Biological Control of Asparagus Pests Using Synthetic Herbivore-Induced Volatiles

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Abstract

Natural enemies can be important regulators of pests in agroecosystems, and they often rely on volatile chemical cues to find hosts. Herbivore-induced plant volatiles (HIPVs) have been a focal point in many studies that seek to increase the efficacy of biological control programs by increasing recruitment and retention of natural enemies. Our research sought to explore the role of HIPVs in asparagus agroecosystems to answer the following questions: 1) What is the composition of HIPV produced by asparagus ferns following feeding by a chewing herbivore? 2) Do field deployed lures baited with synthetic asparagus HIPVs attract natural enemies? and 3) Can HIPV lures increase biological control of asparagus pests? Volatiles were field collected from the headspace of healthy asparagus ferns, mechanically damaged ferns, and ferns fed upon by asparagus beetle larvae (Crioceris asparagi L.) (Coleoptera: Chrysomelidae). We found that asparagus beetle damaged asparagus had significantly higher concentrations of (E)-α-farnesene, (E,E)-α-farnesene, and (1)-tetradecanol than healthy or mechanically damaged ferns. Field experiments demonstrated that lures baited with isolates of ocimene and farnesene attracted parasitoids without attracting pests, but had no impact on predator recruitment. Finally, we determined that overall parasitism rates were not increased by synthetic HIPV lures but found evidence that lures may increase parasitism of asparagus miner (Ophiomyia simplex Loew) (Diptera: Agromyzidae) by pteromalids.

Keywords: asparagus miner, common asparagus beetle, biological control, lure, kairomone

Biological control is one of the foundations of sustainable pest management and can effectively complement other pest management strategies such as cultural and chemical control (Van Driesche and Bellows 1996, Gurr and Kvedaras 2010). Herbivore-induced plant volatiles (HIPVs) are indirect plant defenses that can attract biological control agents, such as arthropod natural enemies, to plants when damaged by pests (Turlings et al. 1990, Dicke and van Loon 2000, Van Loon et al. 2000). Although plants produce low levels of volatile chemicals constitutively, herbivore feeding can result in changes in the production of both constitutive and novel volatiles (Vet and Dicke 1992, Paré and Tumlinson 1997). The information provided by HIPVs to natural enemies can be reliable signals serving as infochemical webs that influence natural enemy foraging behavior and chemotaxis (Vet and Dicke 1992). Although our understanding of these interactions is improving, the applications in agriculture for pest management are still largely lacking and research focused on the development of lures baited with HIPVs, the use of genetically modified crops to express attractive HIPVs, and the use of systemic inducers of plants to enhance biological control programs and support pest management should be the next step to engage crop producers to use HIPV technologies (Turlings and Ton 2006, Kaplan 2012, Bisen et al. 2016, Salvagnin et al. 2018).

Much of the published research on HIPVs to attract natural enemies has been conducted in the laboratory; however, some field experiments deployed lures effectively in agroecosystems (Hunter 2002, Kaplan 2012). Natural enemy attraction to lures baited with HIPVs has been successful in perennial agroecosystems, such as apples (Jones et al. 2016), cotton (Yu et al. 2008), cranberries (Rodriguez-Saona et al. 2011), grapes (James and Price 2004, James and Grasswitz 2005), hops (James 2003a, 2003b, 2005), pears, and walnuts (Jones et al. 2016). Promising results from these types of studies lead to the development of commercially available arthropod predator lures containing the plant volatiles methyl salicylate (PredaLure®, AgBio Inc., Westminster, CO) and 2-phenylethanol (Benallure®, MSTRS Technologies, Ames, IA), which were effectively used in some crops (Sedlacek et al. 2009, Rodriguez-Saona et al. 2011). However, many challenges still face successful development of lures baited with HIPVs in agroecosystems for attracting natural enemies.
HIPVs produced by plants are often complex and can include hundreds of compounds making selection of HIPVs for experimentation challenging (Mumm and Dicke 2010, Kaplan 2012). In addition, lack of food web knowledge, determination of effective HIPVs concentrations, chemical release rates and non-target effects, logistics of field scale testing of lures, and identification of natural enemy responses to lures that are predictable and reliable are important to understand when developing these technologies (Kaplan 2012). Additionally, increased attraction of natural enemies to areas baited with HIPV lures does not necessarily improve host or prey location. In some cases, parasitism or predation may increase in the presence of lures, while in others, attraction may lead to no improvements in pest management (Williams et al. 2008, Ferry et al. 2009, Mallinger et al. 2011). Further complicating matters, volatile signals can also serve as attractants for pests resulting in negative outcomes for pest management (Boelter et al. 1997, Halitschke et al. 2008). Therefore, to narrow the scope of inquiry and address many of these issues it is important for researchers to focus on specific agroecosystems with targeted management goals.

In the United States, specialty crops make up 40% of the total value of the agricultural market, but account for only 1.5% of the total hectares farmed (USDA 2015, USDA ERS 2017). Due to the small total area of these crops, compared with field/row crops, agro-chemical companies often have little financial incentive to register pesticides that target obligate pests in smaller specialty crop sectors, which in turn motivates growers to seek alternative pest management options, such as biological control (Miller and Leschewski 2012). This is particularly true in Michigan asparagus (Asparagus officinalis L.) where obligate pests affecting productivity and crop longevity, the asparagus miner (Ophionymia simplex Loew) (Diptera: Agromyzidae) and the common asparagus beetle (Crioceris asparagi L.) (Coleoptera: Chrysomelidae), are not managed effectively with currently registered insecticides and the crop makes up less than 0.02% of the land occupied by vegetables in the United States (USDA-NASS 2018). Asparagus miner feeding can increase incidents of Fusarium crown rot leading to 50% reductions in field longevity (Elmer et al. 1996, Tuell and Hausbeck 2008, Morrison et al. 2011), and common asparagus beetle management costs and estimates for damage have been reported between $1.4 and 1.6 million per year for Michigan, Washington, and Illinois (Hendrickson et al. 1991).

Our research aimed to understand the use of HIPV lures to enhance biological control of the asparagus miner and the common asparagus beetle in the field. We explored this topic by: 1) identifying HIPVs of asparagus; 2) examining natural enemy responses to lures baited with asparagus HIPVs; and 3) determining if HIPV lures increase biological control of asparagus miner or common asparagus beetle.

Materials and Methods

Experiment 1: HIPV Collection and Analysis

Investigation of asparagus HIPVs were conducted using common asparagus beetle larvae in field trials at the Entomology Research Farm, Michigan State University (East Lansing, MI), from July to August 2014. Beetle larvae were chosen as a target subject because they are one of the key pests of asparagus (Morrison and Szendrei 2014), they are voracious feeders, easy to collect and handle, and co-occur with asparagus miner on field edges of post-harvest commercial asparagus fields (Ingrao et al. 2017).

Sixteen insect exclusion cages (183 x 183 x 183 cm, 32 x 32 mesh Lumite® screen, BioQuip, Rancho Dominguez, CA) were set up in a 0.2 ha fallow field. Cages were spaced 5 m apart in all cardinal directions to create a 4 x 4 randomized block design. Six, 1-yr-old asparagus crowns (cv. ‘Guelph Millennium,’ Oomen Farms Ltd, Hart, MI) were planted at 25 cm depth into each cage in two rows running north to south in a 2 x 3 design with 90 cm row spacing and 60 cm crown spacing within rows. Plants grew under natural conditions, without supplemental fertilizer or irrigation for the duration of the experiment, and they were monitored twice weekly for pests using visual scouting and yellow sticky traps (13 x 8 cm, Great Lakes IPM, Inc., Vestaburg, MI) and any insects found were removed from the cages. Plants were used in experiments when at least one stem reached the fern stage with all cladophylls fully expanded, approximately 6 wk after planting.

Herbivore treatments to induce the plants were assigned to cages and administered to one randomly selected asparagus plant within each cage, and the other plants in the cages were used in later replications. Four replications were conducted over July–August and treatments consisted of: 1) empty collection bag (used to identify background contamination); 2) control (undamaged healthy asparagus plant); 3) mechanically damaged plant; and 4) common asparagus beetle larvae damaged plant. Mechanical damage was inflicted on ferns by removing 8 cm of plant tissue from the terminal end of five randomly selected branches using a scalpel, 48 and 24 h prior to volatile collection. Preliminary tests determined that 20 asparagus beetle larvae (2nd–4th instar) removed approximately the same amount of plant tissue in 48 h of feeding as our mechanical damage treatment. Common asparagus beetle larvae damage treatments were inflicted upon plants with 20 larvae (2nd–4th instars). Larvae were hand collected from a 5-yr-old, 0.2 ha asparagus field (cv. ‘Guelph Millennium’) located at Michigan State University and were used within 3 h of collection for experiments. Asparagus beetle larvae were randomly placed on axillary branches of a caged asparagus fern with a fine tipped paintbrush and were allowed to feed ad libitum for a 48 h period prior to volatile collection. All beetle larvae were removed from plants 1 h prior to volatile collection.

Plant volatiles were collected (1 liter/min) for 24 h on a volatile trap (30 mg HayeSep Q®, Sigma Aldrich, St. Louis, MO) on 4–5 July 2014 (mean ± SEM: 17.3 ± 6.6°C, 60.3 ± 30.9 % RH), 19–20 July 2014 (mean ± SEM: 19.7 ± 5.0°C, 71.8 ± 21.7 % RH), 2–3 August 2014 (mean ± SEM: 21.0 ± 6.0°C, 66.1 ± 23.9 % RH), and 15–16 August 2014 (mean ± SEM: 16.5 ± 6.0°C, 63.0 ± 23.4 % RH). Headspace was sampled by enclosing the entire plant in a collection bag (polyvinyl fluoride film collection bag 56 × 40 cm, Tedlar®, DuPont Inc., Wilmington, DE). The volatile trap was inserted into the bag while being attached to a vacuum pump (Model 5R1110-101-1049, Gast Manufacturing, Benton Harbor, MI). An activated charcoal filter constructed of a modified Pasteur pipette tip (#14762, VWR, Radnor, PA) filled with activated charcoal (Pro-Carb™,PennPlax® Inc., Hauppauge, NY) was placed into the end of the collection bag and sealed with garden wire to allow ambient air to be filtered while entering the bag during volatile collection. Preliminary tests using empty collection bags demonstrated that bags maintained full expansion during a 24 h collection period and the activated charcoal filtered background contamination effectively from the surrounding environment using passive ambient air filtration. Pumps were powered by a 12V battery (Model UB1280, Universal Power Group Inc., C每隔, TX) in a water proof case (Seahorse SE-300F, The Waterproof Case Company LLC., La Mesa, CA).

Volatiles were eluted from each volatile trap using 150 μl dichloromethane and then tetradecane (500 μM) sample was added as an internal standard to each sample. Volatile extractions were analyzed using an Agilent 7890A gas chromatograph (GC) paired with an Agilent 5975C mass spectrometer (MS) (Agilent Technologies, Santa
Clara, CA). The GC–MS was equipped with an Agilent HP-5 column (30 m length, 0.320 mm ID, film thickness 0.25 μm). Helium was used as the carrier gas at 30 cm/s flow velocity. Aliquots (1 μl) of each sample were injected into the GC–MS and separated with a program of 1 min at 40°C followed by increasing temperature at a rate of 10°C/min to 260°C. The reagent gas used for chemical ionization was isobutane. Ion source temperature was 250°C in chemical ionization mode and was 220°C in electron impact mode. GC–MS results were analyzed using MSD ChemStation v.2.00 (Agilent Technologies, Santa Clara, CA). All detected compounds were tentatively identified by comparing the mass spectrum of each compound to those in reference libraries: Adams 2 terpenoid/natural product library (Adams 1995) and NIST 11 (National Institute of Standards and Technology; Springfield, VA). Compound identifications were confirmed by comparing calculated Kovats Indexes (KI) to reference KI (Adams 1995, De Marques et al. 2000, Da Silva et al. 2003). Ocimene, farnesene (≥90 purity, Sigma Aldrich) and 1-tetradecanol (95% purity, Matrix Scientific, Columbia SC) identifications were confirmed by comparing retention times to synthetic reference standards.

Prior to statistical analysis, background contamination identified in the empty collection bag treatment and rare compounds, appearing in less than three samples, were removed from sample profiles. The amount of individual volatile compounds released from each treatment were calculated relative to the hours of collection and the biomass of the plant (volatile (ng) / plant tissue (g) / collection (h)) and were analyzed to determine their relative contributions to the overall headspace profile of asparagus. Differences between treatments among individual compounds were determined using a Kruskal–Wallis test (package = ‘STATS’). When significant differences were found between treatments, a post hoc Dunn’s multiple comparisons test with Bonferroni correction was conducted (α = 0.05; package = ‘DUNN.TEST’). All statistical analyses were conducted using R software (R Core Development Team 2015).

**Experiment 2: HIPV Lures**

Lures were developed for experiments to test the attraction of synthetic asparagus HIPVs to arthropods in the field using ocimene and farnesene (ocimene mixture of isomers and farnesene mixture of isomers, Sigma Aldrich) because of their presence in herbivore-induced asparagus plants, commercial availability, and low cost. Lures comprised a cotton ball (~0.28 g, Covidien LLC, Mansfield, MA) placed in a 2 ml microcentrifuge vial (Denville Scientific Inc., Hollliston, MA) and wrapped with black tape (Scotch Duct Tape, The 3M Company, St. Paul, MN) to prevent photolysis of compounds. Ocimene and farnesene lures were tested at different concentrations either as isolates or as mixtures of the two compounds. The following lures were evaluated: no lure (negative control), blank lure (positive control), farnesene high (1,000 μl farnesene), farnesene low (750 μl farnesene), ocimene high (500 μl ocimene), ocimene low (300 μl ocimene), mixture high (1,000 μl farnesene + 500 μl ocimene), and mixture low (750 μl farnesene + 350 μl ocimene). Vials were opened and attached horizontally to the top of a 1 m tall metal pole with garden wire, directly below a yellow sticky trap (13 × 8 cm, Great Lakes IPM, Inc., Vestaburg, MI). Lure field-release rates were established by collecting volatiles from each lure type over a 7-d period on Days 1, 4, and 7, for 2–7.5 h (Supp Table 1 [online only]). Average release rates per hour were calculated from the weekly mean of three replications with the same headspace collection equipment described in Experiment 1.

Field testing of lures was initially conducted for 5 wk, from July to August 2016, in six commercial asparagus fields (one replication per field) in Oceana County (MI). Field sites were all within 8 km of Lake Michigan and had a consistent eastwardly prevailing wind from the lake. All fields used in the experiment had eastern field edges that were along unmanaged forested borders (mixtures of conifers and deciduous hardwoods) with a ~5 m drive row between the border habitat and field edge. Lures were placed on the eastern crop edges ~5 m from the forested border habitat and 10 m apart so that the prevailing wind carried volatile signals into the wooded field border to attract natural enemies into the asparagus field from these natural habitats. Lures were only placed on the field edge because asparagus miner and common asparagus beetle both congregate on field edges in post-harvest asparagus (Morrison and Szendrei 2013, Ingrao et al. 2017). Sticky traps and lures were replaced weekly and pests, predators, and parasitoids collected on the traps were identified to lowest possible taxonomic level and quantified (Stehr 1987, Goulet and Huber 1993, Arnett 2000, Arnett and Thomas 2000, Arnett et al. 2002, Ubick et al. 2009, Bradley 2012).

To test the effect of field position on the efficacy of lures, we continued sampling for an additional 3 wk from August to September 2016, adding six research sites with lures on the southern field edge of asparagus fields with forested southern margins. Following the same protocol outlined earlier, we collected sticky traps and determined abundance of pests, predators, and parasitoids weekly.

The effect of field position on the number of arthropods trapped was determined; however, since it had no effect, position was dropped as a fixed factor and total abundance for pests, predators, and parasitoids were analyzed with a mixed effects model (GLMER, package = ‘LME4’) with Poisson distribution. This analysis can account for an unbalanced experimental design since lures and traps were sometimes run over by farm equipment and destroyed. Lure treatments were fixed effects, and field and date were random effects. Full and reduced models were considered and models were selected for best fit based on Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). Least squares means multiple comparisons, with Bonferroni correction, were conducted post hoc (α = 0.05; package = ‘MULTCOMP’).

**Experiment 3: Effects of Lures on Biological Control of Key Pests**

In 2017, performance of the lure that attracted the most parasitoids in Experiment 2 (ocimene high; 500 μl) was investigated in the field to understand the impact of the ocimene lure on the biological control of asparagus miner and common asparagus beetle. Lures were constructed and deployed as described in Experiment 2; however, yellow sticky cards were not used. Lures were deployed in four commercial asparagus fields (one replication per field) in Oceana County, MI. Lures were distributed on the asparagus field edge at two densities: control (no lures), low density (three lures on the field edge), and high density (three lures on the field edge and three lures 5 m into the field) (Supp Fig. 1 [online only]). Treatments were separated by 20 m and lures within treatments were spaced at 10 m intervals. Lures were replaced weekly for 6 wk.

Arthropods were collected from a 1 × 20 m transect on the field edge of each treatment area for 5 wk from July to August, starting 1 wk after lure deployment (Supp Fig. 1 [online only]). Within each collection transect, we hand collected 3rd–4th instar asparagus beetle larvae and asparagus miner pupae. Collected larvae were placed in a plastic bag and transported to the laboratory where they were reared in a climate-controlled room (25 ± 0.5°C, 70 ± 5% RH, 16:8 (L:D) h) to determine larval parasitization. Larval rearing cages comprised an asparagus axillary branch cut from a mature fern with the
cut end inserted through a small hole in the bottom of a plastic cup (59 ml, Solo®), Dart Container Corp., Mason, MI) that was filled with potting soil (SureMix Perlite, Michigan Grower Products Inc., Galesburg, MI) to allow larvae to fall to the soil to pupate. The bottom of the asparagus stem was inserted into 4 x 4 x 3 cm piece of saturated wet foam (FloraCraft®, Letica® Corp., Rochester, MI). Larvae (1–10) were placed on the asparagus stems with a fine tipped paint brush and were covered with a 30 x 10 cm cylindrical chamber constructed of plastic transparency film (ACCO Brands, Inc., Lincolnshire, IL), covered with a 160 μm screen mesh at the top to allow for ventilation (Supp Fig. 2 [online only]). Once larvae dropped from the plant and began pupating in the soil, the asparagus stem was removed and the soil filled cups were capped with a perforated lid. Cups were then monitored daily and emerged asparagus beetles and parasitoids were quantified and identified to species using reference vouchers from the A.J. Cook Arthropod Research Collection (Michigan State University).

Asparagus miner pupae were collected by randomly cutting 20 stems/collection transect, ~6 cm below the soil surface and at the highest mine on the stem. Samples were placed in plastic bags and returned to the lab. All asparagus miner pupae were excised from each of the mined stems and placed individually into ventilated plastic cups (59 ml, Solo®), Dart Container Corp., Mason, MI). Rearing cups were then held in a controlled growth chamber (26.0 ± 1.0°C, 80 ± 5.0% RH, 16:8 (L:D) h) until an asparagus miner or parasitoid hatched. Samples were discarded if nothing hatched after 5 wk. Asparagus miners and parasitoids that emerged from pupae were quantified and identified to genus or species using voucher specimens from the A.J. Cook Arthropod Research Collection.

Due to the absence of asparagus beetles on all collection dates except August 14th (41 larvae collected and reared) and August 21st (three larvae collected and reared), statistical analysis on the number of asparagus beetles and the proportion of asparagus beetle parasitized are not presented here. For asparagus miners and its associated parasitoids, the hatch rates were analyzed with a generalized linear mixed model with binomial distribution where treatment was a fixed factor and date and field were random factors (package = ‘LMEM4’). When significant main effects were detected, a post hoc least squares means comparison with Bonferroni correction was used to determine differences between treatments (package = ‘MULTCOMP’). The total number of parasitoids that hatched from asparagus miner pupae were summed over the season and were analyzed with a Pearson’s chi-squared test with post hoc multiple pairwise comparisons (α = 0.05; package = ‘STATS’).

Results

Experiment 1: HIPV Collection and Analysis

We detected 21 volatile compounds that were produced by asparagus ferns in response to herbivory by asparagus beetle larvae (Table 1). Healthy asparagus ferns produced 20 volatile compounds in the headspace ((E)-β-ocimene not present), while mechanical damaged plants produced 18 compounds (undecane, dodecane, and 1-tetradecanol not present). Herbivory by asparagus beetle larvae significantly upregulated the production of (E)-β-ocimene ($\chi^2 = 9.30$, df = 2, $P = 0.01$) and 1-tetradecanol ($\chi^2 = 12.83$, df = 2, $P < 0.01$) in beetle damaged plants when compared with mechanically damaged or healthy plants. Asparagus beetle damaged plants also had significantly higher concentrations of (E,E)-α-farnesene compared to undamaged plants, but had similar concentrations to that of mechanically damaged plants ($\chi^2 = 16.43$, df = 2, $P < 0.01$; Table 1, Fig. 1).

Ocimene was not present in any of the control plants’ headspace, but it made up 1 and 3% of the mechanical and asparagus beetle damaged plants’ profiles, respectively. Farnesene was found in all treatments, but asparagus beetle damaged plants had an eightfold increase in its production over healthy plants and a fourfold increase over mechanically damaged plants. Tetradecanol was not found in the headspace of mechanically damaged treatments and comprised <1% of the headspace of healthy plants; however, it made up 14% of the headspace of asparagus beetle damaged plants. Overall, the three compounds upregulated by asparagus beetle feeding comprised 25% of the overall headspace profile collected from asparagus beetle damaged plants, but only 4% of the mechanically damaged plants and 1% of the control plants’ headspace.

Experiment 2: HIPV Lures

All lures developed from volatile compounds found in the headspace of asparagus beetle damaged plants attracted more parasitoid wasps to yellow sticky traps over the 8 wk sampling period than controls ($\chi^2 = 316.14$, df = 7, $P < 0.01$; Fig. 2). High concentration ocimene lures attracted significantly more parasitoids (primarily Braconidae, Pteromalidae, Eulophidae, and Tachinidae) than all other treatments ($z ≤ -4.55$, $P < 0.01$), except low farnesene concentration lures ($z = -1.99$, $P = 0.48$). Low farnesene concentration lures attracted 19% more parasitoids than high concentration lure mixtures of ocimene + farnesene ($z = 3.84$, $P < 0.01$), and at least 37% more than controls ($z < -10.17$, $P < 0.01$); however, they performed similar to high farnesene ($z = -2.58$, $P = 0.16$), low ocimene ($z = 1.82$, $P = 0.61$), and mixtures of low ocimene + farnesene lures ($z = 2.58$, $P = 0.17$). High farnesene, low ocimene, and both mixture lures all performed similarly and all attracted significantly more parasitoids than the control treatments ($z ≤ -6.24$, $P < 0.01$). Predatory arthropods did not respond differently to our lures compared with the control treatments ($\chi^2 = 5.71$, df = 7, $P = 0.57$). Likewise, key obligate asparagus pests, common asparagus beetle and asparagus miner, showed no significant attraction to any of the lures tested compared with the controls ($\chi^2 = 4.88$, df = 7, $P = 0.68$).

Experiment 3: Effects of Lures on Biological Control of Key Pests

Although common asparagus beetle abundance was low throughout the season (44 individuals collected), 32% (14 individuals) of the larvae we collected were parasitized. Of those, 86% were parasitized by Tetrastichus asparagi (Crawford) (Hymenoptera: Eulophidae) and 14% were parasitized by Pteromalus infernalis (Townsend) (Diptera: Tachinidae). All parasitoids reared from asparagus beetles were collected from high density ocimene treatments, except one P. infernalis which was collected from the low density treatment.

Of the 251 asparagus miner pupae excised from asparagus stems collected in 2017, 54% (136 individuals) were parasitized. Asparagus miner hatch rates were significantly higher in high-density ocimene treatments than in low-density treatments ($\chi^2 = 7.95$, df = 2, $P = 0.02$), but neither were significantly different from the controls (Fig. 3a). Hatch rates of parasitoids were similar across treatments ($\chi^2 = 4.27$, df = 2, $P = 0.12$) (Fig. 3a). Asparagus miner was parasitized by Braconidae, Pteromalidae, Eulophidae, and Eupelmidae. Choresbus rondani (Giard) (Hymenoptera: Braconidae) accounted for 49% of all parasitoids hatched from asparagus miner pupae and was the most common parasitoid found in this study; however, the seasonal
The total of braconids reared from parasitized pupae was not affected by lure treatments ($\chi^2 = 1.91$, df = 2, $P = 0.39$) (Fig. 3b). Pteromalids were the second most common family found parasitizing the miner, accounting for 46% of all parasitoids hatched. Three pteromalid species were found parasitizing the miner: *Thinodytes cephalon* (Walker) (Hymenoptera: Pteromalidae) (92% of all pteromalids), *Cyrtogaster vulgaris* (Walker) (Hymenoptera: Pteromalidae) (5%), and *Sphegigaster gracilis* (Heydon and LeBerge) (Hymenoptera: Pteromalidae) (3%). The seasonal total of pteromalids parasitizing miners was significantly higher in low-density ocimene treatments when compared with all other treatments ($\chi^2 = 7.97$, df = 2, $P = 0.02$) (Fig. 3b); however, pteromalids were only found in two

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>K.I. (c)</th>
<th>K.I. (r)</th>
<th>Plant volatile release ng/g/h</th>
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<td></td>
<td></td>
<td></td>
<td>Mean ± SEM</td>
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<td>1. $\alpha$-Pinene</td>
<td>941</td>
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<td>1005</td>
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<td>1200</td>
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<td>11. Decanal</td>
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</tr>
<tr>
<td>19. Methyl tetradecanoate</td>
<td>1727</td>
<td>1723</td>
<td>127.92 ± 33.08 a</td>
</tr>
<tr>
<td>20. Unknown 3</td>
<td>-</td>
<td>-</td>
<td>3.16 ± 2.19 a</td>
</tr>
<tr>
<td>21. 1-Tetradecanol</td>
<td>1813</td>
<td>1813</td>
<td>3.13 ± 3.13 b</td>
</tr>
</tbody>
</table>

Compounds that significantly differed among treatments are indicated in bold letters ($\alpha = 0.05$).

*K.I. = Kovats indices calculated


* From Da Silva et al. 2003.

* Significant Kruskal-Wallis test with Dunn’s post hoc multiple comparisons and Bonferroni correction ($n = 16$, $\alpha = 0.05$).

* From De Marques et al. 2000.

**Fig. 1.** Representative GC/MS headspace profiles collected in the field from 1-yr-old asparagus ferns treated with either 20 asparagus beetle larvae, fed ad libitum for 48 h, or an undamaged asparagus plant. Arrows indicate compounds that were upregulated in response to beetle feeding. Mechanically damaged ferns had profiles similar to undamaged asparagus (data not shown).
of the four fields we sampled over the entire season. Other parasitoids attacking miners in low numbers were: *Neochrysocharis* sp. (Hymenoptera: Eulophidae) (4%) and *Eupelmus vesicularis* (Retzius) (Hymenoptera: Eupelmidae) (1%).

**Discussion**

Successful use of HIPVs for improving biological control in agroecosystems partly depends on identifying plant volatiles that are attractive to natural enemies of key pests but are not attractive to pests. Here, we identified three plant volatiles from asparagus that had elevated emissions in response to chewing herbivore damage, allowing us to focus on these as potential targets for use in pest management. In field trials, we confirmed that pests and predators were not attracted to synthetic HIPV lures, but parasitoids demonstrated attraction to lures that may lead to increased biological control of the asparagus miner.

Previous studies have indicated that parasitoids often use volatile cues for host location which makes them ideal targets for biological control programs (De Moraes et al. 1998, Du et al. 1998, De Moraes and Lewis 1999). Our results from field experiments support this, with parasitoids significantly more attracted to farnesene and ocimene lures, but other natural enemies and pests not recognizing these as attractive cues. Interestingly, in our research, HIPVs resulting from a specialist chewing pest, attracted parasitoids of a specialist stem mining insect. While we were not able to compare asparagus volatile profiles induced by both herbivores, it is possible that there are similarities in the HIPV profiles induced by the two types of pests and that natural enemies use these as generic host recognition cues. On the other hand, insect stem mining causes minimal emissions of HIPVs compared with chewing (Turlings et al. 1998), thus parasitoids of mining pests might rely on cues emitted by other co-occurring specialist herbivores that cause prominent but reliable cues (Vet and Dicke 1992). In our system, it is common to find asparagus beetles and asparagus miners feeding on the same plants simultaneously, thus asparagus beetle feeding might lead to associational susceptibility of asparagus miners, which should be tested in future studies.

Two families of pupal parasitoids dominated the parasitoid community of the asparagus miner in our study and these groups have been previously reported in the literature in asparagus fields from our region (Morrison et al. 2014). One of these two groups of parasitoids, the pteromalids, had significantly more individuals emerge from asparagus miner pupae collected in the presence of ocimene lures at low density. While braconids are known to be attracted to some HIPVs, we did not observe this with ocimene lures (Ngumbi et al. 2005, Takemoto and Takabayashi 2015, Zimba et al. 2015, Giunti et al. 2016). It is interesting to note that the pteromalid species present in our system generally have broad host ranges while the one braconid species is a specialist on asparagus miner, which might explain the lack of the braconid's response to the ocimene lure (Morrison et al. 2014). While the generalist pteromalids are able to use the volatile induced by a chewing herbivore as a host recognition cue, the specialist braconid might not be able to use it in host finding. Our work highlights the importance of resolving certain insect traits, such as diet breadth, that may explain behavioral responses of parasitoids to plant volatiles.

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**Fig. 2.** Volatile lures were deployed in commercial asparagus fields in Michigan to determine attraction of pests, parasitoids and predator arthropods to baited yellow sticky traps. Lure treatments consisted of: no lure (negative control), blank lure (positive control), farnesene high (1000 μl farnesene), farnesene low (750 μl farnesene), mixture high (1000 μl farnesene + 500 μl ocimene), mixture low (750 μl farnesene + 350 μl ocimene), ocimene high (500 μl ocimene), and ocimene low (300 μl ocimene).

**Fig. 3.** Ocimene lures (500 μl ocimene) deployed in high and low densities in asparagus fields in Michigan were used to determine biological control of asparagus miner by parasitoids with the mean hatch rate of asparagus miner and all parasitoids reared from asparagus miner pupae (a) and the seasonal total of parasitoids from Braconidae and Pteromalidae (b).
From a pest management perspective, it is fortunate that pteromalids are typically three times more abundant in Michigan commercial asparagus fields than braconids (Morrison et al. 2014). Therefore, our ocimene lures were increasing the abundance of the most prominent group of parasitoids in our system. However, despite their abundance in our study, we only collected pteromalids from two of the four fields we sampled. Interestingly, these two fields had similar border habitat compositions with one field border habitat that was forested, two that were asparagus, and one that was a nonasparagus crop. Conversely, the two fields without pteromalids had three forested field border habitats and one that was a nonasparagus crop. Habitat simplification is typically associated with decreases in natural rates of biological control in agricultural systems (Rusch et al. 2016); however, our data seems to support the hypothesis that pteromalids rely more on resources provided by crops than natural habitats (Tschamke et al. 2016). Specific border habitats may also harbor alternative hosts which might explain why some fields had pteromalids and others were void of them. Future studies should focus on investigating the connections among pteromalid abundance, alternative host availability, and habitat complexity near asparagus fields.

Temporal and spatial relationships between pests and natural enemies are important to consider when developing volatile lures to support biological control programs (Braasch and Kaplan 2012). In our system, the two key pests co-occur and congregate on asparagus field edges, post-harvest, while natural enemies are primarily found in the field margins, ~10 m outside the field (Morrison and Szendrei 2013, Ingrao et al. 2017). This spatial arrangement provides a unique opportunity to strengthen the relationship between these two groups in space and time using volatile lures. Lures can be deployed on asparagus field edges to attract natural enemies from field margins, but they should only be deployed when the pest is in a vulnerable life stage, and reaches a management threshold, otherwise lures should be removed to release natural enemies from a habitat devoid of their hosts (Kaplan 2012). Pest phenology is particularly important to consider with HIPV-based biological control because pests are often only vulnerable to particular natural enemies during certain life stages. As HIPV-driven pest management tactics are explored in specialty crop systems, the use of pest degree day models to inform deployment timing will provide important information in developing ‘attract and release’ strategies that consider pest phenology and target life stage.

While the bioactive range of plant volatile lures is variable (Mallinger et al. 2011, Rodriguez-Saona et al. 2011, Braasch and Kaplan 2012), our findings indicate that the concentration of volatiles emitted by lures against the natural background of plant volatiles can have an impact on the abundance of natural enemies (Dicke et al. 2003, Schroder and Hilker 2008). In our study, the low-density deployment of the ocimene lure was more attractive for parasitoids than when we doubled the number of lures on the field edge, suggesting that otherwise attractive plant volatiles can become repellent for insects at high concentrations (Whitman and Eller 1992, Hilker and McNeil 2008, Kaplan 2012). In addition, the spatial arrangement of lures may also have a profound effect on attraction, for example, we may need to consider increasing the space among lures to adjust the concentration of ocimene in the air. Although the bioactive range, deployment density, and spatial arrangement of lures needs further study, our research provides evidence that ocimene lures may increase parasitoid abundance in this system.

Specialty crops, such as asparagus, have high economic value per hectare but are often limited in pest management tools. This requires that alternative pest management tactics, such as HIPV lures for improving biological control, are given greater research attention. One of the significant challenges for specialty crops is that alternative pest management strategies must be developed and tested for each crop–pest combination due to the variability across systems. Coordinated efforts among specialty crop producers, pest managers, and chemical ecologists could facilitate meaningful pest management solutions and further our understandings of the role semiochemicals play in pest management.

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