Biotic and Abiotic Factors Affect Green Ash Volatile Production and Emerald Ash Borer Adult Feeding Preference

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ABSTRACT The emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an exotic woodborer first detected in 2002 in Michigan and Ontario and is threatening the ash resource in North America. We examined the effects of light exposure and girdling on green ash (*Fraxinus pennsylvanica* Marsh) volatile production, and effects of light exposure, girdling, and leaf age on emerald ash borer adult feeding preferences and phototaxis. Green ash seedlings grown under higher light exposure had lower amounts of three individual volatile compounds, (*Z*)-3-hexenol, (*E*)-β-ocimene, and (*Z*,*E*)-α-farnesene, as well as the total amount of six detected volatile compounds. Girdling did not affect the levels of these volatiles. Emerald ash borer females preferred mature leaves, leaves from girdled trees, and leaves grown in the sun over young leaves, leaves from nongirdled trees, and leaves grown in the shade, respectively. These emerald ash borer preferences were most likely because of physical, nutritional, or biochemical changes in leaves in response to the different treatments. Emerald ash borer females and males showed positive phototaxis in laboratory arenas, a response consistent with emerald ash borer preference for host trees growing in sunlight.

KEY WORDS light intensity, girdling, insect–plant interactions, Buprestidae, Coleoptera

Many biotic and abiotic factors affect plant nutritional and defensive chemistry and subsequently insect feeding and oviposition preferences (McKey 1979; Takabayashi et al. 1994; Hemming and Lindroth 1999; Chen and Ruberson 2008; Chen et al. 2008a, b, 2009; Chen and Poland 2009). Changes in environmental conditions affect the physiological state of plants. Growing plants under different light, temperature, fertilization, and watering conditions leads to different growth rates and production of volatiles and direct chemical defenses (Gouingené and Turlings 2002). For example, increased nitrogen fertilization of cotton (*Gossypium hirsutum* L.) reduced many volatile and nonvolatile defensive compounds (e.g., hemigossypolone and heliocides; Chen et al. 2008b). Given choices, beet armyworm *Spodoptera exigua* (Hübner) preferred plants grown with higher over lower fertilization (Chen and Ruberson 2008).

Plant and animal survival depends on sunlight. Light levels affect plant phytochemistry (Loughrin et al. 1994, Hemming and Lindroth 1999, Chen and Poland 2009) including the emission of volatile organic compounds (VOCs) (Gouingené and Turlings 2002). VOCs are known to be exploited by a variety of phytophagous, predacious, and parasitic insects for location of host plant and host/prey (Dickens 2000, Choh et al. 2004). The release of volatile terpenoids in cotton is shown to follow a diurnal pattern (Loughrin et al. 1994), which parallels the foraging pattern of the parasitoid *Cardiochiles nigriceps* Viereck (Hymenoptera: Braconidae) (Lewis et al. 1972). Manchurian ash seedlings, *F. mandshurica* Rupr., emitted higher levels of VOCs during daylight hours (0900–1800 hours) than at night (1800–0900 hours) (Rodriguez-Saona et al. 2006).

Girdling (i.e., removal of a ring of bark and phloem around the outer circumference) is a practice used worldwide in agriculture, horticulture, and forestry, with various purposes (Lahav et al. 1986; Obeso 1998). Plants are also known to induce defensive chemicals after mechanical wounding and insect herbivory (Stout et al. 1998, Reymond et al. 2000). Therefore, girdling might alter plant resistance to herbivory because of changes in nutritional qualities and defense. Girdling may also alter the emission of VOCs. Crook et al. (2008) found that emission of sesquiterpenes from the bark of ash trees was significantly increased 24 h after girdling.

Phytochemistry varies in leaves of various ages. Young and expanding tissues are generally believed to contain high concentrations of nutrients because these tissues require more resources to support their rapid growth (Slansky and Scriber 1985). Young leaves are also better defended because of their higher fitness values compared with mature or senescing leaves (McKey 1979). The greater fitness value of young expanding leaves over older leaves has been experimentally shown in some plants (McKey 1979, Strauss et al. 2004), as has the elevated accumulation of chem-

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ical defenses in these leaves (Ohnmeiss and Baldwin 2000, Chen and Ruberson 2008).

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is an exotic species first detected near Detroit, MI, and Windsor, Ontario, Canada, in 2002, and is threatening the ash resource in North America (Haack et al. 2002, Poland and McCullough 2006). Since discovery, its host range (Anulewicz et al. 2007, 2008), biology (Bauer et al. 2004), behaviors (Lelito et al. 2007, Rodriguez-Saona et al. 2007, Pureswaran and Poland 2009a), and chemical ecology (Rodriguez-Saona et al. 2006, Eyles et al. 2007, Crook et al. 2008, de Groot et al. 2008, Pureswaran and Poland 2009b) have been intensively studied. Girdled ash trees and open-grown ash trees that are fully exposed to the sun are significantly more attractive to emerald ash borer than healthy ash trees and trees that are partially or fully shaded (McCullough et al. 2009). Host preferences may be related at least in part to differnces in host volatiles. Several antennally active volatile compounds were found to be elevated in Manchurian ash seedlings that were stressed by emerald ash borer feeding or exposure to methyl jasmonate, which elicits production of stress-induced volatiles (Rodriguez-Saona et al. 2006). Adult female emerald ash borer were also attracted to volatiles from seedlings damaged by beetles or treated with methyl jasmonate. In laboratory-rearing programs, emerald ash borer also seems to feed more readily and perform better (i.e., increased longevity and fecundity) on mature ash leaves than on newly expanded ash leaves (D. Miller, personal communication). The mechanisms underlying variation in emerald ash borer preference and performance on ash trees of different condition are only recently being explored, and little is known about the effects of biotic and abiotic factors on VOCs of ash trees and emerald ash borer responses to host plants subjected to these factors.

In this study, effects of light intensity and girdling on green ash (*Fraxinus pennsylvanica* Marsh) volatile production were examined, along with the effects of light intensity, girdling, and leaf age on adult emerald ash borer feeding preference. Emerald ash borer phototaxis was also investigated. Specifically we tested the following hypotheses: (1) increased light exposure will elevate green ash VOC production; (2) emerald ash borer adults will prefer foliage from trees grown in the sun over those in the shade; (3) tree girdling will increase green ash VOC emission; (4) emerald ash borer will be observed more often on foliage from girdled than nongirdled trees; (5) emerald ash borer will choose young over mature leaves for feeding because young foliage is more nutritious than mature foliage; and (6) emerald ash borer adults will more often orient to higher than lower light intensity.

Materials and Methods

Plants and Insects

Plants. Green ash (*F. pennsylvanica*) seedlings (2 yr old and ≈15–30 cm tall) were purchased from Lawyer Nursery (West Plains, MT). They were potted with TPOT2 tree pots (width: 15 cm; height: 41 cm; volume: 6.23 liters; Stuewe & Sons, Corvallis, OR) using BACCTO High Porosity Professional Planting Mix (Michigan Peat, Houston, TX) and soil at a ratio of 3:1 as the planting medium. Seedlings were grown from August to September 2008 in a greenhouse set at ≈25 ± 2°C and supplemented with light from 400-W high-pressure sodium lamps from 2200 to 0600 hours. Before potting, trees were stored in a cold room at 4°C. Osmocote 14–14-14 Classic fertilizer (Scotts Company, Marysville, OH) was mixed with the planting medium at ≈4 kg/m^3 soil. Trees were watered as needed.

Approximately 5 wk after potting when seedlings had four to six fully expanded compound leaves, seedlings were matched for size, and one half of the trees were girdled (i.e., a 2.5-cm-wide band of bark and phloem was removed using a knife) at a height of 15 cm from the soil. One half of the girdled and nongirdled trees were kept in 1.2 by 0.7 by 1.1-m (L by W by H) screen cages covered with 80% TapeKnit shade cloth (American Nettings & Fabric, Ferndale, WA). The shade cloth reduced the amount of sunlight available to the trees by ≈70–80%. The remaining trees were fully exposed to the light intensity in the greenhouse.

Insects. Ash trees naturally infested with emerald ash borer were felled at the end of 2007 and early 2008 in Ingham County, MI, and cut into 90-cm-long logs. Logs were held in cold storage at 4°C. As beetles were required for experiments, logs were removed from cold storage to allow beetles to emerge in rearing tubes at 25°C. They were separated by sex and kept in 295-ml plastic beverage containers with an evergreen ash, *F. uhdei* (Wenzig) Linglesh, leaf in a vial of water for feeding. Containers with beetles were stored in growth chambers at 25°C with a photoperiod of 14 L:10 D and >70% RH with fresh food that was replaced twice a week, until they were used in experiments.

Headspace Leaf Volatile Collection and Analysis

The experimental design was a 2 (light intensity: sun and shade) × 2 (wound: nongirdled and girdled) factorial, with 10 replicates per treatment combination. Leaf volatiles were collected for 24 h from each tree 2 wk after girdling in the same greenhouse where trees were grown. Because light and herbivory treatments are known to have limited effects on foliar chemistry of already-mature leaves (Nichols-Orians 1991), a 2-wk treatment period was chosen to ensure that at least some young ash leaves matured under the different light conditions. The terminal part of a branch containing 5–10 compound leaves was chosen from each tree for volatile collection. The selected branches from different treatments were generally matched for age. Each selected branch was enclosed in a large-size Reynold oven bag (Reynolds Kitchens, Richmond, VA). A Super-Q volatile collection trap (Analytical Research Systems, Gainesville, FL) containing 30 mg Alltech Super-Q adsorbent material and a glass tube filled with activated charcoal were placed

against the stem of the enclosed branch. The opening of the oven bag was secured tightly around the branch stem, Super-Q trap, and charcoal filter using twist-ties. The Super-Q trap was connected with plastic tubing to a battery-operated pump (Sensidyne, Clearwater, FL). Air was cleaned as it entered the bag through the charcoal tube, and headspace volatiles inside the bag were pumped out through the Super-Q trap and absorbed by the adsorbent. The aerated portion of the seedling was clipped, and dry weights of aerated tissue were recorded.

Collected volatiles were extracted from the Super-Q adsorbent by flushing with 250 μl of pentane-hexane (both high-performance liquid chromatography grade; purity: pentane >99%; hexane: ≥95%) mixture containing 1 ng/μl of heptyl acetate (internal standard; purity >98%) with a stream of nitrogen gas. Two microliters of each sample was injected into a Thermo Scientific Trace GC equipped with a DSQ-II MS and a 30 m by 0.25 mm ID, 0.25-μm-thick TR-1MS column using splitless injections and helium as the carrier gas. The oven temperature began at 60°C for 1 min and increased at 10°C/min to 190°C and then at 35°C/min to 300°C and was held for 5 min. Compounds previously found to elicit antennal activity in gas chromatography-electroantennographic detection analyses, hexanal, (*E*)-2-hexenol, (*Z*)-3-hexenol, (*Z*)-3-hexenyl acetate, hexyl acetate, linalool, (*E*)-β-ocimene, nonanal, and (*Z*,*E*)-α-farnesene (Rodriguez-Saona et al. 2006), were quantified based on comparison of peak areas with that of the internal standard and calibration curves generated with synthetic standards. The amount of each compound of interest was calculated in nanograms per gram dry weight of tissue. All reagents and solvents used in this study were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher (Pittsburgh, PA).

Emerald Ash Borer Adult Feeding Preference in Response to Light Intensity, Tree Girdling, and Leaf Age

To examine the capability of emerald ash borer for discriminating foliar chemical changes induced by light, girdling, and leaf age, dual-choice feeding preference tests were conducted in 17 by 6 by 12-cm (L by W by H) plastic cages (Pioneer Plastics, Dixon, KY). Small holes (diameter = 0.2 cm) were punched in the cage lids for air circulation. The stems of excised leaves of the paired treatments (as described below) were inserted into 15-ml glass bottles filled with water and placed at opposite sides of the cage. Position of the treatments was randomly assigned. Five 3- to 4-d-old satiated virgin females were released into the center of the cage, and their positions on leaves were recorded 2, 4, 6, 8, 24, 26, 28, 30, and 32 h later. To examine the effects of light condition, girdling, and age of green ash leaves on emerald ash borer feeding preference, three dual-choice preference experiments were conducted: (1) sunny versus shady (mature leaves from nongirdled trees grown under sunny or shady conditions); (2) girdled versus nongirdled (mature leaves from girdled or ungirdled trees grown under sunny conditions); and (3) young versus mature (young or mature leaves of nongirdled trees grown under sunny conditions). Young leaves were <3 wk old and were still expanding, and mature leaves for all choice tests were ≈3–4 wk after full expansion and not senescent. Leaves of similar total leaf area from two treatments in the sunny versus shady and girdled versus nongirdled choice tests were chosen to avoid possible confounding effects of leaf size on emerald ash borer preference (D. S. Pureswaran and T. M. Poland, unpublished data). Leaf area of each test leaf was measured before and after the experiment to quantify feeding damage. Leaf area was quantified with a digital camera and digital imaging software (Image Processing and Analysis in Java; ImageJ, version 1.34s, available at http://rsbweb.nih.gov/ij/). Each preference experiment was repeated eight times in chambers set at 25 ± 1°C and a photoperiod of 14 L:10 D.

Emerald Ash Borer Adult Phototaxis

To examine emerald ash borer adult phototaxis, a dual-choice experiment was conducted using a cylindrical plastic arena (diameter = 20 cm, height = 8 cm), completely covered with black plastic sheeting. Two holes fitting Teflon tubing (diameter = 2 cm) were made diametrically opposite each other in the side of the plastic box. A 2.5-cm-diameter by 1-cm-long Teflon tube was inserted into each hole and secured with hot glue. The other end of each tube was connected to a collection cup (295-ml sealed, clear plastic beverage container) to capture emerald ash borer adults making a choice to leave the plastic box through either tube. One of the collection cups was covered with two layers of shade cloth described above to create a dimly lit environment (≈1.5 foot candle; BK Precision 615 lightmeter); the other was left uncovered and exposed to full light (≈20 foot candle). Five 6- to 8-d-old emerald ash borer adults were placed in the center of the plastic box and the number of emerald ash borer present in the collection cups and remaining in the test box was recorded 1 h later. The test arena was cleaned and rotated 180° after each replicate to avoid possible positional bias before a new replicate was started. The choice test was repeated eight times (a total of 40 emerald ash borer adults tested). The phototaxis test was conducted separately for females and males.

Statistical Analysis

The amounts of individual volatile compounds and the total volatile amount were analyzed separately by two-way analysis of variance (ANOVA) with light intensity and girdling treatments as factors. Volatile data were unbalanced because the amounts of some compounds in some samples were below the detection level. Means were separated by Tukey's test using LSMEANS in SAS. Data were square root $(x + 3/8)$ transformed to correct for heteroscedasticity before

Table 1. ***Fraxinus pennsylvanica*** **volatile production (mean ± SE ng/g dry mass) in response to light intensity and girdling[a]**

Volatile compounds	Shade[b]		Sun[c]	
	Nongirdled	Girdled[d]	Nongirdled	Girdled
(*Z*)-3-hexenol	$1.17 \times 10^3 \pm 211.97$	$1.36 \times 10^3 \pm 107.91$	938.12 ± 148.06	$1.01 \times 10^3 \pm 175.68$
(*E*)-2-hexenol	725.63 ± 274.98	$1.44 \times 10^3 \pm 349.02$	167.07 ± 172.67	613.75 ± 472.41
(*Z*)-3-hexenyl acetate	576.31 ± 133.10	663.66 ± 39.86	468.43 ± 103.46	595.12 ± 43.29
(*E*)-β-ocimene	$1.97 \times 10^3 \pm 361.69$	$2.11 \times 10^3 \pm 158.38$	$1.25 \times 10^3 \pm 251.45$	$1.62 \times 10^3 \pm 298.77$
Nonanal	$1.73 \times 10^3 \pm 230.66$	$2.16 \times 10^3 \pm 208.03$	$1.34 \times 10^3 \pm 321.53$	$1.65 \times 10^3 \pm 335.41$
(*Z,E*)-α-farnesene	$1.22 \times 10^3 \pm 235.48$	$1.29 \times 10^3 \pm 102.90$	733.99 ± 189.74	793.39 ± 123.46
Total[e]	$5.64 \times 10^3 \pm 1123.96$	$7.62 \times 10^3 \pm 841.58$	$3.58 \times 10^3 \pm 573.12$	$4.70 \times 10^3 \pm 840.98$

[a] In addition to the above six compounds, linalool, hexyl acetate, and hexanal were examined, but amounts were below the detection threshold. Identification was based on comparison of retention times with authentic standards and spectral data with Wiley275.L and Nist98.L libraries (L). Amounts were quantified by comparing peak area to the internal standard and to calibration curves generated with synthetic standards.

[b] Trees were grown in the shade (70–80% sunlight blocked).

[c] Trees were grown in the sun.

[d] A 2.5-cm band of bark and phloem was removed at a height of 15 cm from the soil 2 wk before.

[e] Total amounts of the six detected compounds.

analysis. Feeding damage (leaf area consumed by emerald ash borer) measured at the end of the dual-choice tests was subjected to paired *t*-tests, separately for each dual-choice test. For the two treatments, feeding damage at the end of the experiment was regressed against the total number of emerald ash borer observed on the corresponding leaves throughout the experiment, separately by experiment, and pooled across the three experiments. Data for emerald ash borer adult phototaxis were analyzed using χ^2 tests, separately for females and males. All analyses were conducted in SAS with an α level of 0.05 (SAS Institute 1999).

Results

Headspace Volatiles in Response to Light Intensity and Tree Girdling

Green ash foliar volatile production in response to light and girdling treatments is summarized in Table 1. Among the nine investigated compounds, amounts of linalool, hexyl acetate, and hexanal were below the GC detection level. Shade marginally increased the amount of (*Z*)-3-hexenol ($F = 3.74$; df = 1,36; $P = 0.06$), and significantly elevated (*E*)-β-ocimene, (*Z,E*)-α-farnesene, and total volatile production of the six detected compounds (ocimene: $F = 6.40$; df = 1,32; $P < 0.05$; farnesene: $F = 8.64$; df = 1,34; $P < 0.01$; total: $F = 7.83$; df = 1,36; $P < 0.01$). Light intensity did not affect levels of (*E*)-2-hexenol, (*Z*)-3-hexenyl acetate, and nonanal (all $P > 0.05$). Girdling and the interaction of light and girdling did not affect emission of any individual compound or the total amount of all compounds (all $P > 0.05$).

Emerald Ash Borer Adult Feeding Preferences in Response to Light Intensity, Girdling, and Leaf Age

Sunny Versus Shady. Significantly more emerald ash borer adults were found on leaves grown in the sun than those grown in the shade at 2, 6, and 8 h after beetles were released (2 h: $\chi^2 = 4.77$; df = 1; $P < 0.05$; 6 h: $\chi^2 = 5.00$; df = 1; $P < 0.05$; 8 h: $\chi^2 = 8.05$; df = 1; $P < 0.01$; Fig. 1A). At none of the observation periods were leaves grown in the shade preferred over those grown in the sun, and overall the "sunny" leaves were preferred by 2.1:1 over the shaded leaves (Fig. 1A). Emerald ash borer feeding damage was not significant between treatments ($t = 1.18$; df = 7, $P > 0.05$; Fig. 2). Furthermore, there was a positive linear relationship between foliage consumption and number of emerald ash borers observed on corresponding leaves throughout the sunny versus shady experiment (Fig. 3A). Thus, the number of beetles and time spent on each leaf was a good predictor of feeding damage.

Girdled Versus Nongirdled. More emerald ash borer adults were observed on leaves from trees that had been girdled than on leaves from ungirdled trees at 6, 8, 26, and 28 h after beetles were released (6 h: $\chi^2 = 5.33$; df = 1; $P < 0.05$; 8 h: $\chi^2 = 4.00$; df = 1; $P < 0.05$; 26 h: $\chi^2 = 4.26$; df = 1; $P < 0.05$; 28 h: $\chi^2 = 5.56$; df = 1; $P < 0.05$; Fig. 1B) Except for the first observation at 2 h, there were always more beetles on leaves from girdled trees than on leaves from nongirdled trees, and overall the girdled leaves were preferred by 2.0:1 over the nongirdled leaves (Fig. 1B). Emerald ash borer feeding damage was not significantly different between treatments ($t = 0.64$, df = 7, $P > 0.05$; Fig. 2). Similar to the sunny versus shady experiment, a positive linear relationship between foliage consumption and total number of emerald ash borer observed on corresponding leaves was observed (Fig. 3B).

Young Versus Mature. More emerald ash borer adults were observed on mature leaves than on young leaves at all observation times (2 h: $P < 0.001$; 4 h: $P < 0.0001$; 6, 28, and 32 h: $P < 0.01$; 24, 26, and 30 h: $P < 0.05$), except at 8 h ($P > 0.05$; Fig. 1C). Emerald ash borer leaf consumption was significantly higher on mature leaves compared with young leaves ($t = 4.26$; df = 7, $P < 0.01$; Fig. 2). On average, the mature leaves were preferred by 3.1:1 over the young leaves. As with the sunny versus shady and girdled versus nongirdled experiments, there was a positive linear relationship between foliage consumption and total number of emerald ash borer observed on corresponding leaves

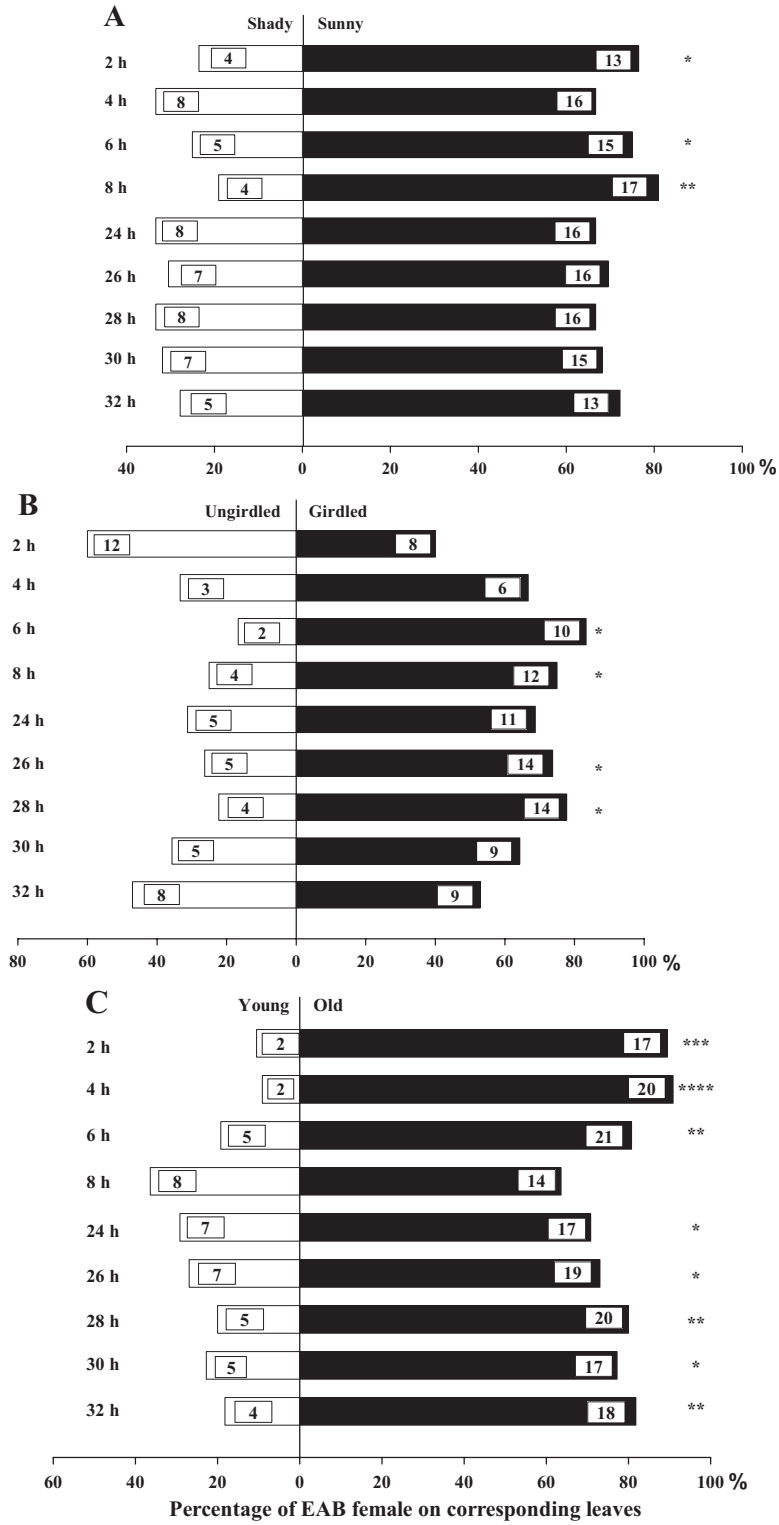

Fig. 1. Distribution of emerald ash borer adults on leaves of two treatments over a 32-h period. (A) Sun versus shade trial. (B) Nongirdled versus girdled trial. (C) Young versus mature trial. Numbers on the bars denote number of emerald ash borer adults observed on corresponding leaves. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$ ($N = 40$ beetles per experiment, eight replicates with five beetles per replicate).

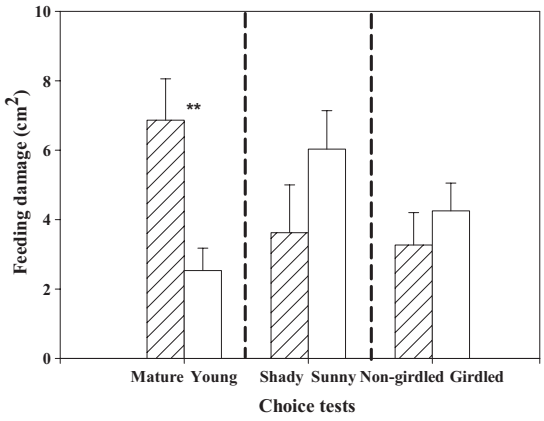

Fig. 2. Amount (cm^2) of foliage consumed by emerald ash borer adult in three dual-choice feeding experiments: Sun versus shade; girdled versus nongirdled; and young versus mature. $^{**}P < 0.01$ between mature and young leaves ($N = 8$ leaves per choice test).

throughout the experiment (Fig. 3C). This relationship for all three experiments combined was also strong (Fig. 3D).

Emerald Ash Borer Adult Phototaxis

Approximately 78% of the emerald ash borer adults in the phototaxis experiment made choices within 60 min. Emerald ash borer adults showed positive phototaxis: 97% of the emerald ash borer females that made a choice moved to the brightly lit treatment ($\chi^2 = 28.13$; df = 1; $P < 0.0001$; Fig. 4), and 87% of emerald ash borer males that made a choice moved to the brightly lit treatment ($\chi^2 = 16.13$; df = 1; $P < 0.0001$; Fig. 4).

Discussion

Plant VOCs are exploited as foraging cues by many phytophagous, predacious, and parasitic insects (Dickens 2000, Choh et al. 2004, Rodriguez-Saona et al. 2006). We found that increased light exposure was associated with reduced emission of the green leaf volatile (Z)-3-hexenol, the monoterpene (*E*)-β-ocimene, and the sesquiterpene (*Z,E*)-α-farnesene. All three compounds elicited emerald ash borer electrophysiological responses in both male and female antennae (Rodriguez-Saona et al. 2006). Both male and female emerald ash borer are attracted to sticky traps baited with (Z)-3-hexenol (de Groot et al. 2008),

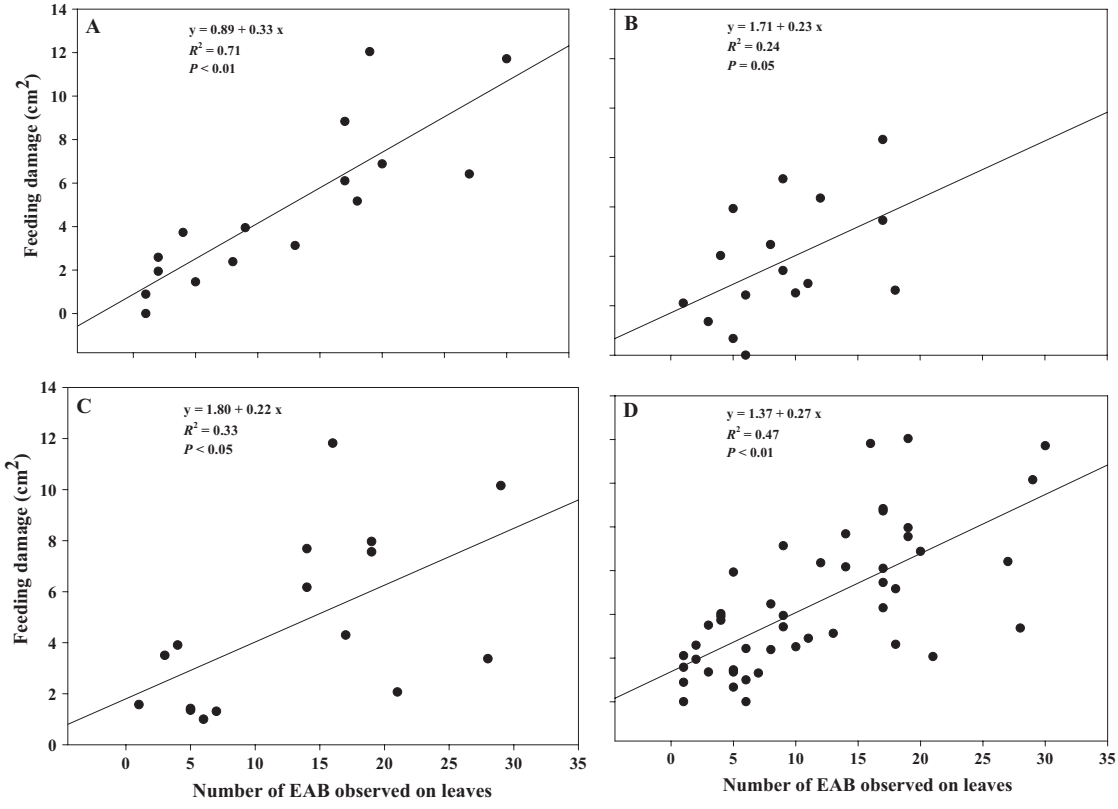

Fig. 3. Linear regression of amount (cm^2) of foliage consumed by adult emerald ash borer at the end of the experiment versus total number of emerald ash borer landing on the two treatments throughout the experiments. (A) Sun versus shade. (B) Nongirdled versus girdled. (C) Young versus mature. (D) Pooled data across the three experiments ($N = 16$ leaves per experiment, $N = 48$ leaves for combined experiments).

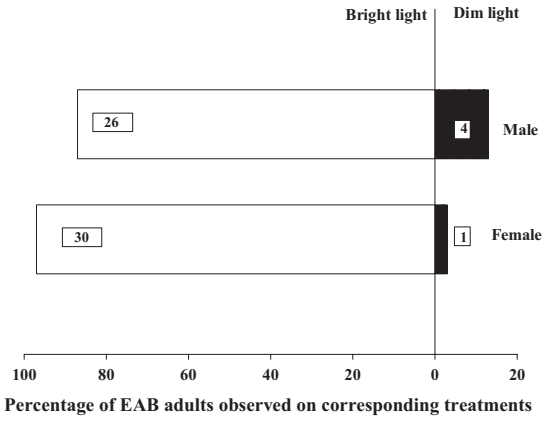

Fig. 4. Phototactic response of male and female emerald ash borer in dual-choice arena.

which elicited the strongest electrophysiological responses among host VOC stimuli (Rodriguez-Saona et al. 2006; de Groot et al. 2008). Emerald ash borer adult females, however, were more often observed on foliage from trees growing in the sun, which produce lower levels of VOCs, including, paradoxically, the attractive (Z)-3-hexenol. Similarly, Pureswaran and Poland (2009b) found an inverse relationship between the amounts of host volatiles emitted and emerald ash borer host preferences and feeding. Of six ash species tested by Pureswaran and Poland (2009b), green ash had the lowest quantity of emitted volatiles, yet was the most preferred species, whereas Manchurian ash had the highest level of emitted volatiles and was the least preferred. In field trapping experiments, attraction of emerald ash borer males was greater to (Z)-3-hexenol released at a low rate (48 mg/d) compared with a high rate (330 mg/d) (de Groot et al. 2008). Green leaf volatiles may have a multifunctional role in emerald ash borer attraction and may be most attractive at lower doses. Furthermore, emerald ash borer host location behaviors observed in laboratory test arenas and artifical trapping experiments may differ from those exhibited for host location in nature, as suggested by Chen et al. (2008b). Insect host plant location in the field involves hierarchical steps as host plant habitat location, host plant suitability, and acceptance. In test arenas, such as those used in this study, the chemical cues needed for host plant habitat location may be incomplete.

Physical or phytochemical changes induced by light exposure might account for emerald ash borer preference for foliage grown in the sun. Other experimental conditions being identical, insect herbivores are documented to prefer leaves from south or light exposed sides over those from north or light-deprived sides (Schoonhoven 1977, Rowe and Potter 2000, Panzuto et al. 2001). For instance, larvae of forest tent caterpillar, *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae), discriminated between sugar maple (*Acer saccharum*) foliage exposed to sun and foliage exposed to shade and chose foliage exposed to sun, partly because of higher amounts of soluble sugars in foliage exposed to the sun (Panzuto et al. 2001). Green ash foliage grown in the sun was found to contain higher levels of total nonstructural carbohydrates compared with foliage grown in the shade (Chen and Poland 2009). Further studies are needed to elucidate the role(s) of sugar in green ash-emerald ash borer interactions.

Effects of leaf excision likely did not affect emerald ash borer attraction in the dual-choice bioassays because leaves from both treatments in each dual-choice bioassay were excised in the same way. Therefore, effects of excision would be similar unless treatment effects interact with leaf detachment.

Girdling did not affect green ash headspace VOC production. This is unexpected because stress is known to induce emission of plants volatiles (Takabayashi et al. 1994, Rodriguez-Saona et al. 2006, Chen and Ruberson 2008). Similarly, Pureswaran and Poland (2009b) found girdling overall did not affect VOC emission of six ash species including green ash. However, girdling mature green ash trees induces increased production of bark sesquiterpenes attractive to emerald ash borer (Crook et al. 2008). The lack of effect of girdling on emission of foliage volatiles from ash seedlings might indicate that ash seedlings do not respond to girdling by inducing VOC emissions or the reactions are below the detection level. Emerald ash borer females in our experiment, however, exhibited a feeding preference for leaves from seedlings that had been girdled. Physical or phytochemical changes induced by girdling might explain emerald ash borer preference. Girdling is known to interrupt the transport of photosynthates to the roots and has a variety of physiological and biochemical effects on plants such as accumulation of carbohydrates (Roper and Williams 1989, Li et al. 2003). Host plant selection by phytophagous insects based on elevated soluble sugars has been documented (Panzuto et al. 2001).

Young and growing tissues generally contain higher concentrations of nutrients (Slansky and Scriber 1985) and defensive compounds (McKey 1979, Chen et al. 2008b) to support rapid growth and protect higher fitness values compared with mature or senescening leaves. Emerald ash borer preference for mature leaves over young leaves might indicate that avoidance of plant secondary metabolites has a greater behavioral effect than nutrient availability for emerald ash borer. Green ash trees possess a variety of phenolic compounds that might confer resistance to emerald ash borer (Eyles et al. 2007). Nutrient deficiency of older leaves can be overcome by compensatory feeding, a phenomenon that is well known for many insect species (Scriber and Slansky 1981).

Some insects (e.g., cockroaches) exhibit negative orientation to light, whereas others (e.g., moths) show positive orientation to light (Jander 1963, Yang et al. 2003, Erber et al. 2006). The positive phototactic response of emerald ash borer adults might be adaptive because emerald ash borer females preferred green ash foliage grown under sunny conditions over foliage grown under shady conditions. Open grown trees are

also significantly more attractive to emerald ash borer adults and have higher larval densities than trees grown under partial or full shade (McCullough et al. 2009). Emerald ash borer is known to be most active in warm, sunny conditions and during sunny periods of the day (i.e., afternoon) (Chinese Academy of Science 1986, Yu 1992, Rodriguez-Saona et al. 2007). Although the underlying mechanism(s) for the preference of emerald ash borer to green ash foliage exposed to sun remain(s) to be examined, the phototaxis of emerald ash borer adults would partially account for higher numbers of beetles on open grown, sunny host trees. As discussed above, other experimental conditions being identical, insect herbivores are documented to prefer leaves from south or light-exposed sides over those from north or light-deprived sides (Schoonhoven 1977, Rowe and Potter 2000, Panzuto et al. 2001). In the case of forest tent caterpillar larvae, *M. disstria* Hübner (Lepidoptera: Lasiocampidae), the preference of sugar maple (*A. saccharum*) foliage exposed to sun over foliage exposed to shade was in part because of higher amounts of soluble sugars in foliage exposed to the sun (Panzuto et al. 2001).

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References Cited

Anulewicz, A. C., D. G. McCullough, and D. L. Cappaert. 2007. Emerald ash borer (*Agrilus planipennis*) density and canopy dieback in three North American ash species. Arb. Urban For. 33: 338–349.

Anulewicz, A. C., D. G. McCullough, D. L. Cappaert, and T. M. Poland. 2008. Host range of the emerald ash borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) in North America: results of multiple-choice field experiments. Environ. Entomol. 37: 230–241.

Bauer, L. S., R. A. Haack, D. L. Miller, T. R. Petrice, and H. Liu. 2004. Emerald ash borer life cycle. P. 8 *In* V. Mastro and R. Reardon (comp.) Emerald Ash Borer Research and Technology Development Meeting, Port Huron, MI, 30 September to 1 October 2003. USDA Forest Service Forest Health Technology Enterprise Team FHTET-2004-02, Morgantown, WV.

Chen, Y., and J. R. Ruberson. 2008. Impact of variable nitrogen fertilization on arthropods in cotton in Georgia, USA. Agric. Ecosys. Environ. 126: 281–288.

Chen, Y., and T. M. Poland. 2009. Interactive influence of leaf age, light intensity, and girdling on green ash foliar chemistry and emerald ash borer development. J. Chem. Ecol. 35: 806–815.

Chen, Y., J. R. Ruberson, and M. O. Dawn. 2008a. Nitrogen fertilization rate affects feeding, larval performance, and oviposition preference of the beet armyworm, *Spodoptera exigua*, on cotton. Entomol. Exp. Appl. 126: 244–255.

Chen, Y., E. A. Schmelz, F. Wäckers, and J. R. Ruberson. 2008b. Cotton plant, *Gossypium hirsutum* L., defense in response to nitrogen fertilization. J. Chem. Ecol. 34: 1553–1564.

Chen, Y., X. Ni, and G. D. Buntin. 2009. Physiological, nutritional, and biochemical bases of corn resistance to foliage-feeding fall armyworm. J. Chem. Ecol. 35: 297–306.

Chinese Academy of Science, Institute of Zoology. 1986. *Agrilus marcopoli* Obenberger, p. 445. *In* Editorial Committee (eds.), Agriculture insects of China (part 1). China Agriculture Press, Beijing, China.

Choh, Y., T. Shimoda, R. Ozawa, M. Dicke, and J. Takabayashi. 2004. Exposure of lima bean leaves to volatiles from herbivore-induced conspecific plants results in emission of carnivore attractants: active or passive process? J. Chem. Ecol. 30: 1305–1317.

Crook, D. J., A. Khrimian, J. A. Francese, I. Fraser, T. M. Poland, A. J. Sawyer, and V. C. Mastro. 2008. Development of a host-based semiochemical lure for trapping emerald ash borer *Agrilus planipennis* (Coleoptera: Buprestidae). Environ. Entomol. 37: 356–365.

de Groot, P., G. G. Grant, T. M. Poland, R. Scharbach, L. Buchan, R. W. Nott, L. Macdonald, and D. Pitt. 2008. Electrophysiological response and attraction of emerald ash borer to green leaf volatiles (GLVs) emitted by host foliage. J. Chem. Ecol. 34: 1170–1179.

Dickens, J. C. 2000. Orientation of Colorado potato beetle to natural and synthetic blends of volatiles emitted by potato plants. Agric. For. Entomol. 2: 167–172.

Erber, J., J. Hoormann, and R. Scheiner. 2006. Phototactic behaviour correlates with gustatory responsiveness in honey bees (*Apis mellifera* L.). Behav. Brain Res. 174: 174–180.

Eyles, A., W. Jones, K. Riedl, D. Cipollini, S. Schwartz, K. Chan, D. A. Herms, and P. Bonello. 2007. Comparative phloem chemistry of Manchurian (*Fraxinus mandshurica*) and two North American ash species (*Fraxinus americana* and *Fraxinus pennsylvanica*). J. Chem. Ecol. 33: 1430–1448.

Haack, R. A., E. Jendek, H.-P. Liu, K. R. Marchant, T. R. Petrice, T. M. Poland, and H. Ye. 2002. The emerald ash borer: a new exotic pest in North America. Newslett. Mich. Entomol. Soc. 47: 1–5.

Gouingené, S. P., and T.C.J. Turlings. 2002. The effects of abiotic factors on induced volatile emissions in corn plants. Plant Physiol. 129: 1296–1307.

Hemming, J.D.C., and R. L. Lindroth. 1999. Effects of light and nutrient availability on aspen: growth, phytochemistry, and insect performance. J. Chem. Ecol. 25: 1687–1714.

Jander, R. 1963. Insect orientation. Annu. Rev. Entomol. 8: 95–114.

Lahav, E., D. Zamet, S. Gazit, and U. Lavi. 1986. Girdling as a means of shortening the juvenile period of avocado seedlings. HortScience 21: 1038–1040.

Lelito, J. P., J. H. Tumlinson, K. Böröczky, and T. C. Baker. 2007. Visually mediated 'Paratrooper Copulations' in the mating behavior of *Agrilus planipennis* (Coleoptera: Buprestidae), a highly destructive invasive pest of North American ash trees. J. Insect Behav. 20: 537–552.

Lewis, W. J., A. N. Sparks, R. L. Jones, and D. J. Barras. 1972. Efficiency of *Cardiochiles nigriceps* as a parasitoid of *Heliothis virescens* on cotton. Environ. Entomol. 1: 468–471.

Li, C., D. Weiss, and E. E. Goldschmidt. 2003. Girdling affects carbohydrate-related gene expression in leaves, bark and roots of alternate-bearing citrus trees. Ann. Botany 92: 137–143.

Loughrin, J. H., A. Manukian, R. R. Heath, T.C.J. Turlings, and J. H. Tumlinson. 1994. Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plants. Proc. Natl. Acad. Sci. U.S.A. 91: 11836–11840.

McCullough, D. G., T. M. Poland, and D. Cappaert. 2009. Attraction of the emerald ash borer to ash trees stressed by girdling, herbicide treatment, or wounding. Can. J. For. Res. 39: 1331–1345.

McKey, D. 1979. The distribution of secondary compounds within plants, pp. 55–133. *In* G. A. Rosenthal and D. H. Janzen (eds.), Herbivores: their interaction with secondary plant metabolites. Academic, New York.

Nichols-Orians, C. M. 1991. Environmentally induced differences in plant traits: consequences for susceptibility ot a leaf-cutter ant. Ecology 72: 1609–1623.

Obeso, J. R. 1998. Effects of defoliation and girdling on fruit production in *Ilex aquifolium.* Funct. Ecol. 12: 486–491.

Ohnmeiss, T., and I. T. Baldwin. 2000. Optimal defense theory predicts the ontogeny of an induced nicotine defense. Ecology 81: 1765–1783.

Panzuto, M., F. Lorenzetti, Y. Mauffette, and P. J. Albert. 2001. Perception of aspen and sun/shade sugar maple leaf soluble extracts by larvae of *Malacosoma disstria.* J. Chem. Ecol. 27: 1963–1978.

Poland, T. M., and D. G. McCullough. 2006. Emerald ash borer: invasion of the urban forest and the threat to North America's ash resources. J. For. 104: 118–124.

Pureswaran, D. S., and T. M. Poland. 2009a. The role of olfaction in short-range mate finding by the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). J. Insect Behav. 22: 205–216.

Pureswaran, D. S., and T. M. Poland. 2009b. Host selection and feeding preference of the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) on ash (*Fraxinus* spp.). Environ. Entomol. 38: 757–765.

Reymond, P., H. Weber, M. Damond, and E. E. Farmer. 2000. Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. Plant Cell. 12: 707–719.

Rodriguez-Saona, C., J. R. Miller, T. M. Poland, T. M. Kuhn, G. W. Otis, T. Turk, and D. L. Ward. 2007. Behavior of adult *Agrilus planipennis* (Coleoptera: Buprestidae). Great Lakes Entomol. 40: 1–16.

Rodriguez-Saona, C., T. M. Poland, J. R. Miller, L. L. Stelinski, G. G. Grant, P. de Groot, L. Buchan, and L. MacDonald. 2006. Behavioral and electrophysiological responses of the emerald ash borer, *Agrilus planipennis,* to induced volatiles of Manchurian ash, *Fraxinus mandshurica.* Chemoecology 16: 75–86.

Roper, T. R., and L. E. Williams. 1989. Net CO_2 assimilation and carbohydrate partitioning of grapevine leaves in response to trunk girdling and gibberellic acid application. Plant Physiol. 89: 1136–1140.

Rowe, W. J., and D. A. Potter. 2000. Shading effects on susceptibility of *Rosa* spp. to defoliation by *Popillia japonica* (Coleoptera: Scarabaeidae). Environ. Entomol. 29: 502–508.

SAS Institute. 1999. SAS/STAT user's guide, version 8th ed. SAS Institute, Cary, NC.

Schoonhoven, L. M. 1977. Feeding behaviour in phytophagous insects, on the complexity of the stimulus situation. Colloques Int. Ctr. Natl. Recherche Sci. 265: 391–398.

Scriber, J. M., and F. Slansky, Jr. 1981. The nutritional ecology of immature insects. Annu. Rev. Entomol. 26: 183–211.

Slansky, F., Jr., and J. M. Scriber. 1985. Food consumption and utilization, pp. 87–163. *In* G. A. Kerkut and L. I. Gilbert (eds.), Comprehensive insect physiology, biochemistry and pharmacology, vol. 4. Pergamon, Oxford, United Kingdom.

Stout, M. J., K. V. Workman, R. M. Bostock, and S. S. Duffey. 1998. Specificity of induced resistance in the tomato, *Lycopersicon esculentum.* Oecologia (Berl.) 113: 74–81.

Strauss, S. Y., R. E. Irwin, and V. M. Lambrix. 2004. Optimal defence theory and flower petal colour predict variation in the secondary chemistry of wild radish. J. Ecol. 92: 132–141.

Takabayashi, J., M. Dicke, and M. A. Posthumus. 1994. Volatile herbivore-induced terpenoids in plant-mite interactions: variation caused by biotic and abiotic factors. J. Chem. Ecol. 20: 1329–1354.

Yang, E.-C., D.-W. Lee, and W.-Y. Wu. 2003. Action spectra of phototactic responses of the flea beetle, *Phyllotreta striolata.* Physiol. Entomol. 28: 362–367.

Yu, C. 1992. *Agrilus marcopoli* Obenberger (Coleoptera: Buprestidae), pp. 400–401. *In* G. Xiao (ed.), Forest insects of China, 2nd ed. China Forestry Publishing House, Beijing, China.

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