated (sum of the number of lace bugs alive each day). The numbers of live insects were used together with totals of eggs and feces to calculate mean eggs per lace bug per day (MED) as well as mean feces per lace bug per day (MFD). Only female ALBs were used to

Table 1. Listing of azalea cultivars used in no-choice and choice laboratory experiments.

Abbrev.[z]	Cultivar[y]	Series	Parentage
AMA	Amaghasa	Satsuki	Seedling of 'Huzan'
AME	EA™ Amethyst	Encore Autumn™	'Karens' × 'Fourth of July'
ANG	EA™ Angel	Encore Autumn™	'Watchet' ('MayBlaine' × '2-72')
BRA	EA™ Bravo	Encore Autumn™	Unknown
CAR	EA™ Carnival (A)	Encore Autumn™	'Watchet' × 'Fourth of July'
CHE	EA™ Cheer (B)	Encore Autumn™	'Pink Cheer' × 'Fourth of July'
COR	EA™ Coral	Encore Autumn™	'Gumpo White' × 'Fourth of July'
DEB	EA™ Debutante (C)	Encore Autumn™	'Watchet' × 'EA Embers'
DVW	Delaware Valley White	S. Indian Hybrid	Mucronatum hybrid
EMB	EA™ Embers (A)	Encore Autumn™	'Watchet' × 'Fourth of July'
EMP	EA™ Empress	Encore Autumn™	'Macrantha Pink' × 'Fourth of July'
FAS	Fashion	Glenn Dale Hybrid	'*Rhododendron indicum*' × '*Momozono*'
FOJ	Fourth of July	Wild-type	Unknown
FOR	Formosa	S. Indian Hybrid	Phoenecium hybrid
GGG	Mrs. G.G. Gerbing	S. Indian Hybrid	Branch sport of George Lindley Taber
GPW	Gumpo White	Satsuki	Unknown
HNG	Hino de Giri	Kurumi Hybrid	Unknown
KOS	Koromo Shikibu	Ryuku	*R. linearifolium* hybrid
KYM	Kelly Marie	Tom Dodd Hybrid	*R. poukhanense* hybrid
MDF	Midnight Flare	Harris Hybrid	Unknown
MON	EA™ Monarch (D)	Encore Autumn™	'May Blaine' × 'Fourth of July'
PRI	EA™ Princess (D)	Encore Autumn™	'May Blaine' × 'Fourth of July'
RDS	Red Slipper	Back Acres Hybrid	('Andros' × 'Parade') × 'Keisetsu'
ROU	EA™ Rouge	Encore Autumn™	'Double Beauty' × 'Fourth of July'
ROY**	EA™ Royalty	Encore Autumn™	'Georgia Giant' × 'Fourth of July'
RUB	EA™ Ruby (B)	Encore Autumn™	'Pink Cheer' × 'Fourth of July'
SAN**	EA™ Sangria	Encore Autumn™	Branch sport of EA Royalty
SNG	Sunglow	Carla Hybrid	'Stewartsonian' × 'Ruffled Giant'
STR	EA™ Starlite	Encore Autumn™	'Pink Cascade' × 'Fourth of July'
SUN	EA™ Sunset	Encore Autumn™	'Watchet' × #2-32 ('Carla' × 'Fourth of July')
SWT	EA™ Sweetheart (C)	Encore Autumn™	'Watchet' × 'EA Embers'
TWI**	EA™ Twist	Encore Autumn™	Branch sport of EA Royalty
WAT	Watchet	Robin Hill Hybrid	'Amagasa' × 'Lady Louise'

[z]Abbreviations used in the body of the text to designate cultivars.
[y]Full siblings within the Encore Autumn™ series are followed by the same letter in parentheses; SAN** and TWI** are branch sports of ROY**.

calculate MED. Eggs were counted under a compound microscope. The leaves, both abaxial and adaxial sides, were digitally scanned into a computer with a desktop scanner (Scanjet 5470C; Hewlett-Packard Co., Palo Alto, CA). The number of excrement droppings and the leaf area in square millimeters (mm^2) was determined by using a digital imaging software program (ImagePro Express 4.0; Media Cybernetics, Silver Spring, MD). Analysis of variance was used for MED and MFD (PROC GLM; SAS, Cary, NC). Mean separations were performed using Fisher's least significant difference (LSD) using a significance level of 0.05.

Choice test. This test was replicated twice; the first began on 26 Aug. 2004 and the second on 8 Nov. 2004. Cuttings from each plant were taken as described previously. All 33 cuttings from each block were placed in an 11.3-L container (Rubbermaid, Wooster, OH) and 200 ALBs were added. The container's lid had a mesh-covered area to allow air exchange. The lid was placed on the container and the edge was wrapped with stretched parafilm to prevent escape. Containers were placed in incubators like in the no-choice test. After 5 d, the experiment was ended.

Eggs were counted under a compound microscope and the leaves were scanned as described for the no-choice test. Analysis of variance was used for mean eggs per leaf area and for mean feces per leaf area (PROC GLM; SAS). Mean separation was performed with using Fisher's LSD using 0.05 as the significance level.

Stomatal measurements

Mean stomatal length (MSL) and area (MSA) were calculated for each cultivar of azalea. These measurements were obtained from averaging 10 stomates from leaves sampled from six blocks. Each block contained a representative plant of the 33 cultivars. This experiment was replicated three times; leaves from cuttings were collected in April, May, and July 2005. Leaves were sampled during the morning at the same time for each replicate to ensure stomates were at uniform physiological conditions. One fully expanded leaf from each cultivar was randomly selected and a 6.8-mm diameter hole was punched out near midleaf adjacent to the midvein using a #3 cork borer (Fisher Scientific, Hampton, NH). The adaxial and abaxial sides of leaf discs were separated using a modified epidermal peel method described by Ghouse and Yunus (1972). Each leaf disc was placed in a test tube with 0.3 mL 50% nitric acid. Test tubes with leaf discs were placed in a water bath (Isotemp 110; Fisher Scientific) at 90 °C for 15 min and gently shaken every 5 min. After cooling, excess nitric acid was decanted and replaced with 0.3 mL deionized water. The water was replaced with 0.3 mL of 1.0% sodium hydroxide to neutralize any residual acid in leaf discs. After another deionized water rinse, leaf discs were stained by adding 0.3 mL of toluidine blue. After 1 h, excess stain was decanted and replaced with 0.3 mL 50% ethanol. Leaf discs were dehydrated with a series of ethanol rinses (50%, 70%, 80%, 90%, and 100%) and stored in 1 mL 100% ethanol until leaf discs were slide-mounted.

Both the adaxial and abaxial sides of each leaf disc were placed in a shallow dish and covered with a glass coverslip. A drop of Euparol (Bioquip Products, Inc., Rancho Dominguez, CA) was placed on a microscope slide and the coverslip with leaf disc was inverted on a drop of Euparol. The coverslip was then pressed down flat. Each slide was labeled with the azalea cultivar name and collection date.

Stomata were observed with a compound microscope (Olympus BX51; Olympus America, Center Valley, PA) with a 60× oil immersion lens. Digital images were taken so that at least 10 stomata were in the field of view with a microscope-mounted digital camera (Micropublisher 3.3; QImaging, Burnaby, BC, Canada). The area of each stomate (delineated by the outer edge of the guard cells) and the length across the stomatal opening were measured using a digital-imaging software program (ImagePro Express 4.0; Media Cybernetics). Length and area of each individual stomate for each cultivar were analyzed using PROC GLM (Version 9.1; SAS) and means were separated with Fisher's LSDusing a 0.05 significance level.

Correlation analysis

All of the experimental variables (eggs per mm^2, feces per mm^2, feces per ALB per day, and eggs per ALB per day) were used to perform a correlation analysis using PROC CORR (SAS Version 9.1) to determine if any of these variables were linked to either stomatal length or area. Pearson's coefficient was used as the measure of correlatedness using a significance level of 0.05.

Results

No-choice laboratory test. Based on variation between cultivars in mean feces per ALB per day (F = 2.07, df = 32,150, P = 0.0019) and mean eggs per ALB per day (F = 2.37, df = 32,150, $P \leq 0.0001$), some azalea cultivars were more suitable food and ovipositional hosts for ALB. 'Fourth of July' (FOJ) had the lowest mean feces per ALB per day value (2.50) but was statistically similar to eight other cultivars (Table 2), including EA™ Sangria (SAN; 6.10), EA™ Rouge (ROU; 6.00), EA™ Royalty (ROY; 5.91), Gumpo White (GPW; 5.60), EA™ Cheer (CHE; 5.36), EA™ Twist (TWI; 5.18), Koromo Shikibu (KOS; 4.82), and EA™ Amethyst (AME; 4.30). The azalea cultivars with the highest mean feces per ALB per day consisted of four cultivars: EA™ Debutante (DEB; 13.00), EA™ Angel (ANG; 11.20), Watchet (WAT; 10.40), and Fashion (FAS; 9.55). Full sibling cultivars were not different from one another. Differences were not detected between ROY and its branch sports in mean feces per ALB per day (Table 2).

'Fourth of July' had the lowest mean eggs per ALB per day (0.0), but was also statistically similar to four other cultivars, including ROU (1.10), SAN (0.50), TWI (0.45), ROY (0.36), and KOS (0.09). The azalea cultivars with the highest mean eggs per ALB per day consisted of 12 cultivars (Table 2): WAT (3.10), EA™ Carnival (CAR; 3.00), SWE (2.73), EA™ Coral (COR; 2.73), FAS (2.64), Kelly Marie (KYM; 2.64), ANG (2.60), DEB (2.55), EA™ Sunset (SUN; 2.50), Red Slipper (RDS; 2.45), Sunglow (SNG; 2.40), and EA™ Princess (PRI; 2.36). The full sibling species were not different from one another. Differences were not detected between ROY and its branch sports in mean eggs per ALB per day (Table 2).

Choice laboratory test. In the choice tests, we also found variation between cultivars among ALB-infested azaleas for both mean feces per mm^2 (F = 8.53, df = 32,143, $P \leq 0.0001$) and mean eggs per mm^2 (F = 9.18, df = 32,143, $P \leq 0.0001$). 'Fourth of July' had the lowest mean feces per mm^2 with 0.01, but it was statistically similar to several other cultivars (Table 3). The cultivars Formosa (FOR; 0.11), EA™ Embers (EMB; 0.10), ROY (0.08), ROU (0.08), TWI (0.08), AME (0.08), and FOJ (0.01) had lower mean feces per mm^2. The cultivars KYM (0.41), FAS (0.38), WAT (0.38), RDS (0.37), and DEB (0.31) had higher mean feces per mm^2. Significant differences in mean feces per mm^2 were not detected between full sibling cultivars, and statistical differences were not detected between ROY and its branch sports (Table 3).

Regarding mean eggs per mm^2, FOJ had no eggs present at any sampling, whereas the cultivars FAS (0.11) and WAT (0.10) had higher mean eggs per mm^2. Significant differences in mean eggs per mm^2 were not detected between full sibling cultivars, and statistical differences were not detected between ROY and its branch sports (Table 3).

Stomata measurements. Both mean stomatal length (F = 35.63, df = 32,164, $P \leq 0.0001$) and mean stomatal area (F = 15.08, df = 32,164, $P \leq 0.0001$) varied among cultivars. RDS (5.97 µm) and KYM (5.93µm) had the longest stomates (Table 4). COR (1.12 µm) had the shortest stomates but was not found to be significantly different from PRI (1.16 µm), SNG (1.23 µm), EMP (1.23 µm), ANG (1.27 µm), CHE (1.28 µm), and SWE (1.28 µm). Full sibling cultivars were not found to be significantly different with respect to mean stomatal length. ROY and its branch sports were clustered and no statistical differences were detected (Table 4). ROY had the highest mean stomatal area (6.62 µm^2) but was not different from TWI

Table 2. Mean feces per lace bug per day (MFD) and mean eggs per lace bug per day (MED) found on 33 cultivars of azalea used in no-choice laboratory bioassays.

Cultivar[z]	MED	SD	T-GROUP[x]	Cultivar[z]	MFD	SD	T-GROUP[x]
WAT	3.10	0.74	A	DEB(C)	13.00	14.86	A
CAR(A)	3.00	1.26	BA	ANG	11.20	3.52	BA
SWE(C)	2.73	0.78	BAC	WAT	10.40	2.31	BAC
COR	2.73	1.27	BAC	FAS	9.55	3.20	BDAC
FAS	2.64	0.92	BDAC	KYM	9.09	6.20	BDEC
KYM	2.64	1.20	BDAC	RDS	9.09	2.70	BDEC
ANG	2.60	0.84	BDAC	SWE(C)	8.91	3.53	FBDEC
DEB(C)	2.55	0.93	BDAC	SUN	8.60	2.59	FBDECG
SUN	2.50	1.17	EBDAC	SNG	8.60	2.50	FBDECG
RDS	2.45	1.13	EBDAC	EMP	8.10	3.44	FBDEHCG
SNG	2.40	0.69	EBDAC	DVW	8.10	3.14	FBDEHCG
PRI(D)	2.36	0.92	EBDACF	CAR(A)	8.09	3.75	FBDEHCG
MON(D)	2.30	1.06	EBDCF	GGG	8.00	2.00	FBDEHCG
MDF	2.30	1.25	EBDCF	FOR	7.60	2.95	FBDIEHCG
EMP	2.20	0.63	EDGCF	MON(D)	7.60	2.80	FBDIEHCG
RUB(B)	2.09	1.04	EHDGCF	COR	7.55	2.29	FBDIEHCG
HNG	2.00	0.82	EHDGICF	MDF	7.30	2.87	FDIEHCG
EMB(A)	1.91	1.14	EHDGIF	AMA	7.10	1.37	FDIEHCG
AMA	1.90	1.28	EHDGIF	HNG	7.00	1.88	FDIEHCG
DVW	1.90	0.99	EHDGIF	STA	7.00	2.19	FDIEHCG
GGG	1.73	0.79	EHGIJF	PRI(D)	6.91	3.70	FDIEHCG
BRA	1.60	0.51	HGIJF	RUB(B)	6.64	2.80	FDIEHG
FOR	1.60	0.69	HGIJF	BRA	6.50	2.06	FDIEHG
GPW	1.50	0.84	HGIJ	EMB(A)	6.27	2.19	FDIEHG
CHE(B)	1.36	0.92	HIJ	SAN[y]	6.10	0.99	FJDIEHG
STA	1.36	1.02	HIJ	ROU	6.00	2.49	FJDIEHG
AME	1.30	0.68	IJ	ROY[y]	5.91	2.25	FJDIEHG
ROU	1.10	0.99	KJ	GPW	5.60	2.31	FJIEHG
SAN[y]	0.50	0.52	LK	CHE(B)	5.36	2.20	FJIHG
TWI[y]	0.45	0.52	LK	TWI[y]	5.18	5.18	JIHG
ROY[y]	0.36	0.50	LK	KOS	4.82	2.22	JIH
KOS	0.09	0.30	L	AME	4.30	2.26	JI
FOJ	0.00	0.00	L	FOJ	2.50	1.43	J

[z]Cultivars followed by the same letter in parentheses are full siblings.
[y]EA 'Twist' (TWI) and EA 'Sangria' (SAN) are both branch sports of EA 'Royalty' (ROY).
[x]T-GROUPS with different letters are significantly different at the 0.05 significance level.

Table 3. Mean feces per area (MFA) and mean eggs per area (MEA) found on 33 azalea cultivars used in choice laboratory bioassays.

Cultivar[z]	MFA	SD	T-GROUP[x]	Cultivar[z]	MEA	SD	T-GROUP[x]
KYM	0.41	0.19	A	FAS	0.11	0.04	A
FAS	0.4	0.13	BA	WAT	0.1	0.02	BA
RDS	0.39	0.12	BAC	RDS	0.1	0.03	BA
WAT	0.38	0.11	BAC	KYM	0.08	0.04	BC
MDF	0.32	0.14	BDAC	MDF	0.08	0.04	BC
SWE(C)	0.31	0.23	BDC	DEB(C)	0.08	0.04	BC
DEB(C)	0.31	0.13	BDEC	SWE(C)	0.08	0.05	BCD
SUN	0.29	0.14	FDEC	SUN	0.07	0.04	CD
CAR(A)	0.26	0.1	FDEG	HNG	0.07	0.03	ECD
SNG	0.25	0.14	HFDEG	DVW	0.06	0.04	EFCD
ANG	0.23	0.1	HFDEGI	STA	0.06	0.02	GEFCD
STA	0.21	0.1	HFJEGI	CAR(A)	0.06	0.03	GEFCD
DVW	0.21	0.14	HFJKGI	ANG	0.05	0.04	GEFD
HNG	0.21	0.08	HFJKLGI	SNG	0.05	0.04	GEFD
MON(D)	0.18	0.11	HMJKLGI	GGG	0.04	0.02	GEFH
BRA	0.17	0.12	HMJKLNI	GPW	0.04	0.03	GEFH
RUB(B)	0.16	0.08	HMJKLNI	BRA	0.04	0.03	GIFH
GGG	0.16	0.04	HMJKLNI	PRI(D)	0.04	0.02	GIFH
COR	0.16	0.1	HMJKLNI	AMA	0.04	0.02	GIH
PRI(D)	0.15	0.1	HMJKLNI	RUB(B)	0.03	0.03	GIH
KOS	0.14	0.15	MJKLNI	MON(D)	0.03	0.01	GIHJ
AMA	0.13	0.06	MJKLNI	CHE(B)	0.03	0.02	GIHJ
GPW	0.13	0.1	MJKLN	COR	0.03	0.03	GIHJ
CHE(B)	0.13	0.07	MJKLN	EMP	0.02	0.02	IKHJ
FOR	0.12	0.07	MKLN	FOR	0.02	0.02	IKHJ
SAN[y]	0.11	0.07	MLN	EMB(A)	0.02	0.02	IKHJ
EMP	0.11	0.07	MON	AME	0.02	0.01	IKJ
EMB(A)	0.1	0.04	MON	SAN[y]	0.02	0.02	IKJ
ROY[y]	0.08	0.06	ON	ROU	0.02	0.01	IKJ
AME	0.08	0.07	ON	ROY[y]	0.01	0.01	KJ
TWI[y]	0.08	0.07	ON	TWI[y]	0.01	0.01	KJ
ROU	0.08	0.04	ON	KOS	0.01	0.01	KJ
FOJ	0.01	0.01	O	FOJ	0	0.01	K

[z]Cultivars followed by the same letter in parentheses are full siblings.
[y]EA ‘Twist’ (TWI) and EA ‘Sangria’ (SAN) are both branch sports of EA ‘Royalty’ (ROY).
[x]T-GROUPS with different letters are significantly different at the 0.05 significance level.
Area = mm^2.

(6.43 μm^2), FOR (6.40 μm^2), WAT (6.23 μm^2), or DEB (6.14 μm^2), whereas ‘Delaware Valley White’ (DVW; 3.86 μm^2) and FOJ (3.63 μm^2) had the lowest mean stomatal area. Statistical differences were not detected between full sibling cultivars for mean stomatal area. No statistical differences were found between ROY and TWI, but SAN had significantly less mean stomatal area (Table 4).

Correlation analysis. No correlations were found between mean stomatal area and length for any of the other experimental variables using 0.05 as the significance level. Positive correlations were detected between mean eggs and feces in both choice (0.95) and no-choice (0.84) laboratory bioassays. ALB eggs are normally found beneath a dome of fecal matter on the leaf, so it was expected that eggs and fecal counts would be correlated.

T *tests on Encore Autumn Series™ azaleas.* We chose to further analyze the Encore Autumn™ series plants to determine if there were clear differences based on the parents used to breed the cultivars. All of the Encore Autumn™ series have FOJ as the male parent and many have WAT as the female seed parent. DEB and SWE are both the result of backcrosses of WAT and FOJ [(WAT* FOJ)*WAT]. All of the Encore Autumn™ cultivars that contain WAT in their pedigree were grouped and compared with those cultivars that do not contain WAT using a two-tailed *t* test. Cultivars without WAT in their lineage had lower mean eggs and feces in both choice and no-choice tests. The presence of WAT as a parent has a negative impact on resistance to ALB feeding; however, based on the mean separations from the choice and no-choice experiments, there is a range of susceptibility among these related plants.

Discussion

Mean stomatal area and MSL have varied effects on feeding preference by ALB adults. The cultivars that had the lowest MSA values differed regarding ALB preference. Fourth of July is not a preferred host to ALB, whereas DVW was moderately preferred by ALB in this study. Past research has also indicated that DVW is susceptible to ALB. The use of DVW as a control in choice studies (Braman and Pendley, 1992) or as a test subject in shading studies (Bentz, 2003) confirms that DVW is susceptible to ALB. ROY had the largest MSA values of all 33 cultivars (6.65) yet it was not preferred by ALB for either feeding or oviposition suggesting that traits other than stomatal area should be attributed to its nonpreference.

Encore Autumn™ cultivars exhibited varied levels of resistance. The cultivars that have WAT in their parentage all had higher ALB feces and eggs in both choice and no-choice feeding, even with a nonpreferred host like FOJ as the male parent. Possible future studies should focus on chemical and genetic characteristics of WAT and FOJ, which appear to be on opposing sides of the ALB resistance spectrum. Within the Encore series, similar levels of ALB preference were found between full sibling cultivars. There was little difference in mean stomatal measurements and ALB preference between branch sports, TWI, SAN, and their parent, ROY. Although it is concluded here that stomate size is not a reliable characteristic for determining ALB resistance, there are several other plant traits that might confer some levels of resistance to ALB feeding. The cultivars evaluated in this study represent only a small percentage of the total population of commercial azalea cultivars. Additional research that focuses on similar traits among a wider number of cultivars would provide more insight into the influence of leaf characteristics on ALB feeding.

Many morphological traits associated with azalea leaves are strongly influenced by environmental conditions. Shrewsbury and Raupp (2000) looked at various landscape characteristics as descriptors for ALB preference and found that light intensity along with habitat structural complexity were major contributing factors to ALB abundance. Higher abundance of ALB was found in afternoons with higher light intensity and

Table 4. Mean stomatal length (MSL) and mean stomatal area (MSA) measured for 33 cultivars of azalea.

Cultivar[z]	N	MSL	SD	T-GROUP[x]	Cultivar[z]	N	MSA	SD	T-GROUP[x]
RDS	170	5.97	1.25	A	ROY[y]	170	6.62	1.46	A
KYM	170	5.93	0.87	A	TWI[y]	170	6.43	0.96	BA
GPW	170	5.68	0.93	B	FOR	140	6.40	1.12	BA
FOJ	80	5.50	2.89	C	WAT	120	6.23	0.75	BC
DVW	150	5.50	2.88	C	DEB(C)	150	6.14	0.97	BCD
MDF	140	5.37	0.97	DC	SAN[y]	150	5.99	0.97	ECD
GGG	160	5.22	0.75	DE	AMA	180	5.84	0.94	EFD
KOS	170	5.06	0.74	FE	FAS	150	5.69	1.06	EFG
HNG	160	4.91	0.77	F	STA	140	5.64	1.09	EFG
ROY[y]	170	1.57	0.30	G	SUN	140	5.61	0.78	HFG
TWI[y]	170	1.56	0.25	HG	ANG	150	5.53	0.82	HFG
FOR	140	1.54	0.29	HG	GGG	160	5.50	2.88	HFG
SAN[y]	150	1.47	0.27	HGI	GPW	170	5.50	2.88	HFG
DEB(C)	150	1.43	0.22	HJGI	HNG	160	5.50	2.88	HFG
AMA	180	1.43	0.27	HJGI	KYM	170	5.50	2.88	HFG
FAS	150	1.42	0.28	HJGI	KOS	170	5.50	2.88	HFG
SUN	140	1.41	0.21	HJGI	MDF	140	5.50	2.88	HFG
STA	140	1.40	0.32	KHJGI	RDS	170	5.50	2.88	HFG
RUB(B)	140	1.39	0.25	KHJLI	CAR(A)	150	5.44	0.75	HG
CAR(A)	150	1.36	0.25	KJLI	SWE(C)	160	5.27	0.99	HI
ROU	170	1.35	0.25	KJLI	BRA	170	5.10	0.75	IJ
BRA	170	1.35	0.20	KJLI	EMB(A)	170	5.10	0.73	KIJ
AME	160	1.34	0.26	KJLI	MON(D)	180	5.08	0.85	KIJ
MON(D)	180	1.33	0.22	KMJLI	RUB(B)	140	5.07	0.87	KJ
WAT	120	1.32	0.19	KMJLI	ROU	170	5.05	0.82	KLJ
EMB(A)	170	1.31	0.21	KMJLI	PRI(D)	160	5.03	0.94	KMLJ
SWE(C)	160	1.28	0.23	KMJLN	EMP	160	4.82	0.76	KMLN
CHE(B)	150	1.28	0.22	KMJLN	CHE(B)	150	4.70	1.01	MLN
ANG	150	1.27	0.24	KMJLN	AME	160	4.68	0.91	MN
EMP	160	1.23	0.23	KMLN	COR	150	4.66	0.73	N
SNG	150	1.23	0.21	MLN	SNG	150	4.49	0.91	N
PRI(D)	160	1.16	0.23	MN	DVW	150	3.87	1.63	O
COR	150	1.12	0.20	N	FOJ	80	3.63	1.66	O

[z]Cultivars followed by the same letter in parentheses are full siblings.
[y]EA 'Twist' and EA 'Sangria' are both branch sports of EA 'Royalty'.
[x]Means with the same letter (T-GROUP) are not significantly different.

lower ALB abundance was found under overstory. In a separate study, Bentz (2003) found that azaleas grown in shaded areas were better hosts for ALB, but also tolerated ALB feeding much better. Mean eggs laid and mean adults reared were positively correlated with increased leaf nitrogen and nitrogen levels increased with shading levels (Bentz, 2003). As shading levels increased, Bentz (2003) also reported that mean leaf area and moisture content increased, whereas trichome density decreased. Trichome density would be a target for resistance studies because these hairs can physically restrict insect access to the underside of the leaf. Although these two studies had contradictory outcomes, their findings indicate that plant resistance to ALB feeding is not only controlled by the plant genetics, but is also influenced by the environment.

Stomate size is only a single trait that most likely works in unison with other plant characteristics such as cuticular waxes (Balsdon et al., 1995; Chappell and Robacker, 2006), leaf pubescence (Schultz, 1993; Wang et al., 1998), and plant exudates to account for the resistance to ALB feeding. Past research has found that additional azalea traits have similar effects on plant preference and feeding. Balsdon et al. (1995) examined epicuticular lipids from the upper and lower leaf surfaces of selected deciduous azalea cultivars and found that certain triterpenoids (more specifically ursolic acid, oleanic acid, and α- and β-amyrin) that might deter ALB feeding and egg-laying occurred in lower concentrations in more susceptible cultivars. Differences were also found between the composition of waxes on the adaxial and abaxial leaf surfaces. Because ALB feeding occurs exclusively on the lower surface, it makes sense that compounds selected to deter ALB feeding would be more prevalent on the lower leaf surface. Chappell and Robacker (2006) later demonstrated that this resistance could be conveyed to nonresistant cultivars by foliar applications of these epicuticular waxes.

Schultz (1993) evaluated host plant acceptance of 20 cultivars of azalea and concluded that both bloom color and abaxial leaf texture did not affect host plant acceptance. The results of this study showed that the cultivar Macrantha Pink had the lowest leaf injury of the 20 cultivars and lower numbers of eggs than all but two cultivars (Elsie Lee and Kathy). 'Macrantha Pink' is the female seed parent of the Encore Autumn™ cultivar Empress (EMP). EA™ 'Empress' received median values in our study; it was neither highly preferred nor resistant to ALB feeding and oviposition. Bloom period was also found to affect ALB oviposition and leaf feeding; newer foliage was initially avoided and the previous year's growth was preferred until the new growth matured. Wang et al. (1998) had insufficient data to conclude that leaf pubescence was involved in ALB resistance of deciduous azaleas, although apparent differences were seen in ALB resistance of pubescent versus nonpubescent cultivars. *Rhododendron canescens* was found highly resistant to ALB feeding and had extremely high trichome density, whereas other resistant taxa had fewer surface hairs. There are clear differences between FOJ and all of the other cultivars in this study regarding leaf pubescence; the upper and lower leaf surface of FOJ has large clusters of trichomes, giving the leaf an extremely rough surface. In addition, the FOJ leaf surface is sticky, suggesting that leaf exudates might be another ALB resistance character. In contrast, WAT, which is preferred by ALB, has fewer trichomes, a smooth leaf surface, and lacks sticky compounds. Further study of leaf pubescence and leaf chemistry may explain the apparent gradient of ALB susceptibility seen in the WAT*FOJ cultivars. However, it should be noted that leaf pubescence is an environmentally influenced plant trait and may prove to be phenotypically plastic. Stomatal length and area are clearly not reliable plant characteristics to determine resistance to ALB feeding and oviposition. In this study, we focused on one physical leaf characteristic to explain differences in ALB feeding on select cultivars of azalea. Future work will examine differences in the chemical composition of leaves of tolerant species compared with susceptible plants to locate reliable plant traits that can be used to improve ALB resistance in azalea germplasm.

This study provides valuable information about the relative resistance of these selected evergreen azalea cultivars to ALB and addresses the relative contribution of stomate size in the context of ALB resistance. The outcomes of this study will help plant breeders in making future decisions when screening germplasm for ALB resistance.

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