Behavioral and Electrophysiological Responses of *Listronotus maculicollis* (Coleoptera: Curculionidae) to Volatiles From Intact and Mechanically Damaged Annual Bluegrass

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ABSTRACT *Listronotus maculicollis* Kirby is a highly destructive pest of low mown, cool-season turfgrasses in the northeastern United States and Canada. Behavioral and electrophysiological assays were conducted to identify compounds that may be useful in developing novel monitoring techniques. In Y-tube assays, males and females responded differently to volatiles from intact and clipped annual bluegrass (*Poa annua* L.). Females were significantly attracted to intact *P. annua* but repelled from clippings; males did not respond significantly to either treatment. Electroantennogram (EAG) recordings from both sexes showed a significant response to volatiles from both treatments. Gas chromatography mass spectrometry (GC-MS) identified 12 volatile compounds from *P. annua* of which nine were common to both intact plants and clippings. On average, seven-fold higher quantities of volatiles were collected from clippings than from intact plants (24.3 versus 3.4 ng/g of tissue/h). Eight compounds were released in significantly greater quantities from clippings of which 50% were the n-C_6 compounds hexanal, (*E*)-2-hexenal, and (*Z*)-3-hexenyl acetate, commonly referred to as "green leaf volatiles" (GLVs). Only octanal was emitted in greater amounts from intact plants than clippings. These nine compounds were tested individually against male and female antennae. Both sexes displayed greatest sensitivity to nonanal, octanal, and (*E*)-2-hexenal, but a significant dose–response relationship was observed with all compounds tested. These studies indicate that both sexes respond physiologically and that *L. maculicollis* females exhibit behavioral responses to host-plant volatiles. Future studies will need to assess the effects of individual compounds and component mixtures on *L. maculicollis* behavior in the field.

KEY WORDS annual bluegrass weevil, EAG, GC-MS, green leaf volatiles, *Listronotus maculicollis*

The weevil *Listronotus maculicollis* Kirby is a native pest of highly maintained cool season turfgrasses in the northeastern United States and eastern Canada (Vittum et al. 1999). Extensive damage from larval populations can be found in high valued, low mown areas on golf courses (e.g., greens, tees, fairways) with high percentages of annual bluegrass (*Poa annua* L.). The reported distribution of damaging *L. maculicollis* populations continues to expand from its epicenter around the metropolitan New York City area, with damage now reported from all New England and mid-Atlantic states north into the provinces of Quebec and New Brunswick, south into North Carolina, and west into Ohio (Simard et al. 2007).

Much of the effort to suppress larval populations involves preventive applications of broad spectrum insecticides to intercept adults moving from overwintering sites onto low mown playing surfaces before egg laying. The absence of an effective monitoring method for the relatively small (4.5 mm in length) and cryptic adults and the possibility of extended emergence periods of overwintering populations (McGraw and Koppenhöfer 2009a) has led to confusion in the timing of preventive insecticide applications. As a consequence, it is not unusual that multiple insecticide applications are made against single generations (McGraw and Koppenhöfer 2007). The overuse of synthetic insecticides, particularly of the pyrethroid class, has led to the development of insecticide resistance in some areas of the weevil's distribution (Ramoutar et al. 2009).

Monitoring *L. maculicollis* populations is laborious and hence often not performed by turfgrass managers (McGraw and Koppenhöfer 2009a). More commonly, golf course superintendents rely upon plant phenological indicators (Tashiro et al. 1978) or calendar dates to time preventive insecticide applications. Anecdotal evidence suggests that full bloom of *Forsythia*

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spp. coincides with the movement of adults from overwintering sites to low mown playing surfaces and that full bloom of flowering dogwood (*Cornus* spp.) indicates that all adults have emerged. However, because of variability in plant phenology across sites and the unrelatedness of the plants and insect this method is not precise enough. Thus, better monitoring tools for *L. maculicollis* are needed, ideally relying on pest behavior, to reduce labor costs and avoid unnecessary insecticide applications.

Little is known about *L. maculicollis* foraging behavior, and the role of plant odors in weevil orientation and host selection remains unstudied. Recent field studies have observed edge biases to the distribution of and damage from first generation larvae (Diaz et al. 2008, McGraw and Koppenhöfer 2009 a, b). The spatial distribution of emerging overwintering adults and subsequent first generation larvae suggest that adults, particularly ovipositing females, may be attracted to low mown hosts. Rothwell (2003) observed more larvae in low mown (≤1.27-cm) grasses (*P. annua, Agrostis stolonifera* L., and *Lolium perenne* L.) in choice and no-choice laboratory and small plot trials.In addition, larvae were larger and developed faster in low mown grasses than in higher mown grasses. Diaz and Peck (2007) observed weevils overwintering considerable distances from low mown playing surfaces (up to 60 m); yet, 6.4–9.2-fold higher populations of spring-generation larvae and adults were found in low mown fairway turf than in higher mown grasses (Diaz et al. 2008). Taken together, these finding indicate that adult weevils may traverse substantial distances in search of host plants. Plant cues from mechanically damaged turfgrasses (i.e., volatiles) could aid in *L. maculicollis* orientation.

The objectives of this study were to 1) determine if *L. maculicollis* adults demonstrate behavioral and antennal electrophysiological (EAG) responses to volatiles released from *P. annua*, 2) determine whether mechanically damaged *P. annua* (clippings) differs in the volatile emissions from intact plants, and 3) determine if males and females respond differently to these volatiles. We hypothesized that *L. maculicollis*, particularly females, would be attracted to volatiles released from mechanically damaged *P. annua* given ovipositional biases in the field (Rothwell 2003, Diaz et al. 2008). We also hypothesized that mechanically damaged (i.e., clipped or mown) *P. annua* would differ from undamaged *P. annua* in the composition or quantities of released volatiles. It is our hope that our findings will help improve our understanding of *L. maculicollis* behavior as it relates to foraging strategies and host selection and will aid in the development of more effective monitoring techniques.

Materials and Methods

Insects. Overwintering *L. maculicollis* adults were collected from Pine Brook Golf Course in Manalapan, NJ in November 2008 and 2009 from beneath forest leaf litter adjacent to untreated, infested fairways. The weevils were extracted in the field by taking the top 2–3 cm of soil, placing the soil in 19-liter buckets and adding lukewarm water (25–30°C). A piece of paper towel was placed on the water surface to collect weevils as they walked across it. Buckets were monitored for up to 1 h. Weevils were transported back to the laboratory, identified, and placed into plastic boxes (12 × 20 × 32 cm) containing overwintering substrates (soil, leaf litter, and pine needles). The containers were held at 8°C (55% RH), with a photoperiod of 10:14 (L:D) h until the day before bioassays, to simulate overwintering conditions. Before bioassays, weevils were extracted by hand followed by lukewarm water (if necessary), sexed (Cameron and Johnson 1971), and placed individually in 24-well plates containing sand. The plates were fit with Parafilm over the top, capped, and held in place with rubber bands to prevent weevils from escaping. The plates were then returned to the incubator until the following day.

Plant Material. *P. annua* used in bioassays was collected from established, uniform fields in New Brunswick, NJ in December 2008. Cores (10 cm in diameter) were removed to a depth of 15 cm and placed into pots. Pots were kept in a greenhouse (22°C, 40–60% RH, 16:8 (L:D) h) until used in bioassays. Plants were clipped two times per week to maintain an average height of ≈1.27 cm.

Y-tube Assays. The responses of males and females to *P. annua* turf cores, *P. annua* clippings, and combinations of the two were tested by using a Y-tube olfactometer (Analytical Research Systems Inc., Gainesville, FL). The Y-tube consisted of two arms 8 cm in length and one 12-cm arm held together by 2-cm ground glass joints. Air was filtered through activated charcoal and was split into two 1.6 liters/min air streams. Each air stream was delivered through one of the 8-cm arms of the Y-tube via an odor source in a glass tube (14 cm long, 2 cm in diameter). Odor sources consisted of either 5 g (wet weight) of *P. annua* turf cores (above ground plant material and thatch), 1.5 g of *P. annua* clippings (wet weight), or a blank. The weight of the odor source was selected based on the maximum amount of plant material that could be placed into the glass tube of the olfactometer arm. Before each bioassay, purified air was passed through the Y-tube for 10 min to purge the system. At the start of the assay, fresh plant material was used as an odor source. No plant material was used for longer than 30 min.

Weevils were placed individually in the bottom of the Y-tube in a glass inlet fit with a screen to prevent weevils from leaving the test arena. Each weevil was observed for a maximum of 10 min. Weevils failing to make a decision after 10 min were recorded as nonresponding. Each individual was used only once. After each replicate, the arm which held the odor source was switched to exclude directional bias in the experiment. After each observation, the Y-tube and all glass connections (with the exception of the odor sources) were rinsed with methanol, followed by hexane, and oven dried at 80°C for 5 min to avoid any possible contamination. Each sex was observed for two consecutive replicates before the position of the olfactometer arm holding the odor source was reversed.

Treatments were changed after four runs (two observations for each sex). In total, 40 responding weevils of each sex were observed for each treatment. All experiments were performed between 0800–1600 hours at 24 ± 2°C, and 200–300 lux.

Volatile Collections and Analyses. Volatiles were collected from the headspaces above intact *P. annua* and *P. annua* clippings via a pull collection system (Tholl and Röse 2006). Treatments consisted of 5 g (wet weight) of unclipped *P. annua* and thatch or 1.5 g of *P. annua* clippings (wet weight). Clippings were used in place of clipped plant-soil cores to eliminate the high ratio of soil and thatch to leaf material that would be needed in the collection vials, and to ensure adequate quantities of volatiles were collected for analysis. Plant materials were housed in 25-ml containers. Air was pulled with the aid of a 12-volt vacuum pump (Sensidyne, Clearwater, FL) through the top of the container at a constant rate of ≈2 liters/min, through a charcoal filter, and collected on a Super-Q adsorbent filter trap (Analytical Research Systems Inc., Gainesville, FL). Volatiles were collected continuously between 1530 and 0800 hours. Volatiles were extracted from the Super-Q filter by rinsing with 150 μl dichloromethane, and forced through with N_2 gas. An internal standard (400 ng of *n*-octane) was added to each extract to quantify volatiles emissions in plant extracts.

The volatiles eluted from Super-Q traps were separated and quantified using a Hewlett–Packard 6890 Series gas chromatograph fitted with an Agilent HP-1 column (10 m × 0.53 mm × 2.65 μm; Palo Alto, CA). Helium was used as a carrier gas at a flow rate of 5 ml/min. The oven temperature was programmed at 40°C for 1 min, increased at 14°C/min to 180°C, held for 2 min, then increased at 40°C/min to 200°C and held for 2 min. Volatile compounds (ng/g of wet material/h) were quantified based on comparison of peak areas with those of the internal standard, and identified by mass spectral data, NIST library, retention index, and comparing of the retention times with commercially available compounds as described in Rodriguez-Saona et al. (2009) and Szendrei et al. (2009).

Electroantennogram (EAG) Recordings. The antennal responses of male and females to volatiles from intact and clipped *P. annua* were studied using electroantennogram analyses. EAG analyses were performed by excising antennae in the middle of the scape from live weevils. The excised end of the antenna was fit into a glass capillary with a reference electrode filled with a saline solution (7.5 g NaCl, 0.21 g $CaCl_2$, 0.35 g KCl, 0.2 g $NaHCO_3$ in 1 liter H_2O) while the club end of the antenna was fit into the capillary with the recording electrode. Purified air was directed over the antenna through a 10-mm-diameter glass tube. Headspace volatile extracts (collected as described above) from each treatment were prepared in 10 μl aliquots on filter paper (Whatman No. 1, Whatman International Ltd., Maidstone, United Kingdom). Extracts were air dried before they were inserted into a Pasteur pipette and placed directly into an opening in the glass tubing providing airflow to the excised antenna. EAG signals from the antennae were fed onto a signal connection interface box (IDAC-2; Syntech, Hilversum, The Netherlands) and processed on a PC using Syntech software. A stimulus flow controller (CS-05; Syntech) was used to generate 1-s-long stimuli at 1-min intervals. In each test, two controls (hexane, air) were used to ensure that the weevils were responding to volatiles in plant extracts and not a mechanoreceptor effect of the air puffs or solvent. The order of the treatments tested was randomized for each insect tested. In total, eight female and six male antennae were tested.

Male and female antennal response to nine individual compounds: (+)-β-pinene, (*E*)-2-hexenal, 6-methyl-5-hepten-2-one, (Z)-3-hexenyl acetate, decanal, hexanal, nonanal, octanal, and phenyl ethyl alcohol, were further studied using similar EAG techniques as described above. These compounds were identified in this study as being major components of *P. annua* and significantly influenced by treatment (whole-plant versus clippings) (see Results). All compounds were purchased from Sigma-Aldrich (St. Louis, MO). Four concentrations (0.02, 0.2, 2, and 20 mg) of purified (>95%) synthetic compounds were diluted in hexane before testing in dose–response assays. Each compound dilution series was tested against five female and four male antennae, with the exception of octanal (two male antennae). Each replicate began with testing the antennae against the control (hexane only), followed by four dilutions of each chemical in increasing concentration. A standard [20 mg of (*E*)-2-hexanal], known to elicit a strong antennal response (see Results), was presented at the end of each replicate round to ensure that the antenna was still responsive. The stimuli were presented with 30-s intervals between the treatments. Each antenna was subjected to three rounds as described, and an average response was recorded for statistical analysis.

Statistical Analyses. Statistical analyses were conducted using Statistix (2003). Data from Y-tube behavioral assays were analyzed using a G-test with William's Correction (Sokal and Rohlf 1995). The numbers of males or females making a choice were tested against a null hypothesis of no preference. Differences in volatile emissions between intact and clipped *P. annua* were analyzed by Wilcoxon Rank Sum tests, because the data did not conform to a normal distribution and treatment observations were unbalanced (whole plant = 6; clippings = 8). EAG responses to treatments (measured in volts [V], a negative value), were converted to amplitude and scaled as mV, and then transformed (ln[(-x * 1000) +0.5]) before two-way Analysis of Variance (analysis of variance [ANOVA]) with sex and extract treatment (whole plant, clipping, solvent, air) as factors. One-way ANOVA analyses were performed when significant effects of factors were found, followed by Tukey's honestly significant difference (HSD) all-pairwise comparisons of means ($\alpha = 0.05$).

Average EAG response to individual compounds was normalized relative to the standard, first by subtracting the solvent, and then reporting the value as a proportion of the response to the standard. Standardized data were square root transformed (0.5* [$\sqrt{}$ x +

Table 1. Response of *L. maculicollis* males and females to *Poa annua* whole plant and clippings in Y-tube olfactometer bioassays

P. annua treatment	Sex	N/total[a]	Choice	No. respond.	G test-Williams	*P* value[b]
1. Whole plant	Female	40/49 (81.6)	*P. annua*	27	4.94	0.03
			Blank	13		
	Male	40/44 (90.9)	*P. annua*	20	0.0	1.00
			Blank	20		
2. Clippings	Female	40/42 (95.2)	Clippings	11	8.29	0.004
			Blank	29		
	Male	40/43 (93.0)	Clippings	16	1.59	0.21
			Blank	24		
3. Whole plant vs clippings	Female	40/41 (97.6)	Whole plant	22	3.96	0.53
			Clippings	18		
	Male	40/43 (93.0)	Whole plant	24	1.59	0.21
			Clippings	16		

[a] n = The total number of weevils responding to an odor source; total divided by the total number of weevils tested (responding and non-responding).
[b] Bold values indicate significant responses ($df = 1$, $P < 0.05$).

1]) to stabilize the variance and conform to a normal distribution. One-way ANOVA analyses were performed when significant effects of factors were found, followed by Tukey's HSD all-pairwise comparisons of means ($\alpha = 0.05$). Dose–response relationships to individual synthetic compounds were analyzed by simple linear regression followed by comparison of slopes and intercepts to determine if male and female antennae responded differently to the compounds. For each synthetic compound, *t*-tests were used to determine if sexual differences existed at individual dilutions. EAG not conforming to a normal distribution (as determined by Shapiro-Wilks tests of normality) was subjected to nonparametric Kruskall–Wallis one-way ANOVA, followed by Wilcoxon Rank Sums test to determine sexual differences in response to the compound and individual dilution.

Results

Y-tube bioassays. A high percentage of both males (92%) and females (90%) made a choice in Y-tube bioassays in the allotted time. However, significant differences were detected in the choices made by each sex (Table 1). Significantly more females were attracted to undamaged *P. annua* than to the blank. In addition, females significantly avoided clipped *P. annua* when opposite of clean air. Surprisingly, when offered a choice between intact and clipped *P. annua*, female response was not significant toward either treatment. Males' response was not significant when presented either intact or clipped *P. annua* opposite clean air, or when both *P. annua* treatments were offered simultaneously.

Plant Volatile Identification. GC-MS determined the identities of 12 volatiles emitted from *P. annua;* the identity of one compound remains unknown (Table 2). Nine of these compounds were common to both clippings and intact plants, with an additional four only identified from clippings. Furthermore, intact *P. annua* and *P. annua* clippings differed quantitatively in their volatile emissions (Table 2). On average, total volatile emissions from clipped *P. annua* were approximately seven-fold higher than that of intact turfgrass cores. Extracts from clipped *P. annua* contained significantly higher quantities of hexanal, (*E*)-2-hexenal,

Table 2. Comparison of volatiles from whole plant and *Poa annua* clippings as identified by GC-MS

		Whole plant		Clippings			
Peak	Chemical name	Mean ± SE[a]	% total	Mean ± SE	% total	*U*[b]	*P*[c]
1	Hexanal	0.12 ± 0.09	3.54%	1.26 ± 0.33	5.17%	8, 40	0.03
2	(*E*)-2-hexenal	0.14 ± 0.14	3.94%	3.00 ± 0.82	12.38%	0, 48	<0.001
3	(+)-β-Pinene	n/d[d]	0.00%	1.00 ± 0.25	4.12%	6, 42	0.01
4	6-Methyl−5-hepten-2-one	n/d	0.00%	1.68 ± 0.54	6.92%	3, 45	0.004
5	Octanal	0.75 ± 0.17	21.91%	0.12 ± 0.12	0.51%	8.5, 39.5	0.02
6	(Z)-3-hexenyl acetate	0.57 ± 0.24	16.52%	7.80 ± 1.53	32.16%	6, 42	0.02
7	Limonene	0.19 ± 0.15	5.39%	0.25 ± 0.18	1.01%	23, 25	0.93
8	Undecanal	0.08 ± 0.08	2.47%	0.53 ± 0.27	2.19%	30.5, 17.5	0.33
9	Nonanal	0.14 ± 0.11	4.12%	2.35 ± 0.44	9.70%	0, 48	<0.001
10	Phenyl ethyl alcohol	0.84 ± 0.29	24.35%	2.64 ± 0.42	10.89%	5, 43	0.01
11	Decanal	0.61 ± 0.20	17.68%	2.89 ± 0.64	11.92%	2, 46	0.003
12	Unknown	n/d	n/d	0.46 ± 0.18	1.89%	12, 36	0.08
13	Dodecanal	n/d	n/d	0.28 ± 0.14	1.14%	15, 33	0.20
	Total	3.44 ± 1.46		24.28 ± 5.88		0, 48	<0.001

[a] Average peak size measured in ng/g of wet tissue/h.
[b] Figures in bold indicate significant differences between intact and clipped *P. annua* ($\alpha = 0.05$).
[c] Wilcoxon Rank Sum U statistic (whole plant, clippings).
[d] n/d = not detected.

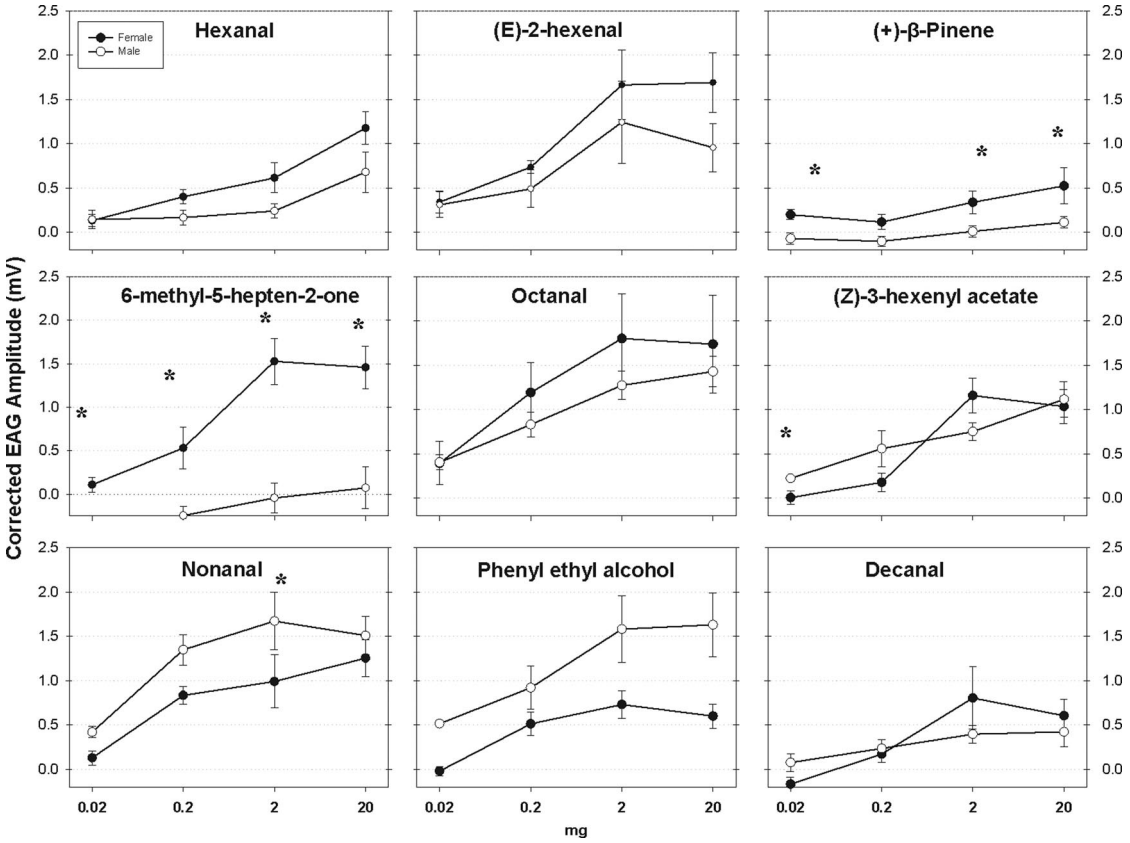

Fig. 1. Dose–response curves for electroantennogram (EAG) responses of female and male *L. maculicollis* to selected synthetic compounds identified as important *P. annua* volatiles. Data are presented as solvent corrected values (mV ± SE). (*) denotes a significant difference in the response between the sexes ($P < 0.05$).

(+)-β-pinene, 6-methyl-5-hepten-2-one, (Z)-3-hexenyl acetate, nonanal, phenyl ethyl alcohol, and decanal (Table 2). Octanal was the only volatile found in significantly greater quantities in intact *P. annua* than in clipped *P. annua* extracts (Table 2). (Z)-3-hexenyl acetate (32.2%) and (*E*)-2-hexenal (12.4%) comprised the largest volatile emission from clipped *P. annua*, while phenyl ethyl alcohol (24.4%) and octanal (21.9%) comprised the largest percentages of volatiles in extracts of undamaged plant material (Table 2).

Electroantennogram Responses. Strong significant differences were observed in the magnitude of weevil EAG responses to *P. annua* volatiles in whole plant (1.06 ± 0.07 mV) and clippings extracts (1.80 ± 0.26 mV) when compared with the solvent (0.34 ± 0.07 mV) or air (0.26 ± 0.04 mV) alone ($F = 40.12$; df = 3; $P < 0.0001$). No differences were detected in EAG responses between the sexes ($F = 0.18$; df = 1; $P = 0.78$), and no interactions were observed between sex and treatment ($F = 0.47$; df = 3; $P = 0.71$). However, one-way ANOVA and means separation of treatments within each sex revealed differences in male and female antennal responses. Female response was significantly stronger to both *P. annua* treatments compared with solvent extracts and clean air ($F = 26.0$; df = 3; $P < 0.0001$). Furthermore, significantly greater EAG amplitudes were observed to volatiles in clippings compared with whole plant extract. Male antennae also demonstrated strong differences in EAG responses to plant extracts when compared with controls ($F = 32.9$; df = 3; $P < 0.0001$), but responses did not differ significantly between plant extract treatments.

Male and female EAG responses to individual synthetic compounds increased in a significant dose–response manner with each compound tested (Fig. 1). EAG magnitudes were highest for nonanal, octanal, and (*E*)-2-hexenal. There were no significant differences between the sexes in the sensitivity when the responses to all compounds were averaged ($F = 2.54$; df = 1; $P = 0.11$), though regression slopes differed significantly between the sexes ($F = 12.64$; df = 1, 348; $P = 0.0004$). No differences between the sexes were observed when regression slopes of individual compounds were compared, though significantly higher intercepts were found for females with (+)-β-pinene ($F = 23.06$, df = 1, 41; $P < 0.0001$) and 6-methyl-5-hepten-2-one ($F = 43.47$, df = 1, 37; $P < 0.0001$), and for males with phenyl ethyl alcohol ($F = 43.47$, df = 1, 37; $P < 0.0001$). Female antennal response was significantly greater than males for responses averaged across concentrations, for the compounds (+)-β-pinene ($F = 21.84$; df = 9; $P < 0.0001$) and 6-methyl-

5-hepten-2-one ($F = 67.4$; df = 9; $P < 0.0001$). Male antennae were more sensitive than female antennae to (Z)-3-hexenyl acetate ($F = 9.57$; df = 9; $P < 0.005$) and phenyl ethyl alcohol ($U = 8.63$; df = 7; $P < 0.007$). For single concentrations of each compound (Fig. 1), female antennae were significantly more sensitive than male antennae to all concentrations of 6-methyl-5-hepten-2-one and to three of the four concentrations of (+)-β-pinene. Male antennae were more sensitive than female antennae at one concentration each of (Z)-3-hexenyl acetate and nonanal.

Discussion

This study is the first investigation into the chemical ecology of *L. maculicollis* and an initial attempt to discern the possible importance of turfgrass (*P. annua*) volatiles in *L. maculicollis* orientation and host selection. We observed significant behavioral responses of *L. maculicollis* females to volatiles emitted from *P. annua* and differential responses to *P. annua* treatments. However, contrary to our hypothesis, females significantly avoided *P. annua* clippings and were more attracted to the intact *P. annua* when assayed opposite to clean air. One explanation is that the amount of volatiles in the Y-tube olfactometers may not have been representative of those volatiles likely to be encountered by the insect in the field. In fact, high quantities of volatiles, as observed with clippings, in close proximity to the insect may cause repellency where attraction at longer ranges typically occurs. Sensitivity to low doses of host-plant volatiles has been demonstrated in several beetle species (Hansson et al. 1999, Larsson et al. 2001, de Groot et al. 2008). Females may have been attracted to the lower quantities of plant odors released from intact plants, but repelled by the seven-fold higher emissions from clippings. Unnaturally high quantities of volatiles in this case may cause saturation of the antenna, creating a repellent effect. Clippings contained significantly higher quantities of green-leaf volatiles (GLVs), such as hexanal, (*E*)-2-hexenal, and (Z)-3-hexenyl acetate, which have been shown to be attractive to several weevil species; however, examples of weevil repulsion to GLVs exist (Parra et al. 2009). Another factor complicating the results from behavioral assays was that females failed to respond when both *P. annua* treatments were offered simultaneously, despite significant and opposite responses when each stimulus was offered individually. Szendrei et al. (2009) observed similar results in Y-tube olfactometer experiments with the cranberry weevil (*Anthonomus musculus* Say), leading the authors to speculate that the positive response toward one stimulus was antagonized by the other when volatiles were mixed in the notch of the Y-tube.

Unlike *L. maculicollis* females, males did not show significant behavioral responses to *P. annua* volatiles in Y-tube bioassays, suggesting that the sexes may differ in their foraging strategy. Behavioral differences between sexes in response to host-plant volatiles are commonly observed among insects, with females typically having a greater response than males (Szendrei and Rodriguez-Saona 2010). To date, field studies have not indicated protandry or sex ratio bias toward colonization of low mown playing surfaces in *L. maculicollis* (Rothwell 2003, B.A.M., unpublished data). Diaz et al. (2008) observed a skewed male sex ratio in higher mown grasses, but not on the low mown playing surfaces where oviposition typically occurs. Though evidence for sex-biased colonization has not been found for *L. maculicollis* at the scale of tens of meters, additional olfactory cues (e.g., aggregation or sex pheromones) may play a role in the attraction of the opposite sex at a more localized level. In some weevil species, males produce aggregation pheromones, which may have synergistic effects on the attractiveness of plant volatiles, including GLVs (Rochat et al. 2000, Reddy and Guerrero 2004). Male boll weevil, *Anthonomus grandis* Boheman are believed to release an aggregation pheromone after feeding upon cotton (Dickens 1989). Because *L. maculicollis* oviposition is likely to occur at feeding sites, males may randomly search for hosts before signaling females to hosts, and therefore could be less responsive to plant volatiles. Conversely, males may not be the colonizers of patches, but may require additional odor cues such as female-produced sex pheromones to locate hosts or mates. Future physiological studies will be conducted to determine if *L. maculicollis* either produce an aggregation or sex pheromone.

Volatile emissions from intact *P. annua* and clippings demonstrated significant quantitative differences from intact plants. We identified 13 volatile peaks in our GC-MS analyses, 13 from intact *P. annua* turf cores, nine from clippings, with nine common to both. Though the number of peaks and compounds were similar in both treatments, the average volatile emissions from clippings were approximately seven-fold higher than from intact plants. This information was not surprising because damaged plants, whether damaged mechanically or by insect herbivory, have been shown to produce higher quantities of volatiles (Paré and Tumlinson 1997), particularly of n-C_6 GLV compounds. GLVs are general plant volatiles (alcohols, aldehydes, and acetates) emitted by all green plants, though the composition and proportions may be species-specific (Visser et al. 1979). Eight individual peaks were significantly higher in clipped plants, yet only one (octanal) was found to be significantly higher in intact plant extracts. Of the eight volatiles released at significantly greater rates from clippings, most were GLVs. (Z)-3-hexenyl acetate, a GLV previously associated with mechanically damaged turfgrasses (Watkins et al. 2006), comprised 32.2% of the total volatile emissions in clippings. GLV extracts containing (Z)-3-hexenyl acetate elicit behavioral responses in the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Visser and Avé 1978, Visser et al. 1979). However, when applied as single compounds, no attraction is observed. The similarities in the compounds detected in both intact and clipped *P. annua* by GC-MS suggest that not only the quantity, but also the proportion of compounds may be

important if *L. maculicollis* is able to differentiate cues between mown (mechanically damaged) and intact plants.

Despite differences in the behavioral response to host-plant volatiles between sexes in *L. maculicollis*, we generally observed similar male and female EAG responses to extracts containing volatiles emitted from intact and clipped *P. annua.* These results demonstrate that both sexes possess receptor neurons for volatiles released by *P. annua.* Although no sexual differences were detected in the absolute EAG response to volatiles released by either treatment, female antennae responded more strongly to volatiles in clipping extracts. These differences in the response between the two treatments reflect either a difference in the number of receptor neurons activated by each treatment or in the degree of activation (Reinecke et al. 2005). Because 8 compounds, particularly the GLVs, were emitted in greater quantities in clipped *P. annua*, it is most likely the latter. Many weevils, including the boll weevil, *A. grandis* (Dickens 1984), the cabbage seed weevil, *Ceutorhynchus assimilis* (Paykull) (Blight et al. 1995), the black vine weevil, *Otiorhynchus sulcatus* (F.) (van Tol and Visser 2002), and the cranberry weevil, *A. musculus* (Szendrei et al. 2009) have shown strong antennal responses to GLVs. Because of the differential response of female antennae to clipped and intact *P. annua* additional behavioral studies will be necessary before we can fully understand the significance of these findings.

Antennae of both sexes demonstrated strong responses to many of the GLVs in EAG assays of individual synthetic compounds, including (*E*)-2-hexenal, and (Z)-3-hexenal acetate. As with whole plant extract EAGs, few differences were detected between sexes. Female antennae displayed greater sensitivity than male antenna to 6-methyl-5-hepten-2-one at all concentrations tested and to three of four concentrations of (+)-β-pinene (though depolarization amplitudes were generally low for both sexes). The lack of significant differences between male and female antennal responses to whole plant extract and individual synthetic compounds is not surprising given that both insects feed upon the same hosts species and therefore are likely to detect similar plant volatile cues in foraging. Surprisingly, male and female antennae were both highly sensitive to octanal, which was detected in higher quantities in intact plants, yet only female behavior was significantly affected in Y-tube bioassays. These results demonstrate the importance of using multiple methodologies to assess the importance of semiochemicals in orientation and host selection.

The physiological state of the test insects and plant material may have interfered with our ability to observe significant male response to plant odors, despite positive antennal responses. The physiological condition of insects, including starvation, has been documented to affect the response to host volatiles in several taxa (Bernays and Chapman 1994, Davidson et al. 2006). The weevils used in our bioassays were collected from overwintering sites >2 mo before testing. The weevils were kept in overwintering conditions, and food was not provided before bioassay. Furthermore, because all weevils were field collected, we were unable to determine the mating status in females. However, apart from the potential effects of mating status on female choice, the test insect conditions are unlikely to be much different from that of emerging insects in the field. Another explanation for lack of strong responses in behavioral assays may relate to the quality or composition of plant odors. *P. annua* is a winter annual, which germinates from seed in the fall, grows through the winter, and completes its life cycle in the following summer (Vargas and Turgeon 2004). Though the plant material used in the experiments was collected during the active growth phase, the physiological state may have played a role in its attractiveness toward females. In New Jersey, *P. annua* produces flowers in late April to early May, a period when females are depositing the majority of their eggs (McGraw and Koppenhöfer 2009a,b). Future research should investigate the effects of *P. annua* phenological and physiological conditions on released volatiles and their effects on *L. maculicollis* behavior.

This is the first study to examine the role that plant semiochemicals play in *L. maculicollis* orientation and foraging behavior. Little is known about *L. maculicollis* behavior because of the difficulty of observing the rather cryptic insects in the field. However, examining the behavioral and electrophysiological responses of *L. maculicollis* to plant odors in the laboratory may allow insights into how populations move onto low mown grasses (i.e., playing surfaces) on golf courses and could aid in developing monitoring traps or even novel management strategies (e.g., lure-and-kill technologies, mass trapping, deterrents). Our behavioral and EAG studies indicate that 1) *L. maculicollis* females exhibit both behavioral and physiological (EAG) responses to host-plant volatiles, and 2) both sexes possess receptors sensitive to volatiles emitted by *P. annua.* However, more behavioral assays and field studies will be required to clarify the response to individual compounds and ratios of volatile mixes, as well as to develop a better understanding of their ecological significance before novel monitoring strategies can be developed.

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