

# Identification and Field Evaluation of Attractants for the Cranberry Weevil, *Anthonomus musculus* Say

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**Abstract** Studies were conducted to develop an attractant for the cranberry weevil, *Anthonomus musculus*, a pest of blueberry and cranberry flower buds and flowers in the northeastern United States. In previous studies, we showed that cinnamyl alcohol, the most abundant blueberry floral volatile, and the green leaf volatiles (*Z*)-3-hexenyl acetate and hexyl acetate, emitted from both flowers and flower buds, elicit strong antennal responses from *A. musculus*. Here, we found that cinnamyl alcohol did not increase capture of *A. musculus* adults on yellow sticky traps compared with unbaited controls; however, weevils were highly attracted to traps baited with the *Anthonomus*

*eugenii* Cano aggregation pheromone, indicating that these congeners share common pheromone components. To identify the *A. musculus* aggregation pheromone, headspace volatiles were collected from adults feeding on blueberry or cranberry flower buds and analyzed by gas chromatography-mass spectrometry. Three male-specific compounds were identified: (*Z*)-2-(3,3-dimethyl-cyclohexylidene) ethanol (*Z* grandlure II); (*Z*)-(3,3-dimethylcyclohexylidene) acetaldehyde (grandlure III); and (*E*)-(3,3-dimethylcyclohexylidene) acetaldehyde (grandlure IV). A fourth component, (*E*)-3,7-dimethyl-2,6-octadien-1-ol (geraniol), was emitted in similar quantities by males and females. The emission rates of these volatiles were about 2.8, 1.8, 1.3, and 0.9 ng/adult/d, respectively. Field experiments in highbush blueberry (New Jersey) and cranberry (Massachusetts) examined the attraction of *A. musculus* to traps baited with the male-produced compounds and geraniol presented alone and combined with (*Z*)-3-hexenyl acetate and hexyl acetate, and to traps baited with the pheromones of *A. eugenii* and *A. grandis*. In both states and crops, traps baited with the *A. musculus* male-produced compounds attracted the highest number of adults. Addition of the green leaf volatiles did not affect *A. musculus* attraction to its pheromone but skewed the sex ratio of the captured adults towards females. Although the role of plant volatiles in host-plant location by *A. musculus* is still unclear, our studies provide the first identification of the primary *A. musculus* aggregation pheromone components that can be used to monitor this pest in blueberry and cranberry pest management programs.

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## Introduction

The genus *Anthonomus* contains more than 100 North American species (Borror et al., 1989), many of which are considered among the most destructive pests of cultivated plants. One such example is the cranberry weevil, *Anthonomus musculus* Say (Coleoptera: Curculionidae), a pest that causes major economic losses annually to highbush blueberries, *Vaccinium corymbosum* L., and cranberries, *Vaccinium macrocarpon* Aiton (Ericales: Ericaceae), in the northeastern United States (Marucci, 1966; Averill and Sylvia, 1998; Long and Averill, 2003). Native to North America, *A. musculus* is distributed from Ontario (Canada) and New England (United States) to the Rocky Mountains and Florida (United States) (Lacroix, 1926; Averill and Sylvia, 1998). Although it utilizes many host plants within the family Ericaceae (Mechaber, 1992), *A. musculus* is considered a major pest only on cultivated *Vaccinium* spp. In New Jersey, this insect is commonly recognized as one of the top pests of highbush blueberries, whereas in Massachusetts it is considered a pest of cranberries (Doehlert and Tomlinson, 1947; Averill and Sylvia, 1998). *Anthonomus musculus* rarely occurs on cranberries in New Jersey (Mechaber, 1992).

*Anthonomus musculus* feeding and oviposition activity causes damage to blueberry and cranberry flower buds and flowers. Females lay their eggs inside unopened flowers, and the larvae complete their development within the flower buds. Most *A. musculus* adults overwinter outside commercial blueberry and cranberry fields, under debris and fallen leaves in surrounding wooded areas. Mechaber (1992) suggested that *A. musculus* can complete two generations a year, but a single generation is thought to be typical (Lacroix, 1926; Doehlert and Tomlinson, 1947). In New Jersey, adults become active in late April and move from overwintering sites to cultivated blueberries before the buds start to swell (Doehlert and Tomlinson, 1947). The adults emerge in late May–June (summer generation), and feed on tender leaves and young fruit. The majority of these adults eventually move out of the blueberry fields to alternative hosts in the surrounding woods, where they have been observed to mate (Mechaber, 1992; Z. Szendrei, personal observation). In Massachusetts, overwintering adults move from wild hosts (e.g., wild *Vaccinium* spp.) to cranberries in late May–June, mate, and lay eggs on the developing flower buds (Mechaber, 1992; Averill and Sylvia, 1998). Summer generation adults appear from late June to early July, and these adults will overwinter (Lacroix, 1926).

Current monitoring methods for *A. musculus* are labor-intensive and inaccurate. Because adults overwinter in wooded areas surrounding blueberry and cranberry fields, pest pressure is typically highest close to field edges; thus,

monitoring efforts focus along these areas. Monitoring for adults is initiated when buds begin to swell. In highbush blueberries, overwintered *A. musculus* adults are monitored by using beating trays or by visually inspecting buds and flowers for injury; whereas in cranberries, adults are monitored by using sweep nets (Averill and Sylvia, 1998). In addition, adults are active almost exclusively on warm days. They drop to the ground when disturbed, and their populations are patchily distributed, causing inconsistent scouting results. Thus, a cost-effective and consistent sampling method for detecting *A. musculus* adults is critical to better estimate population occurrences and densities.

Previous studies have identified semiochemical-based attractants, i.e., aggregation pheromones, host-plant volatiles, and their combinations, which in some cases have been used successfully to monitor *Anthonomus* species (e.g., Tomlinson et al., 1969; Dickens, 1989; Eller et al., 1994; Innocenzi et al., 2001; Tinzaara et al., 2007). For example, Dickens (1989) showed that the green leaf alcohols (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, and 1-hexanol in combination with the boll weevil, *Anthonomus grandis* Boheman, aggregation pheromone enhances trap captures of boll weevils compared to the pheromone alone. Hence, our objective was to develop similar attractants to monitor more precisely *A. musculus* populations in blueberries and cranberries. In a recent study, we showed that female *A. musculus* are attracted to blueberry flowers, whereas males are attracted to undamaged flower buds and are repelled by damaged flower buds (Szendrei et al., 2009). In that study, volatile compounds from blueberry flower buds and flowers were identified; of these, cinnamyl alcohol and the green leaf volatiles (GLVs) (*Z*)-3-hexenyl acetate and hexyl acetate elicited strong antennal responses from both male and female *A. musculus*. Here, we conducted studies to: a) investigate the response of *A. musculus* to these blueberry volatiles and to the pepper weevil, *Anthonomus eugenii* Cano, and boll weevil, *A. grandis*, aggregation pheromones; b) isolate and identify the major components of the *A. musculus* aggregation pheromone; and c) evaluate the response of *A. musculus* to its aggregation pheromone alone and in combination with the antennally-active green leaf acetates.

## Methods and Materials

*Insects* Field-collected *A. musculus* adults were used for headspace volatile collections. Adult *A. musculus* were collected in March–April 2008 and 2009 from highbush blueberries in Hammonton (Atlantic Co.) and Chatsworth (Burlington Co.), New Jersey, USA and transported to the laboratory. In the laboratory, adults were held in environmental chambers (16:8 hL:D, 15°C), in screen cages (30×30×30 cm), and were fed fresh highbush blueberry (cv. Bluecrop)

buds and foliage collected from an unsprayed field located at the P.E. Marucci Blueberry & Cranberry Center (Chatsworth, NJ, USA). Adult weevils were separated by sex under a stereomicroscope based on characters of the pygidium (Szendrei et al., 2009). The pygidium is fused with the last tergite in females, but these two segments are separate in males.

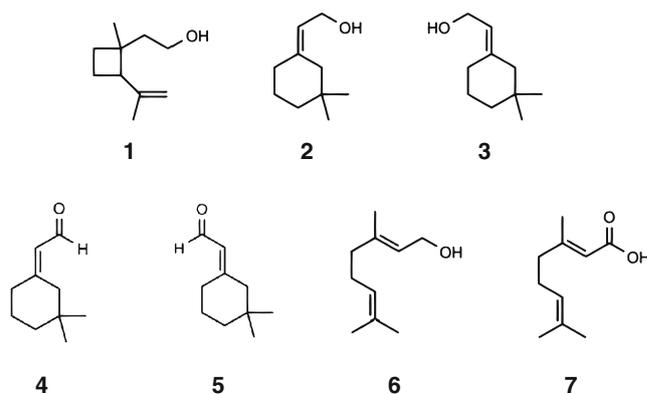
**Volatile Collections** Headspace volatiles were collected from *A. musculus* males and females in the laboratory (16:8 hL:D, 25°C), in 6 ml clear scintillation glass vials (Thermo Fisher Scientific Inc., Pittsburgh, PA, USA). The cap of each vial held an adsorbent trap, filled with 30 mg Super Q (80/100 mesh; Alltech, Deerfield, IL, USA), and a Pasteur pipette (Thermo Fisher Scientific Inc.), containing 1.25 g of activated charcoal (Alltech) to clean the incoming air. Volatiles from inside the vials were drawn through the trap by pulling air at a flow rate of ca. 1 l/min for 72 h. During volatile collections, weevils were provided daily with 0.3 g field collected blueberry or cranberry flower buds. Males and females were separated prior to volatile collections, and 20 adults of each sex were placed in a vial. In addition, volatile emissions were compared between males feeding on either blueberry or cranberry flower buds ( $N=4$  replicates of 20 adults each per crop). After collections, traps were washed with 150  $\mu$ l methylene chloride, an internal standard [400 ng of nonyl acetate (Sigma-Aldrich; St. Louis, MO, USA)] was added to each extract, and samples were stored in a freezer until used in gas chromatography-mass spectrometry (GC-MS) analysis.

**Chemical Analyses** Samples and synthetic standards were analyzed by GC-MS (Instruments 7890 and 5975 C; Agilent, Palo Alto, CA, USA) in both EI and CI (isobutane) modes by using a 30 m $\times$ 0.25 mm i.d.  $\times$  0.25  $\mu$ m film thickness medium polar DB35 (35%-Phenyl-methylpolysiloxane) and non-polar DB-1 (100% Dimethylpolysiloxane) capillary column (Agilent). Samples were introduced with cold on-column injection into a 1 m deactivated fused silica retention gap between injector and analytical column. For both columns, the oven temperature was kept at 30°C for 2.5 min after injection and then temperature programmed 20°C/min to 90°C and then 2°C/min to 125°C followed by 20°C/min to 240°C. The He carrier gas flow rate was 30 cm/sec (constant flow), and the MS transfer line temperature was 250°C. The ion source temperature was 220°C in EI mode and 250°C in CI mode. The presence of known pheromone components was confirmed by analyzing synthetic standards (compounds 1–7; Fig. 1), as well as selected natural samples in EI- and CI-mode with both GC columns. For quantitative analyses on the DB-1 column, the synthetic standards and the internal standard nonyl acetate were analyzed in CI mode to establish diagnostic

ions: for compounds 1, 2, 3, and 6 they were  $m/z$  155 ( $M + 1$ ) and  $m/z$  137 ( $M + 1 - 18$ ); for compounds 4 and 5 it was  $m/z$  153 ( $M + 1$ ); for compound 7 it was  $m/z$  169 ( $M + 1$ ); and finally, for nonyl acetate it was  $m/z$  187 ( $M + 1$ ). The MS was set to collect data for all five ions during the whole analysis. In that way, known compounds could be selectively detected in the complex natural samples while at the same time making it possible to detect the presence of related compounds. Response factors for all compounds were calculated by injecting known amounts of the standards (for the synthetic geranic acid the areas for both isomers were added prior to conducting calculations).

**Synthetic Chemicals** (*Z*)-2-isopropenyl-1-methylcyclobutaneethanol (grandlure I; compound 1, Fig. 1), (*Z*)-2-(3,3-dimethyl-cyclohexylidene)ethanol (*Z* grandlure II; compound 2, Fig. 1), (*E*)-2-(3,3-dimethyl-cyclohexylidene)ethanol (*E* grandlure II; compound 3, Fig. 1), a mixture of (*Z*)-(3,3-dimethylcyclohexylidene) acetaldehyde (grandlure III; compound 4, Fig. 1) and (*E*)-(3,3-dimethylcyclohexylidene) acetaldehyde (grandlure IV; compound 5, Fig. 1), (*E*)-3,7-dimethyl-2,6-octadien-1-ol (geraniol; compound 6, Fig. 1), and (*E*)-3,7-dimethyl-2,6-octadienoic acid [geranic acid (including neric acid); compound 7, Fig. 1] were purchased from Bedoukian Research Inc. (Danbury, CT, USA) (all >95% purity). Cinnamyl alcohol, (*Z*)-3-hexenyl acetate, and hexyl acetate were purchased from Sigma-Aldrich (all  $\geq$ 97% purity).

**2008 Field Experiments** Field studies were conducted to test the response of *A. musculus* to yellow sticky traps (23 by 28 cm Unbaited Pherocon AM; Trécé Inc., Adair, OK,



**Fig. 1** Structures of seven common aggregation pheromone components in *Anthonomus* spp. weevils (Coleoptera: Curculionidae). **1**: (*Z*)-2-isopropenyl-1-methylcyclobutaneethanol (grandlure I); **2**: (*Z*)-2-(3,3-dimethyl-cyclohexylidene) ethanol (*Z* grandlure II); **3**: (*E*)-2-(3,3-dimethyl-cyclohexylidene) ethanol (*E* grandlure II); **4**: (*Z*)-(3,3-dimethylcyclohexylidene) acetaldehyde (grandlure III); **5**: (*E*)-(3,3-dimethylcyclohexylidene) acetaldehyde (grandlure IV); **6**: (*E*)-3,7-dimethyl-2,6-octadien-1-ol (geraniol); and **7**: (*E*)-3,7-dimethyl-2,6-octadienoic acid (geranic acid)

USA) baited with cinnamyl alcohol and commercial *A. eugenii* pheromone (Trécé Inc.). Preliminary studies determined that *A. musculus* were caught successfully in yellow sticky traps placed at canopy level in highbush blueberry fields, whereas no weevils were caught in black cone traps placed on the ground for capturing walking insects (Z. Szendrei, unpublished data); thus, we used yellow sticky traps placed at canopy level in our studies. Cinnamyl alcohol is the main blueberry flower volatile (Szendrei et al., 2009), and was tested in three different types of release devices: plastic bubble (average release rate: 2.2 mg/d; ChemTica Internacional S.A., San José, Costa Rica), plastic 2-ml centrifuge vials (average release rate: 2 µg/day for 100 mg of cinnamyl alcohol in 1 ml mineral oil; ISCA Technologies, Riverside, CA, USA), and SPLAT<sup>®</sup> (Specialized Pheromone and Lure Application Technology, ISCA Technologies) at three different doses: 0, 30, 100 mg of cinnamyl alcohol per lure (mean release rate: 0.1 µg/d/mg lure). The responses to 10 treatments were compared to the response to the unbaited yellow sticky traps. All lures were attached to the sticky traps by using 22-gauge green florist wire. Traps were placed at four commercial highbush blueberry farms in New Jersey. One of the farms was located in New Lisbon (Burlington Co., NJ, USA), one in Chatsworth (Burlington Co., NJ, USA), and two in Hammonton (Atlantic Co., NJ, USA). Traps were positioned along the field edges near wooded borders, 10 m apart from each other, and were hung from a metal pole within the blueberry canopy ca. 1.6 m above ground. Treatments were set up in a randomized complete block design with four replications per farm. The position of the treatments within blocks was rotated weekly and the number of *A. musculus* on the traps was recorded weekly from April through June (11 wk). Lures were replaced with new ones every 3 wk.

**2009 Field Experiments** Field studies were conducted to test the attraction of adult *A. musculus* to a mixture of prospective components of the *A. musculus* aggregation pheromone at four commercial highbush blueberry farms in New Jersey (same locations as described above) and four cranberry farms in Massachusetts (three in East Wareham and one in West Wareham, Plymouth Co., MA, USA) by using the same type of yellow sticky traps as in 2008. The *A. musculus* aggregation pheromone lures contained compounds **2**, **4**, **5**, and **6** loaded at a ratio of 4: 1: 1: 0.1, at a dose of 30 mg per lure, and a release rate of ca. 1 mg/day (ChemTica Internacional S.A., San José, Costa Rica). Because green leaf volatiles often synergize with insect pheromones (Reddy and Guerrero, 2004), we also tested the attraction of *A. musculus* to the GLVs (*Z*)-3-hexenyl acetate and hexyl acetate alone or in combination with the aggregation pheromone components. Both (*Z*)-3-hexenyl

acetate and hexyl acetate are emitted from blueberry flower buds and flowers and elicit strong antennal responses from *A. musculus* (Szendrei et al., 2009). All custom-made lures were formulated into plastic bubble release devices (ChemTica Internacional S.A.) that were stapled to the yellow sticky traps. In addition to these lures, we also tested the commercially available *A. eugenii* and *A. grandis* pheromones (Trécé Inc.). Thus, we tested the following treatments: 1) geraniol (compound **6**); 2) (*Z*)-3-hexenyl acetate; 3) geraniol and (*Z*)-3-hexenyl acetate; 4) a blend of *Z* grandlure II (compound **2**), grandlure III (compound **4**), and grandlure IV (compound **5**); 5) a blend of *Z* grandlure II, grandlure III, grandlure IV, and geraniol (the *A. musculus* aggregation pheromone; see results); 6) a blend of *Z* grandlure II, grandlure III, grandlure IV, (*Z*)-3-hexenyl acetate and hexyl acetate; 7) a blend of *Z* grandlure II, grandlure III, grandlure IV, geraniol, and (*Z*)-3-hexenyl acetate; 8) the commercial *A. eugenii* pheromone lure; 9) the commercial *A. grandis* pheromone lure; and 10) an unbaited control. The GLV lures were loaded with 30 mg of (*Z*)-3-hexenyl acetate or hexyl acetate. Treatments were set up in a randomized complete block design with four replications at each of the eight farms. Treatments were rotated weekly among the positions in each block. In blueberries, traps were placed at canopy level as described above; and in cranberries traps were placed 1 m above canopy level. Traps were checked weekly between April and June (9 weeks) in blueberries and in May (3 weeks) in cranberries. Lures were replaced with new ones every 3 wk. All weevils captured on 27 April (overwintered adult generation) and 26 May (summer adults) in blueberry fields were removed from sticky traps and the sexes were separated.

**2010 Field Experiments** To further evaluate the *A. musculus* pheromone, an experiment was conducted to test the attraction of adult *A. musculus* to its aggregation pheromone in eight fields at four commercial highbush blueberry farms in New Jersey (same locations as described above). Yellow sticky traps were either baited with the ChemTica pheromone lures (containing compounds **2**, **4**, **5**, and **6** loaded at the ratio, dose, and release rate described above) or unbaited (controls). Trap position was the same as that described above, and the numbers of weevils on traps were recorded weekly from 1 April to 22 June.

**Statistical Analyses** For the 2008 and 2009 field data, the number of *A. musculus* captured in yellow sticky traps was analyzed by using a generalized linear mixed model (SAS Institute, Version 9.1, Cary, NC), with lure treatment as fixed factor, farm and block as random factors, and week as the repeated variable in the model. This analysis was used to determine the effects of lure types on attractiveness of traps to *A. musculus*. Differences among treatment means

were assessed by using the lsmeans statement with the pdiff option, and differences were considered significant at an  $\alpha$  level of 0.05. Numbers of *A. musculus* were  $\log_{10}$ -transformed prior to analyses. Data were analyzed separately by years and states. For the 2010 data, a paired *t*-test was conducted to compare the number of *A. musculus* captured in pheromone-baited versus unbaited traps. We used a *Chi-square* test to determine whether there was a significant difference between the quantities of compounds emitted by male *A. musculus* feeding on cranberries versus blueberries and to test whether the sex ratio among captured weevils was different from 1:1 ( $\alpha=0.05$ ).

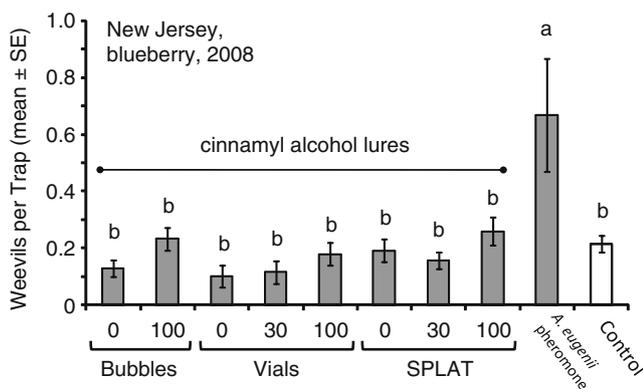
## Results and Discussion

This study reports the first identification and field activity of components of the *A. musculus* aggregation pheromone. Our 2008 field experiment showed that there was a significant treatment effect on the number of *A. musculus* caught on yellow sticky traps among cinnamyl alcohol, *A. eugenii* aggregation pheromone, and unbaited control traps ( $F=3.80$ ,  $df=9$ , 339,  $P<0.01$ ) (Fig. 2). The *A. eugenii* pheromone-baited traps caught more *A. musculus* adults than all other treatments ( $t>3.12$ ;  $df=9$ ;  $P<0.01$ ) (Fig. 2). These results are likely due to the fact that these two *Anthonomus* species share common pheromone components (Table 1). Subsequent headspace analysis of adult *A. musculus* showed that males emit (*Z*)-2-(3,3-dimethylcyclohexylidene) ethanol (*Z* grandlure II; compound 2, Fig. 1), (*Z*)-(3,3-dimethylcyclohexylidene) acetaldehyde

(grandlure III; compound 4, Fig. 1), and (*E*)-(3,3-dimethylcyclohexylidene) acetaldehyde (grandlure IV; compound 5, Fig. 1) (Fig. 3a). Female *A. musculus* did not emit any of these grandlure components (Fig. 3b); however, geraniol [(*E*)-3,7-dimethyl-2,6-octadien-1-ol; compound 6, Fig. 1] was detected in similar quantities in male and female *A. musculus* samples when feeding on cranberry flower buds (Fig. 3b). This suggests that cranberry flower buds release geraniol in response to damage. When insects were fed blueberry buds, we did not detect geraniol in female samples (data not shown), but small quantities were found in male collections (Fig. 4), suggesting that this compound may be a part of the aggregation pheromone. Similarly, *A. eugenii* males emit compounds 2, 4, 5, and 6, but they also emit compounds 3 and 7 (Table 1; Eller et al., 1994). In the 1999 study, however, geraniol was emitted only by males; thus, all six compounds were designated as “male-specific” (Eller et al., 1994). So far, geraniol has been reported only from *A. eugenii* and *A. musculus* (Table 1), but it has been suggested as a possible host precursor for the different grandlures in *Anthonomus* spp. (Tumlinson et al., 1970). Compound 2 (the most abundant component of both *A. musculus* and *A. eugenii* pheromones; Table 1) as well as compounds 4 and 5 are also found in *A. grandis*; the strawberry blossom weevil, *Anthonomus rubi* Herbst. (Innocenzi et al., 2001); and the pecan weevil, *Curculio caryae* (Horn) (Hedin et al., 1997) (Table 1); however, compounds 4 and 5 were found in trace amounts in *A. rubi* and had no effect on their behavior (Innocenzi et al., 2001).

The emissions of the four *A. musculus* pheromone components: 2, 4, 5, and 6 were  $2.8\pm 0.4$  (mean $\pm$ SE),  $1.8\pm 0.2$ ,  $1.3\pm 0.2$ , and  $0.9\pm 0.1$  ng/male/d, respectively. The total amount of all grandlures (i.e., sum of 2, 4, and 5) emitted by male *A. musculus* was  $5.6\pm 0.7$  (mean $\pm$ SE) ng/male/d. This amount is >50-fold lower than those reported for *A. grandis* ( $4.2\ \mu\text{g}/\text{male}/\text{d}$ ; Chang et al., 1989), *A. eugenii* ( $15\ \mu\text{g}/\text{male}/\text{d}$ ; Eller et al., 1994), *A. rubi* ( $8.1\ \mu\text{g}/\text{male}/\text{d}$ ; Innocenzi et al., 2001), and *C. caryae* ( $0.3\ \mu\text{g}/\text{male}/\text{d}$ ; Hedin et al., 1997). Our quantitative assessment of the pheromone components may have been influenced by the field-collected weevils of unknown age and sexual maturity that we used in our assays. Once methods of maintaining these insects in laboratory culture have been worked out, we intend to control the developmental and reproductive variables to achieve a more precise determination of the quantity of pheromone components emitted.

There were minimal differences in volatile collections from *A. musculus* feeding on different host plants (blueberries versus cranberries). Qualitatively, the headspace of male *A. musculus* that were feeding on blueberry flower buds did not differ from those feeding on cranberry flower buds (Fig. 4). Quantitatively, grandlure components in the headspace of male *A. musculus* sampled on blueberry



**Fig. 2** Average number of *Anthonomus musculus* adults on yellow sticky traps baited with different lures at four commercial highbush blueberry farms in New Jersey in 2008. Cinnamyl alcohol was formulated in three different types of release devices at three different concentrations (numbers on x-axis represent cinnamyl alcohol concentrations in mg). The *Anthonomus eugenii* pheromone lure was purchased from a commercial source. Control yellow sticky traps were unbaited. Treatments with different letters above columns are significantly different ( $P\leq 0.05$ )

**Table 1** Comparison of the composition and relative abundance of the aggregation pheromone in grandlure-emitting weevil species<sup>a</sup>

Compound <sup>b</sup>	<i>Anthonomus musculus</i>	<i>Anthonomus eugeni</i> <sup>c</sup>	<i>Anthonomus grandis</i> <sup>d</sup>	<i>Anthonomus rubi</i> <sup>e</sup>	<i>Curculio caryae</i> <sup>f</sup>
	(Cranberry Weevil)	(Pepper Weevil)	(Boll Weevil)	(Strawberry Blossom Weevil)	(Pecan Weevil)
1			36	15	25
2	43	48	35	75	55
3		32		trace	
4	23	3	14	trace	10
5	20	2	15	trace	10
6	14	2			
7		13			
Lavandulol				10	

<sup>a</sup> Table entries are percentages of amounts

<sup>b</sup> Names of compounds are provided in Fig. 1

<sup>c</sup> Eller et al. (1994)

<sup>d</sup> Chang et al. (1989)

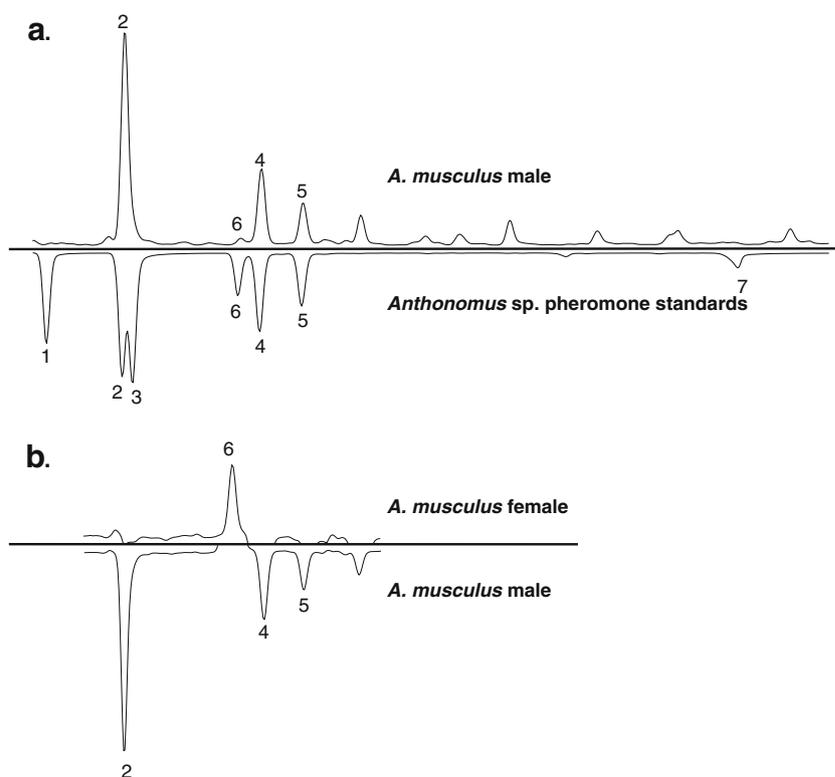
<sup>e</sup> Innocenzi et al. (2001)

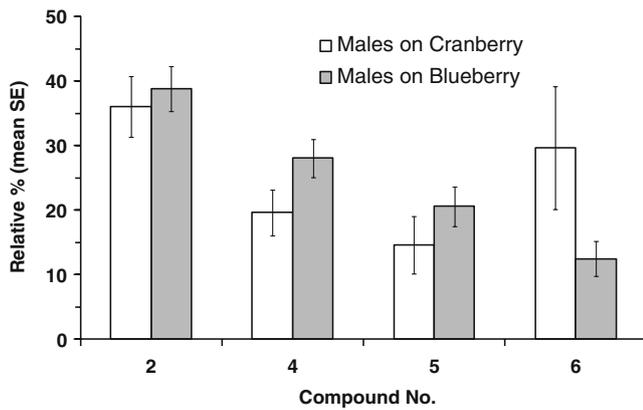
<sup>f</sup> Hedin et al. (1997)

versus cranberry hosts were similar, but geraniol (compound **6**; Fig. 1) was significantly ( $\chi^2=19.25$ ,  $df=3$ ,  $P<0.01$ ) more abundant in the headspace from males feeding on cranberries (Fig. 4). Although the reason for this difference remains unknown, it is possible that host plant might affect the production of geraniol in male *A. musculus* or its emission after herbivore feeding damage. Geraniol is

a monoterpenoid alcohol commonly emitted from plants, particularly flowers (Schiestl, 2010), that is induced by herbivore feeding (Han and Chen, 2002), and with known activity on insects, including mosquitoes (e.g., Qualls and Xue 2009) and bees (e.g., Williams et al., 1981). Besides *Anthonomus* spp., it is a component of the honey bee pheromone (Boch, 1962; Pickett et al., 1980). Geraniol is a

**Fig. 3** Comparison between a representative chromatogram trace of male *Anthonomus musculus* (top) and a blend of standard pheromone components from *Anthonomus* spp. (bottom) **a**; and between male and female *A. musculus*, showing a selective ion trace of female samples when male compounds were subtracted from the trace (top) and for male samples when female peaks were subtracted (bottom) **b**. Names of compounds 1–7 are provided in Fig. 1





**Fig. 4** Emissions of the four identified *Anthonomus musculus* aggregation pheromone components from males fed on blueberry and cranberry flower buds. Amounts of compound 6 (geraniol) were significantly greater in males fed on cranberry flower buds compared with those fed on blueberry flower buds ( $P < 0.05$ ); host plant did not affect any of the grandlure compounds 2, 4, and 5 ( $P > 0.05$ ). Names of compounds 2, 4, 5, and 6 are provided in Fig. 1.  $N = 4$  replicates (of 20 males each) per crop

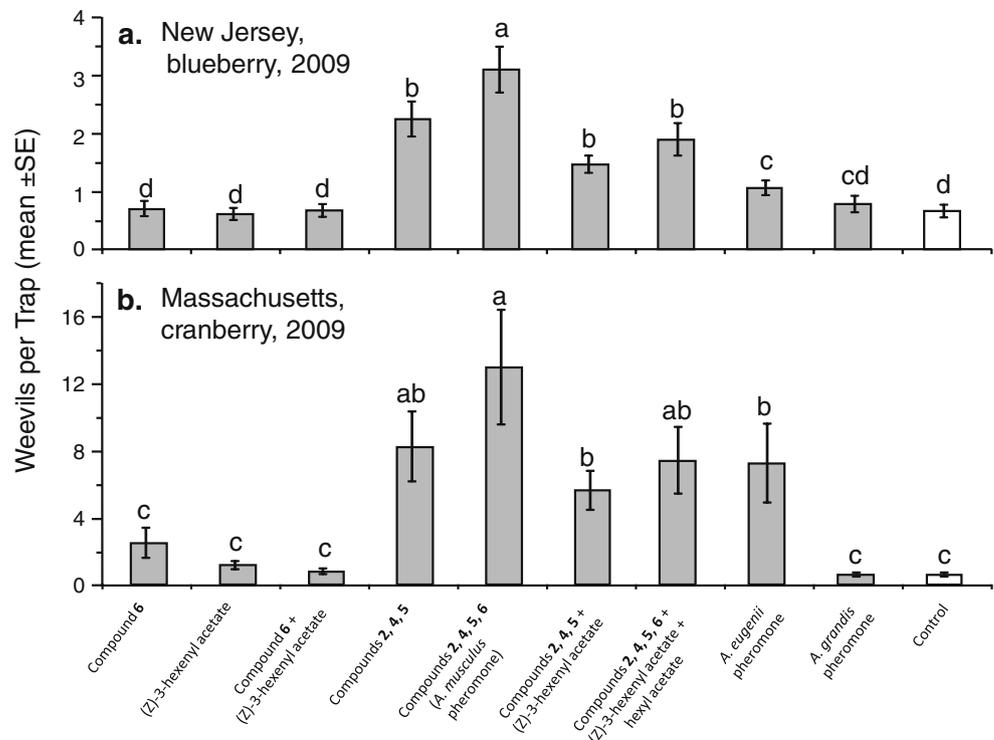
possible precursor of the *A. grandis* pheromone (Tumlinson et al., 1970; Thompson and Mitlin, 1979), and can be either derived from the host plant or produced *de novo* by male *Anthonomus* spp. Blueberry flower buds and flowers, however, did not emit detectable amounts of geraniol even after damage by *A. musculus* adults (Szendrei et al., 2009); thus, it is likely that *A. musculus* produces this compound. We will conduct more studies to elucidate the importance of

host plant parts and phenology on pheromone production in *A. musculus*.

To support our behavioral data from field flight trapping (see below), we also attempted to evaluate headspace volatiles from *A. musculus* by gas chromatography-electroantennal detection (GC-EAD). However, this was a major technical challenge because of the small size of the insects and the relatively small amount of pheromone components in the extracts (see above). Similar problems have been encountered with the plum curculio, *Conotrachelus nenuphar* (Herbst) (Curculionidae) (T. Leskey and Aijun Zhang, USDA-ARS, Beltsville, MD, USA, personal correspondence). We plan to continue to pursue electrophysiological assays of this system in collaboration with Drs. Leskey and Zhang.

In 2009, the attractiveness of the identified main four-component pheromone blend to *A. musculus* was evaluated in commercial blueberry and cranberry farms. In New Jersey blueberry fields, the numbers of adult *A. musculus* caught on yellow sticky traps with different lure treatments were significantly different ( $F = 15.02$ ;  $df = 9, 315$ ;  $P < 0.01$ ) (Fig. 5a). Yellow sticky traps baited with compounds 2, 4, 5, and 6 (*A. musculus* aggregation pheromone components) caught more adult *A. musculus* ( $t > 2.21$ ;  $df = 9$ ;  $P < 0.03$ ) than any of the other lure treatments. Relative to the unbaited control, this treatment attracted ~2.9 times more weevils over the course of the field season. All treatments that contained the *A. musculus* species-specific lure increased attraction of weevils to traps relative to the unbaited control, lures

**Fig. 5** Mean ( $\pm$  SEM) trap catch of *Anthonomus musculus* on yellow sticky traps baited with different compounds in the 2009 field experiment in New Jersey **a** and Massachusetts **b**. Names of compounds 2, 4, 5, and 6 are provided in Fig. 1. Treatments with different letters above bars are statistically different ( $P \leq 0.05$ )



containing only plant volatiles, and related *Anthonomus* species pheromones ( $t > 3.39$ ;  $df = 9$ ;  $P < 0.05$ ). Although the number of weevils in blueberry fields was lower in 2010 than in 2009, our results from 2009 were confirmed in 2010: i.e., ~ 2 times more adult *A. musculus* were captured in pheromone-baited compared with unbaited traps (mean number of weevils ( $\pm$  SE) in: unbaited traps =  $4.9 \pm 1.3$ ; pheromone-baited traps =  $9.3 \pm 1.7$ ;  $t = 2.57$ ,  $P = 0.037$ ). Lures baited with the *A. musculus* pheromone (compounds **2**, **4**, **5**, and **6**) caught similar numbers of males and females in blueberry fields (Table 2). However, females from the summer generation but not from the overwintered generation were significantly more attracted to lures baited with compounds **2**, **4**, and **5** (Table 2), indicative that newly emerged, virgin females might be more responsive to the male-produced grandlures. Similar variation in male:female ratio in response to pheromone-baited traps was observed for *A. rubi* during the trapping season (Innocenzi et al., 2001).

In Massachusetts cranberries, the numbers of *A. musculus* caught on yellow sticky traps were also different among lure treatments ( $F = 9.02$ ;  $df = 9, 90$ ;  $P < 0.01$ ), (Fig. 5b). The most attractive trap for *A. musculus* weevils in this state and crop was the same as in New Jersey, i.e., traps baited with the lure containing compounds **2**, **4**, **5**, and **6**. The response of *A. musculus* weevils to the other *Anthonomus* pheromones was similar in the two states and crops (Fig. 5). Compared to the unbaited control, the *A. eugenii* pheromone but not the *A. grandis* pheromone attracted more *A. musculus* weevils. Four of six pheromone components are shared by *A. musculus* and *A. eugenii*; whereas, three of

four components are shared between *A. musculus* and *A. grandis* (Table 1). Key differences are the presence of compound **1** in the *A. grandis* pheromone blend and compounds **3** and **7** in the *A. eugenii* blend; these compounds are absent in the *A. musculus* blend and can account at least in part for the specificity in *A. musculus* attraction to its aggregation pheromone. Overall, the abundance of weevils in yellow sticky traps was approximately 4 times higher in Massachusetts than in New Jersey.

In our 2009 and 2010 field studies, 30 mg of compounds **2**, **4**, **5**, and **6** were loaded at a 4:1:1:0.1 ratio. This ratio was estimated based on preliminary data collected from a few males. Subsequent analysis (Table 1; Fig. 4) with more replications revealed that the ratio of these compounds is closer to 2:1:1:0.7. Further studies are needed to determine whether this latter ratio is more attractive to *A. musculus*. Despite using a potentially suboptimal ratio, our field study clearly shows that a lure containing compounds **2**, **4**, **5**, and **6** attracts adult *A. musculus*. Whether all components in the *A. musculus* pheromone blend are needed for this attraction requires further investigation; however, a blend of compounds **2**, **4**, and **5** was less attractive than the full blend, and compound **6** alone did not attract *A. musculus* (Fig. 5). Most recent analysis of the headspace emissions from adult *A. musculus* indicate the presence of two previously undetected minor compounds (an acid similar to nerolic/geranic acid and an alcohol similar to nerol/geraniol; data not shown) released by males only, which might have behavioral significance. Future studies will identify these compounds and determine their biological activity.

**Table 2** Trap catch and sex ratio of male and female *Anthonomus musculus* captured in response to synthetic lures in New Jersey blueberry fields

Lure <sup>a</sup>	27 April 2009			26 May 2009			Totals		
	(Overwintered Adults)			(Summer Generation Adults)			(Both Generations)		
	Female	Male	Ratio	Female	Male	Ratio	Female	Male	Ratio
Control	16	15	1.1	2	5	0.4	18	20	0.9
<i>Anthonomus grandis</i> pheromone	16	26	0.6	2	2	1.0	18	28	0.6
<i>Anthonomus eugenii</i> pheromone	23	21	1.1	8	15	0.5	31	36	0.9
Compounds <b>2</b> , <b>4</b> , <b>5</b> , <b>6</b> + (Z)-3-hexenyl acetate + hexyl acetate	30	20	1.5	55	26	2.1**	85	46	1.8**
Compounds <b>2</b> , <b>4</b> , <b>5</b> + (Z)-3-hexenyl acetate	23	13	1.8	40	7	5.7**	63	20	3.2**
Compounds <b>2</b> , <b>4</b> , <b>5</b> , <b>6</b> ( <i>Anthonomus musculus</i> pheromone)	30	18	1.7	85	83	1.0	115	101	1.1
Compounds <b>2</b> , <b>4</b> , <b>5</b>	30	27	1.1	57	34	1.7*	87	61	1.4*
Compound <b>6</b> + (Z)-3-hexenyl acetate	20	20	1.0	4	4	1.0	24	24	1.0
(Z)-3-hexenyl acetate	12	15	0.8	5	0		17	15	1.1
Compound <b>6</b>	15	12	1.3	10	4	2.5	25	16	1.6

<sup>a</sup> Names of compounds **2**, **4**, **5**, and **6** are provided in Fig. 1

\*  $0.05 \geq P \geq 0.01$

\*\*  $P < 0.01$

In addition to the aggregation pheromone, volatiles from host plants may inform foraging *A. musculus* about suitable feeding and oviposition sites. Several species related to *A. musculus*, e.g., *Anthonomus pomorum*, *A. grandis*, and *A. rubi*, have shown electrophysiological antennal (EAG) responses to host plant volatiles (Dickens, 1990; Kalinova et al., 2000; Bichao et al., 2005) and several species of weevils, such as the pine weevil, banana weevil, vine weevil, and pepper weevil (Budenberg et al., 1993; Wibe et al., 1997; van Tol and Visser, 2002; Adesso and McAuslane, 2009), responded to host-plant volatiles in behavioral bioassays. In Y-tube olfactometer assays, Mechaber (1992) found that adult *A. musculus* were more attracted to conspecific-damaged cranberry vines compared with undamaged vines, as well as to undamaged and damaged flower buds compared to clean air (Mechaber, 1992). Recently, we found that female *A. musculus* are attracted to blueberry flowers, whereas males are attracted to undamaged flower buds and are repelled by damaged flower buds (Szendrei et al., 2009). These studies indicate that *A. musculus* adults might use host-plant volatiles during host or mate location, but did not identify the specific volatile cues responsible for this attraction. In this study, we tested two potential host-plant attractants for *A. musculus*. In 2008, we tested the main blueberry floral component, cinnamyl alcohol, at different doses and formulations. However, regardless of the dose and formulation, cinnamyl alcohol baited traps caught similar numbers of *A. musculus* when compared to the control trap (Fig. 2). In 2009, we tested the GLV (*Z*)-3-hexenyl acetate. GLV compounds are highly ubiquitous in plants and play important roles in insect-plant interactions (Visser, 1986; Szendrei and Rodriguez-Saona, 2010). For example, a mixture of plant volatiles that includes (*Z*)-3-hexenyl acetate attracts the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Visser, 1986), and the scarab beetle *Anomala octiescostata* Burmeister (Leal et al., 1994). Although (*Z*)-3-hexenyl acetate was found in headspace collections from both blueberry flower buds and flowers and elicited significant EAG responses in *A. musculus* (Szendrei et al., 2009), it did not increase adult weevil attraction compared with unbaited control traps (Fig. 5). Host-plant volatiles, particularly GLVs, can enhance the response of insects to their pheromone (Dickens et al., 1990; Reddy and Guerrero, 2004). For example, (*Z*)-3-hexenyl acetate enhanced the response of the diamondback moth, *Plutella xylostella* (L.), to its sex pheromone (Reddy and Guerrero, 2000). A synergistic effect has also been reported between GLVs and the *A. grandis* aggregation pheromone (e.g., Dickens, 1989). Here, we tested the response of *A. musculus* to its aggregation pheromone in combination with (*Z*)-3-hexenyl acetate and hexyl acetate. Our results suggest an interruptive effect of these GLVs:

addition of (*Z*)-3-hexenyl acetate to a blend of compounds 2, 4, and 5 reduced numerically (but not significantly) *A. musculus* trap captures (Fig. 5). Similarly, addition of (*Z*)-3-hexenyl acetate and hexyl acetate to a blend of compounds 2, 4, 5, and 6 reduced (significantly in New Jersey and numerically in Massachusetts) *A. musculus* trap captures (Fig. 5). A reduction in the number of insects caught in traps was observed when the GLVs (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, and (*Z*)-3-hexen-1-ol were added to lineatin, the aggregation pheromone of the striped ambrosia beetle, *Trypodendron lineatum* Olivier (Borden et al., 1997), as well as when the GLVs 1-hexanol, (*Z*)-3-hexen-1-ol, and (*E*)-3-hexen-1-ol were added to the pheromone of the spruce bark beetle, *Ips typographus* (L.) (Zhang et al., 1999). Furthermore, addition of the GLV acetates to the *A. musculus* aggregation pheromone skewed the sex ratio of the captured weevils towards females in the summer (Table 2).

Similarly to our results, Cha et al. (2008) failed to find a significant change in the number of male grape berry moths captured between host-plant volatile-baited and control traps. Plant volatile attractants for codling moth failed under certain cases in the field (Knight and Light, 2005). The failure of plant volatiles to attract insects to traps in our field experiments could be because the tested volatiles were already present in the environment at the time when lures were placed in the field, and the background cues may have masked odors from the lures (Schröder and Hilker, 2008). In the case of cinnamyl alcohol, habituation or lack of sensitivity may also explain the low response rate, since this volatile stands out prominently from the background during bloom in blueberry fields. It is also possible that male *A. musculus* were repelled by the GLV plus pheromone baits as a result of odor camouflaging, where an odor is less detectable when presented in a combination with other odors. In our case, the quality or quantity of GLVs may have disrupted the perception of pheromone compounds by the insect and thus lead to lower or non-significant response.

Although the role of plant volatiles in host-plant location by *A. musculus* remains unclear, the *A. musculus* aggregation pheromone identified here can be an easy-to-use and cost-effective tool for monitoring this pest in blueberry and cranberry pest management programs. In highbush blueberries, an attractant for this weevil will be most useful for early detection of overwintered adults. The *A. musculus* pheromone was more attractive to the newly-emerged summer generation adults than to the overwintered adults (Table 2). Considering that the overwintered adults are the most damaging to the crop, the pheromone needs to be optimized by testing different pheromone doses, formulations, and ratios. Then, traps must be optimized by testing, trap colors, designs, and placements. A refined pheromone

blend that may contain plant volatiles and other minor components is required to optimally monitor *A. musculus* populations. Once optimized, this pheromone can also be used to develop an attract-and-kill formulation to manage *A. musculus*, similarly to the ones tested for *A. grandis* (e.g., McKibben et al., 1990).

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