Response of Cranberry Weevil (Coleoptera: Curculionidae) to Host Plant Volatiles

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ABSTRACT The oligophagous cranberry weevil, *Anthonomus musculus* Say, causes economic losses to blueberry growers in New Jersey because females deposit eggs into developing flower buds and subsequent larval feeding damages buds, which fail to produce fruit. A cost-effective and reliable method is needed for monitoring this pest to correctly time insecticide applications. We studied the behavioral and antennal responses of adult *A. musculus* to its host plant volatiles to determine their potential for monitoring this pest. We evaluated *A. musculus* response to intact and damaged host plant parts, such as buds and flowers in Y-tube bioassays. We also collected and identified host plant volatiles from blueberry buds and open flowers and performed electroantennograms with identified compounds to determine the specific chemicals eliciting antennal responses. Male weevils were more attracted to blueberry flower buds and were repelled by conspecific-damaged buds compared with clean air. In contrast, females were more attracted to open flowers compared with flower buds. Nineteen volatiles were identified from blueberry buds; 10 of these were also emitted from blueberry flowers. Four of the volatiles emitted from both blueberry buds and flowers [hexanol, (Z)-3-hexenyl acetate, hexyl acetate, and (Z)-3-hexenyl butyrate] elicited strong antennal responses from *A. musculus.* Future laboratory and field testing of the identified compounds in combination with various trap designs is planned to develop a reliable monitoring trap for *A. musculus.*

KEY WORDS highbush blueberry, Y-tube bioassay, headspace analysis, gas chromatography-mass spectrometry, electroantennogram

In the process of host selection, arthropod pests may use plant volatiles to locate hosts (Bruce et al. 2005 and references therein). Host plant volatiles are often used as attractants in traps, thus increasing monitoring and/or trap-and-kill efficiency (Rodriguez-Saona and Stelinski 2009). The oligophagous flower bud feeding cranberry weevil (a.k.a. blossom weevil, *Anthonomus musculus* Say, Coleoptera: Curculionidae) may use specific volatile chemicals to locate a suitable host plant. Within the Curculionidae, i.e., the apple blossom weevil (*A. pomorum* L.), the boll weevil (*A. grandis* Boheman), and the strawberry blossom weevil (*A. rubi* Herbst) respond electrophysiologically to host plant volatiles (Dickens et al. 1990, Kalinová et al. 2000, Bichao et al. 2005); and numerous species, i.e., the white pine weevil [*Pissodes strobi* (Peck)], the banana weevil (*Cosmopolites sordidus* Germar), and the black vine weevil [*Otiorhynchus sulcatus* (Fabricius)] respond to host plant volatiles in behavioral bioassays (Budenberg et al. 1993, Wibe et al. 1997, van Tol and Visser 2002). Previous studies by Mechaber (1992) using a Y-tube olfactometer showed that adult *A. musculus* were attracted to damaged cranberry (*Vaccinium macrocarpon* Ait.) flower buds compared with clean air or healthy plants. However, that single study to date did not test the behavioral response of *A. musculus* to other plant parts that also receive damage in the field, such as leaf buds and open flowers.

Anthonomus musculus is native to North America and ranges from Ontario and New England south to Florida and west to the Rocky Mountains (Dittl 1988). This pest causes economic losses to commercial cranberry and blueberry growers in Massachusetts, New Jersey, Wisconsin, and Michigan. In early spring, eggs are deposited into developing flower buds and subsequent larval feeding prevents the development of fruit that causes significant economic losses to commercial blueberry growers. The immature stages are protected by the buds during their development; therefore, management strategies typically target the mobile adults. Adults feed on the developing buds, flowers, and leaves, and

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they are active during the day. Their peak activity is during the spring when buds and flowers are developing, but they can be found in blueberry fields throughout the growing season (Doehlert and Tumlinson 1947; Z.S., personal observation). Adult weevils actively move around on plants on sunny days and therefore can be monitored in highbush blueberries with beat trays. On cloudy days, visual assessment of blossom damage provides an alternative monitoring technique. These techniques are labor intensive, unreliable, and inaccurate because of the patchy distribution of the pest. Furthermore, weevils tend to drop to the ground when scouts move plants while searching for them. A cost-effective and reliable method is needed for monitoring this pest and timing of insecticide applications.

To date, semiochemical attractants have not been identified for *A. musculus;* therefore, the goal of this study was to assess the responses of this species to host plant volatiles and determine the possibility of their use in behavioral manipulation of this pest. Our specific objectives were to (1) evaluate the behavioral responses of adult *A. musculus* to intact and insect-damaged host plant parts, such as buds and flowers; (2) isolate and identify host plant volatiles from blueberry buds (*A. musculus* damaged and undamaged) and open flowers; and (3) investigate antennal responses of weevils to individual volatile compounds to identify the specific chemicals eliciting an antennal response.

Methods and Materials

Y-Tube Assays. *Anthonomus musculus* were collected with beating trays in March–April 2008 from two commercial highbush blueberry (*Vaccinium corymbosum* L. variety Duke) farms in central New Jersey. After returning them to the laboratory, weevils were maintained in environmental chambers (16:8 L:D, 15°C) in screen cages (30 by 30 by 30 cm) with access to fresh buds and foliage ad libitum collected daily from an unsprayed highbush blueberry field (variety Duke).

The olfactory preferences of *A. musculus* were tested using a Y-tube olfactometer (two 8-cm and one 12-cm arms; 2 cm diameter, ground glass joints; Analytical Research Systems, Gainesville, FL). Air was filtered through activated charcoal and was split into two 1 liter/min air streams. Subsequently, each air stream was delivered through glass tubes (14 cm long, 2 cm diameter) that held odor sources and that were connected to the olfactometer. Odor sources from the plants consisted of 0.3–0.4 g of field-collected plant material (buds: leaf or flower, damaged or undamaged; or fully opened flowers). Before the start of assays, all buds remained attached to branches in water overnight in a 15°C environmental chamber. One half of these buds were exposed to weevils from 1400 to 0900 hours to attain damaged buds (presence of damage was verified by visual observation under a stereomicroscope). Buds were detached from the branches as needed, immediately before use in assays.

Weevils were placed individually at the bottom of the Y and were observed until a choice was made or for a maximum of 10 min. Thereafter, weevils were sexed based on characters of the pygidium (J. Prena, personal communication). Weevils not making a choice within 10 min were recorded as nonresponding; each individual was tested once. After each replicate, the position of the odor sources was randomized to exclude the possibility of positional bias. Treatment types were changed after every two replicate runs, and new odor sources were prepared every 2–3 h. After each weevil and odor source treatment run, the glass tubes were first rinsed with methanol and then with hexane and subsequently baked in a drying oven at 60°C for 15 min. Each experiment was replicated 17–26 times over a period of 7–12 d. All experiments were performed between 0900 and 1700 hours at 21 ± 2°C and 800–900 lux, under laboratory conditions.

Volatile Collection and Analysis. All plant material for the volatile collections was obtained from mature highbush blueberry plants (variety Duke) grown in an experimental field in Chatsworth, NJ. Flower volatiles were collected using a push-pull system (Rodriguez-Saona et al. 2001, 2006). Fresh, field-collected branches with ≈200 fully open flowers were placed inside a 4-liter volatile collection chamber ($N = 3$; Analytical Research Systems). Purified air entered through valves near the top of each chamber at 2 liters/min, and volatiles were collected in Alltech Super-Q adsorbent traps (30 mg/trap; Analytical Research Systems) by pulling purified air from the chambers at a rate of 1 liter/min. Bud volatiles were collected by placing 20 field-collected flower buds in a 10-ml glass vial ($N = 4$). For the weevil-damaged buds, 10 field-collected weevils were added to 20 flower buds in a 10-ml glass vial ($N = 8$). Air was pushed in (1 liter/min) through a charcoal filter and out through a Super-Q trap. Volatiles were collected from 0900 to 1700 hours.

The collected volatiles from the Super-Q traps were eluted with dichloromethane (150 μl), and 400 ng of n-octane (Sigma-Aldrich, St. Louis, MO) was added as an internal standard. Compounds were separated and quantified on a Hewlett–Packard 6890 Series gas chromatograph (GC), equipped with a flame ionization detector (FID) and an Agilent HP-1 column (10 m by 0.53 mm by 2.65 μm), and using He as the carrier gas (constant flow = 5 ml/min, velocity = 39 cm/s). The temperature program was 40°C for 1 min, 14°C/min to 180°C (2 min), and 40°C/min to 200°C, where it was maintained for 2 min. Individual compounds (ng/g of wet material/h) were quantified based on comparisons of peak areas from GC-FID with the internal standard.

Identification of compounds was carried out on a Varian 3400 gas chromatograph (Varian Inc, Palo Alto, CA) coupled with a Finnigan MAT 8230 mass spectrometer (MS; Finnigan MAT, Bremen, Germany),

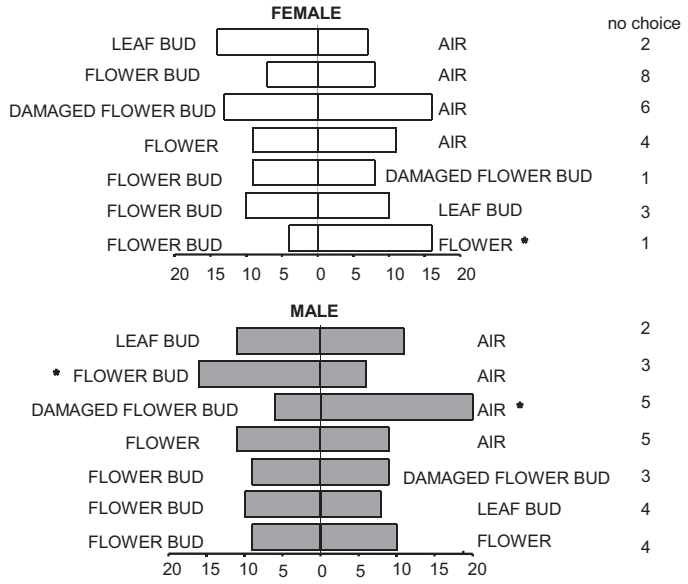

Fig. 1. Responses of *A. musculus* in Y-tube olfactometer choice test with females (top) and males (bottom). Asterisks denote statistically significant preference for a resource at $\alpha = 0.05$ (*G*-test with William's correction).

equipped with a Supelco MDN-5S column (30 m by 0.32 mm by 0.25 μm), with He as the carrier gas. The temperature program was 35°C for 1 min, 4°C/min to 170°C, and 15°C/min to 280°C. The MS data were acquired and processed in a Finnigan MAT SS300 data system. Compounds were identified by comparison of spectral data with those from the NIST library and by the GC retention index (Jennings and Shibamoto 1980, Adams 2001) and confirmed by comparing their retention times with those of commercially available compounds.

Electroantennogram Analysis. Antennal receptivity of adult weevils to 27 selected synthetic compounds found in blueberry buds and flowers was determined by electroantennogram (EAG) analysis. Adult weevils were collected from blueberry fields in Burlington Co., NJ, in 2007. The synthetic products were ≥95% pure, with the exception of linalool oxide and farnesene (mixtures of isomers), and ocimene, which was 70% pure (Sigma-Aldrich and Bedoukian, Danbury, CT).

A working solution (10 μg/μl) of the synthetic compounds was prepared in high-performance liquid chromatography (HPLC)-grade hexane. For each replicate, one *A. musculus* antenna was carefully removed from the insect, and the base was inserted into a reference electrode (a capillary tube filled with physiological saline solution consisting of 7.5 g NaCl, 0.21 g $CaCl_2$, 0.35 g KCl, and 0.2 g $NaHCO_3$ in 1 liter H_2O), whereas the tip of the antenna was inserted into a recording glass capillary electrode. A current of humidified and charcoal-purified air (0.7 liters/min) was constantly directed over the antenna through a 10-mm diameter glass tube. Ten micrograms of each chemical was individually pipetted onto a piece (0.5 by 2 cm) of clean filter paper (Whatman No.1; Whatman International, Maidstone, United Kingdom), which was subsequently exposed to air for 20 s to allow for solvent evaporation. Subsequently, filter paper strips were inserted into a glass Pasteur pipette (sample cartridge), which were used to deliver odorants 100 s thereafter. A new cartridge was prepared for each antennal preparation. The stimulus was created by inserting a sample cartridge through a side hole located at the mid-point of the glass tube that provided the airflow to the antenna (0.5 liters/min). A stimulus flow controller (CS-05; Syntech, Hilversum, The Netherlands) was used to generate a 1-s-long stimulus at 1-min intervals. The signals generated by the antenna were passed through a high-impedance amplifier (NL 1200; Syntech) and displayed on a monitor using Syntech software for processing EAG signals. Hexane (control) was presented at the beginning and end of each trial, and synthetic compounds were tested in a random order. Seven female and six male antennae were tested for their response to each chemical.

Statistical Analyses. For each treatment, a *G*-test with William's correction (Sokal and Rohlf 1995) was conducted on the numbers of insects making a choice, which tested the null hypothesis of no preference. The differences in the amount of chemical compounds, expressed as a percent of total volatile emissions, between damaged and undamaged buds were arcsine transformed [$\text{asin}(x + 0.1)$] and analyzed as a split-plot design (damage – whole plot, chemical compound – subplot), followed by Tukey-adjusted ls-means separation (SAS Institute, Cary, NC). EAG data were transformed [$\ln(x + 0.5)$] to meet the assumptions of normality and homogeneity of variances. The

values of the EAG depolarization amplitude after exposure to the chemical compounds were analyzed (SAS Institute) by two-way analyses of variance (ANOVAs) with sex of insect and type of chemical compound as the two factors. Significant ANOVA results were followed by a Dunnett's test with adjustment for means comparison ($P < 0.05$).

Results

Y-Tube Assays. Eighty-two percent of all the tested weevils made a choice. There were no differential responses based on sex (18% of both males and females did not make a choice). Overall, when a host plant cue was offered opposite clean air, 43% of the weevils chose the host plant cues. Significantly more *A. musculus* females chose open flowers compared with flower buds ($G_{adj} = 7.7$, df = 1, $P < 0.01$; Fig. 1). Female weevils were not attracted to leaf or flower buds compared with clean air, and damaged flower buds were not more attractive than undamaged flower buds. Male weevils were attracted to intact flower buds ($G_{adj} = 4.6$, df = 1, $P < 0.01$) and were repelled by damaged flower buds ($G_{adj} = 7.9$, df = 1, $P < 0.01$) relative to clean air (Fig. 1). Flower buds and leaf buds or flowers elicited similar responses for males, and neither sex showed differential attraction to damaged versus undamaged flower buds.

Identification of Plant Volatiles. Nineteen volatiles were identified by GC-MS from flower buds (Fig. 2; Table 1) and 37 from open flowers (Fig. 3; Table 2). Total volatile emission (ng/g plant tissue/h) from flowers was 2.3-fold higher than from flower buds. Compounds ranged from n-C_6 to n-C_{15} in both developmental stages. From a functional point of view, (C_6) green leaf volatiles were present in both bud and flower samples: (Z)-3-hexen-1-ol and hexanol comprised nearly 30% of intact bud volatiles but only 3% of flower volatiles. (Z)-3-hexenyl acetate represented 20% of the total volatiles in intact buds but only 3% in flowers. The two main undamaged flower bud volatiles, as a proportion of total emission, were (Z)-3-hexen-1-ol and (Z)-3-hexenyl acetate (Table 1). Methyl salicylate was the most abundant volatile in damaged buds (19%), followed by (Z)-3-hexenyl pentanoate (11%) and nonanal (10%) (Table 1). Nonanal and (Z)-3-hexenyl pentanoate were significantly more abundant in damaged than in undamaged bud samples ($t = 5.11$, $P < 0.01$ and $t = 3.98$, $P = 0.04$, respectively). (Z)-3-hexen-1-ol was significantly more abundant in undamaged (4.8%) compared with damaged (10.4%) buds ($t = -4.29$, $P = 0.01$; Table 2). Cinnamyl alcohol was the main flower volatile: it comprised 59% of the total volatiles emitted (Table 2). Ten volatiles were common to both buds and flowers (see italicized text in Tables 1 and 2). Twenty-five volatiles found in flowers were not present in flower buds, and only eight bud volatiles found only in buds were not found in flowers.

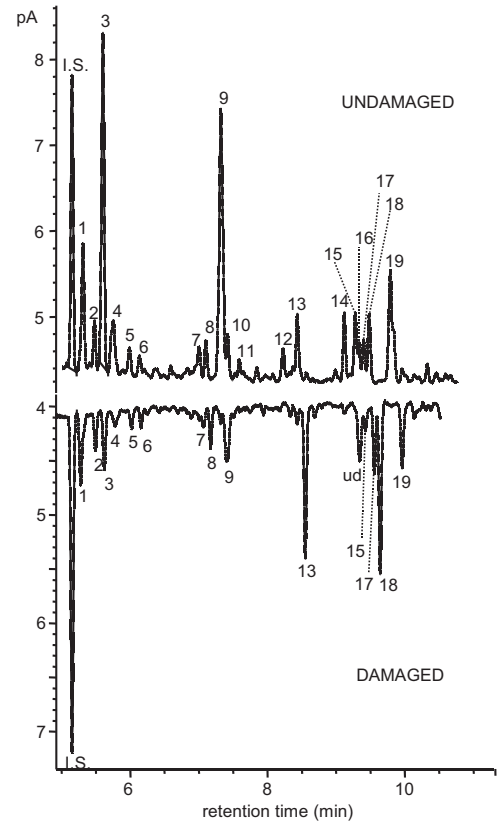

Fig. 2. Representative GC-FID profiles of headspace collected from highbush blueberry (*V. corymbosum* variety Duke) flower buds that were either left undamaged (top) or that were damaged by 10 *A. musculus* (bottom). Peak numbers correspond to chemical compound numbers in Table 1. I.S., internal standard (n-octane). Volatiles were collected from 0900 to 1700 hours. Peak marked "ud" in damaged buds' chromatogram is an undetermined compound.

EAG Analysis. The EAG magnitudes of antennal responses were significantly different among the tested chemicals ($F = 2.14$; df = 27,308; $P = 0.001$). The EAG responses between males and females were similar ($F = 2.52$; df = 1,308; $P = 0.11$), and the interaction between chemical compound and sex of insect was also not significantly different ($F = 0.55$; df = 27,308; $P = 0.96$). Antennal responses were significantly greater compared with air in the case of 12 chemicals: (Z)-3-hexenyl acetate, methyl salicylate, nonanal, hexyl acetate, hexanol, linalool oxide, cinnamyl alcohol, linalool, myrcene, cis-3-hexenyl butyrate, α-humulene, and limonene (Fig. 4). The EAG response was statistically equal to air in tests with the terpenes α-pinene, β-pinene, ocimene, eucalyptol, caryophyllene, caryophyllene oxide, β-farnesene, and farnesene; the esters hexyl butyrate, (Z)-3-hexenyl propionate, hexyl-2-methyl butyrate, (Z)-3-hexenyl hexanoate, and (Z)-3-hexenyl-2-methyl butyrate; the alkane tridecane; and the ketone 2-undecanone (Fig. 4).

Table 1. Volatiles identified by GC-MS from 20 highbush blueberry (variety Duke) flower buds that were either left undamaged or were damaged by 10 *A. musculus*

	Chemical name	Undamaged		Damaged	
		Mean ± SE	Percent total	Mean ± SE	Percent total
1	Diacetone alcohol	31.40 ± 11.47	3.96	31.89 ± 5.50	7.28
2	Ethyl isopentanoate	40.69 ± 12.03	5.13	27.21 ± 3.55	6.21
3	*(Z)-3-hexen-1-ol*	*189.87 ± 14.49*	*23.96*	*38.67 ± 3.28*	*8.83**
4	*Hexanol*	*46.60 ± 5.11*	*5.88*	*17.22 ± 1.51*	*3.93*
5	*2-Heptanone*	*13.92 ± 1.47*	*1.76*	*14.36 ± 0.48*	*3.28*
6	*Ethyl pentanoate*	*13.29 ± 2.11*	*1.68*	*12.54 ± 0.00*	*2.86*
7	6-Methyl-5-heptene-2-one	20.43 ± 3.20	2.58	18.07 ± 1.72	4.12
8	*β-Pinene*	*15.00 ± 2.12*	*1.89*	*16.81 ± 1.42*	*3.84*
9	*(Z)-3-hexenyl acetate*	*159.76 ± 14.84*	*20.16*	*38.79 ± 2.99*	*8.85*
10	*Hexyl acetate*	*18.32 ± 2.06*	*2.31*	*0 ± 0*	*0*
11	*(E)-2-hexenyl acetate*	*15.33 ± 1.59*	*1.93*	*0 ± 0*	*0*
12	Linalool oxide	16.45 ± 2.69	2.08	*0 ± 0*	*0*
13	Nonanal	38.46 ± 2.04	4.85	45.50 ± 8.56	10.38*
14	*(Z)-3-hexenyl butyrate*	*23.60 ± 2.31*	*2.98*	*0 ± 0*	*0*
15	*(E)-2-hexenyl butyrate*	*23.48 ± 3.45*	*2.96*	*20.31 ± 1.04*	*4.63*
16	Decanal	13.29 ± 1.56	1.68	0 ± 0	0
17	Methyl salicylate	22.23 ± 7.63	2.80	30.00 ± 3.48	19.03
18	(Z)-3-hexenyl pentanoate	20.11 ± 4.67	2.54	48.43 ± 10.47	11.05[a]
19	Hexyl pentanoate	70.32 ± 6.44	8.87	25.03 ± 4.35	5.71
	Total	792.56 ± 101.27	100	438.22 ± 81.27	100

Volatiles were collected between 0900 and 1700 hours. Italics indicate compounds that are common to buds and flowers, means ± SE represent ng/g plant tissue/h.

*Significant differences between damaged and undamaged buds in percent total volatiles emitted ($\alpha = 0.05$).

Discussion

This study provides the first record of behavioral and electrophysiological responses of *A. musculus* to blueberry volatiles emitted from buds and flowers. In Y-tube bioassays, Mechaber (1992) detected no difference between male and female *A. musculus* response to cranberry vines, but in our Y-tube studies, *A. musculus* sex determined the behavioral outcome. The results of our Y-tube bioassays indicated that female *A. musculus* were attracted to open

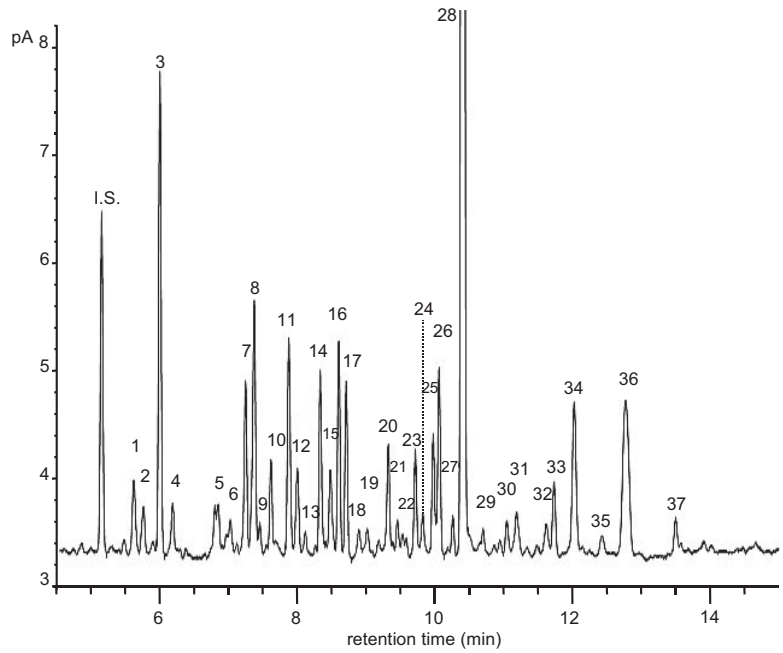

Fig. 3. Representative GC-FID profiles of headspace collected from highbush blueberry (V. corymbosum variety Duke) open flowers. Peak numbers correspond to chemical compound numbers in Table 2. I.S., internal standard (n-octane). Volatiles were collected from 0900 to 1700 hours.

Table 2. Volatiles identified by GC-MS from highbush blueberry (variety Duke) open flowers

	Chemical name	Mean ± SE	Percent total
1	*(Z)-3-hexen-1-ol*	*12.23 ± 5.40*	*0.67*
2	*Hexanol*	*8.12 ± 2.64*	*0.44*
3	*2-Heptanone*	*67.63 ± 2.01*	*3.71*
4	*Ethyl pentanoate*	*7.31 ± 0.41*	*0.40*
5	α-Pinene	12.02 ± 4.90	0.66
6	*β-Pinene*	*6.24 ± 3.13*	*0.34*
7	Ethyl-3-hexanoate	48.05 ± 17.15	2.63
8	*(Z)-3-hexenyl acetate*	*48.80 ± 3.73*	*2.67*
9	*Hexyl acetate*	*4.95 ± 1.57*	*0.27*
10	*(E)-2-hexenyl acetate*	*19.84 ± 5.88*	*1.09*
11	Limonene	61.69 ± 18.58	3.38
12	Eucalyptol	21.63 ± 3.59	1.19
13	Ocimene	3.97 ± 0.46	0.22
14	Unknown	37.91 ± 8.89	2.08
15	Linalool	19.91 ± 2.99	1.09
16	Myrcenone	28.02 ± 7.16	1.54
17	Unknown	23.20 ± 4.49	1.27
18	Hexyl ester	3.99 ± 1.75	0.22
19	Hexenyl ester	7.69 ± 1.79	0.42
20	*(Z)-3-hexenyl butyrate*	*15.86 ± 5.09*	*0.87*
21	Hexyl butyrate	6.83 ± 0.91	0.37
22	*Hexenyl butyrate*	*6.24 ± 0.32*	*0.34*
23	(Z)-3-hexenyl-2-methyl butyrate	23.50 ± 3.35	1.29
24	Hexyl-2-methyl butyrate	7.57 ± 1.61	0.41
25	2-Undecanone	28.24 ± 7.30	1.55
26	n-Tridecane	36.22 ± 6.13	1.98
27	Unknown	4.34 ± 0.95	0.24
28	Cinnamyl alcohol	1075.94 ± 303.12	58.96
29	Copaene	4.13 ± 0.78	0.23
30	β-Bourbonene	9.23 ± 1.69	0.51
31	(Z)-3-hexenyl hexanoate	15.08 ± 3.39	0.83
32	Paraffin (impurity)		
33	Paraffin (impurity)		
34	Caryophyllene	35.38 ± 3.75	1.94
35	Humulene		
36	γ-Cadinene	60.54 ±14.57	3.32
37	Farnesene	7.56 ± 0.49	0.41
	Total	1824.82 ± 328.43	100.00

Volatiles were collected between 0900 and 1700 hours. Gray areas indicate compounds that are common to buds and flowers, means ± SE represent ng/g plant tissue/h.

flowers when they were offered opposite to flower buds but not compared with clean air. The lack of attraction to other plant parts could indicate that host plant location is governed by chemical cues emanating from other plant parts or that female *A. musculus* use visual, tactile, and/or conspecific cues in the process of host location. Male *A. musculus* were attracted to undamaged flower buds but were repelled by damaged flower buds compared with clean air. When these two treatments were offered simultaneously, males did not respond significantly to either odor source. This is likely because male response to attractive intact flower bud volatiles was antagonized by repellant damaged flower bud volatiles because of the mixing of volatiles from both odor sources at the juncture of the Y-tube. Furthermore, males showed no significant attraction to open flowers in the Y-tube bioassays. These results indicate that male *A. musculus* might be more attracted to host plant volatiles earlier in the blueberry season than females. Sex-related differences in attraction to host plant volatiles have been documented for other *Anthonomus* species (i.e., *A. grandis* Boheman; Dickens 1986).

The differential responses between the sexes also suggest that both host plant odor and potential *A. musculus* pheromones may serve as attractants for colonization. In a number of related *Anthonomus* species (*A. grandis* Boheman [McKibben et al. 1971]), including *A. eugenii* Cano (Eller et al. 1994) and *A. rubi* Herbst (Innocenzi et al. 2001), male-produced pheromones attract conspecifics to plants where females can find both mates and oviposition sites. If this is true for *A. musculus*, it could also provide an explanation for the males' avoidance of the volatiles from damaged buds: if males locate hosts soon after emergence when only buds are available, it is advantageous for them to be able to discriminate and avoid previously colonized host patches to reduce intraspecific competition. Pheromones and protandry have not yet been studied in *A. musculus*, but protandry is common in several insect species with nonoverlapping generations, a situation in which selection could act on sexual difference (Rutowsky 1997).

Our results indicated that there is a substantial difference in both the number of volatiles and blend proportions of the volatiles released by blueberry plants depending on phenological stage. The spring emergence of *A. musculus* coincides with the first stages of bud development in its host; thereafter, adults continue to damage developing buds, flowers, and leaves (Mechaber 1992). Consequently, host plant volatiles in early and in late phenological stages of the host plant could play a role in *A. musculus* host location. In fact, phenological changes in host plant volatiles and their effect on antennal responses have been documented for the codling moth, *Cydia pomonella* L. (Bengtsson et al. 2001). Kalinová et al. (2000) have shown that intact apple buds from the same variety emit qualitatively and quantitatively different volatiles in different phenological stages. Because *A. musculus* thrives on both the bud and flowering stages, it is reasonable to assume that host plant recognition in this species depends on a blend of more ubiquitous plant volatiles as opposed to the presence or absence of stage specific compounds. Indeed, there are only a few examples of insects that use taxonomically characteristic compounds that are unique to particular host plant species or developmental stage (Visser 1988, Bruce et al. 2005). In the case of blueberries and *A. musculus*, a variable volatile blend of (Z)-3-hexenyl acetate, hexyl acetate, hexanol, 2-heptanone, ethyl pentanoate, and β-pinene (compounds present in both blueberry buds and flowers) may provide information on the location and on the developmental stage of the host plant.

When buds were damaged by *A. musculus*, the absolute volatile emissions were on average one half of that measured from healthy buds; in addition, five chemicals were missing from the damaged bud volatile profile. These differences might explain the observed avoidance of damaged buds by male *A.*

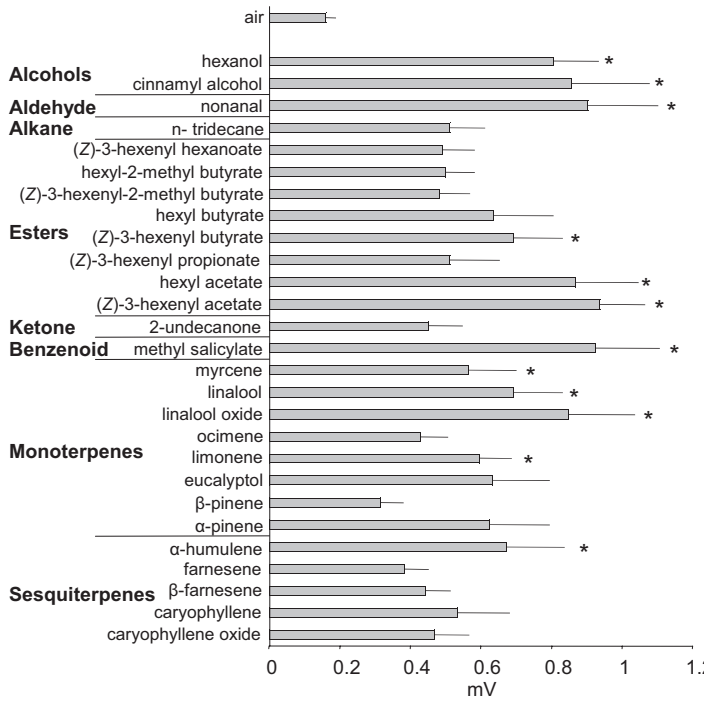

Fig. 4. Mean (±SE) EAG response of *A. musculus* antennae to synthetic compounds (10 μg) selected from highbush blueberry headspace analysis. Asterisks denote significantly greater antennal responses compared with air (α = 0.05, Dunnett's test). Results of male and female weevil responses combined in the graph (N = 11).

musculus in our behavioral assays. Our findings are in contrast with the general tenet of herbivore-induced volatile literature, which states that injury by phytophagous insets increases absolute volatile emissions in plants (Paré and Tumlinson 1997, Dicke 1999). Our results are difficult to compare with previous studies that were based mostly on annual plant systems that examined leaf damage. In some stages of infestation, Hern and Dorn (2001) observed a marked decrease in volatile emission or even the absence of some compounds from *C. pomonella L.*–infested compared with healthy apples. It is therefore possible that fruiting plant parts have considerably different volatile responses than vegetative organs when damaged by herbivores. In our samples, damaged buds emitted significantly greater amounts of nonanal than healthy buds, which concurs with the results obtained from potatoes with *Leptinotarsa decemlineata* (Say) feeding damage (Bolter et al. 1997). Nonanal is a ubiquitous aliphatic aldehyde found in essential oils such as rose, citrus, and certain pine oils, and it induces a range of behavioral responses in scolytid beetles (de Groot and Poland 2003); therefore, it may be an induced response for direct defense in blueberries.

Identification of volatiles specifically released by blueberry buds and flowers combined with EAG screening of these compounds suggests that (Z)-3-hexenyl acetate, hexyl acetate, and hexanol may be used by *A. musculus* for host location. Compounds such as (Z)-3-hexenyl acetate are ubiquitous green leaf volatiles that are perceived by a large number of insects representing most of the major insect orders (Visser 1986, Bruce et al. 2005). Green leaf volatiles may be constitutively produced or induced by herbivory, and they may be involved in host plant location (Loughrin et al. 1996) or they may serves as reliable indicators of nonhost plants (Wilson et al. 1996). Furthermore, green leaf volatiles have been shown to synergize with pheromones in other weevil species (Dickens et al. 1990, Larsson et al. 2001).We will study this for *A. musculus* in the future. Although we found behavioral differences between male and female *A. musculus*, the similarity of EAG responses between the sexes is not surprising given that they share a common host plant. Similarly, Kalinová et al. (2000) did not observe a difference in *A. pomorum* EAG response to apple volatile between males and females.

In summary, this study is a first step toward identification of host plant attractants for semiochemical-based monitoring of *A. musculus* populations in blueberries. Five main conclusions can be drawn from our studies: (1) blueberry buds emit volatiles that are attractive to male *A. musculus;* (2) 19 volatiles were identified from blueberry buds, and (3) 10 of these are also emitted from blueberry flowers; (4) 4 of the volatiles emitted from both blueberry buds and flowers [hexanol, (Z)-3-hexenyl acetate, hexyl acetate, and (Z)-3-hexenyl butyrate] elicit significant antennal responses from *A. musculus;* and (5) *A. musculus*–damaged buds have reduced volatile

emission compared with healthy buds. Our future studies will focus on examining the behavioral responses of *A. musculus* to the synthetic compounds that elicited significant antennal responses. We will quantify *A. musculus* responses to various blends and dosages of these compounds in combination with different traps designs to develop an effective monitoring strategy for this pest. Furthermore, we will examine the role of conspecific cues in the behavioral ecology of *A. musculus.*

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