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ORIGINAL ARTICLE

Beetle herding: Optimizing the biological control of the invasive air potato vine using attractive semiochemical lures

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Abstract

Purposeful manipulation of biological control programs, such as timed releases of biological control agents, can be ephemeral and difficult to expand into new areas impacted by the targeted invasive plant. Integration of chemical ecology through attractive semiochemical lures to aggregate biological control agents to un-colonized areas can help mitigate this challenge. The invasive air potato vine, *Dioscorea bulbifera* L., is native to Asia and Africa with invasive infestations in the southeastern United States, Hawai'i, and Puerto Rico. In 2011, a host specific biological control agent, *Lilioceris cheni* (Coleoptera: Chrysomelidae), was introduced to manage *D. bulbifera*. Synthetic and racemic blends of previously identified attractive herbivory induced plant volatiles (HIPVs), ocimene and farnesene, were first evaluated for antennal response through electroantennography, then deployed as potential attractive lures in field conditions. Electroantennogram results validated the ability of adult male and female *L. cheni* to detect the two compounds. When used in field conditions, adult *L. cheni* beetles showed increased response to plants with ocimene and farnesene lures compared to control plants. The chemically enhanced lures increased *L. cheni* adult densities on *D. bulbifera* plants in the field compared to control plants. Plants with higher densities of *L. cheni* had greater direct herbivore feeding damage and observed cupped leaves, indicating the presence of oviposition and future larval development. The information gathered in this study indicated that the use of attractant semiochemical lures to purposefully aggregate and direct movement of biological control agents can improve the efficacy of invasive plant biocontrol programs.

Keywords Antennal response · Biological control · Coleoptera · Electroantennogram · Invasive plants · Semiochemical

Introduction

Biological control programs of invasive plants utilize specialized herbivores or pathogens to manage the population size of the targeted pest plant. There has been increased use of strategies, such as semiochemicals, to enhance biological control programs using predators and parasitoids (Simpson et al. 2011; Sharma et al. 2019); however, these strategies are rarely integrated into biological control of invasive plants. Despite this, research focused on the integration of semiochemicals into invasive plant biological control programs has shown increased monitoring efficiency, damage to the target plant, and increased establishment success of the biological control agent (Cossé et al. 2006; Gaffke et al. 2018, 2019, 2021).

Dioscorea bulbifera L. (Dioscoreales: Dioscoreaceae), the air potato, is an invasive weed found in the southeastern United States. Primary infestation sites occur in Florida, Georgia, Alabama, Mississippi, Louisiana, Texas, Hawai'i,

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and Puerto Rico (Schultz 1993; EDDMapS 2023). Initially introduced in 1905, *D. bulbifera* quickly escaped cultivation operations and invaded natural areas causing habitat degradation and replacement of native species in both undisturbed and disturbed habitats by growing into the forest canopies creating "vine blankets" (Morton 1976; Simberloff et al. 1997; Horvitz and Koop 2001; Odom et al. 2008; Croxton et al. 2011; Lake et al. 2015). These dense patches of vines block sunlight from reaching native flora and smother seedlings on the ground (Simberloff et al. 1997; Horvitz and Koop 2001). Outside of its native range of Asia and Africa, *D. bulbifera* is extremely problematic due to asexual reproduction through aerial bulbils that drop to the ground during the winter and sprout into new vines the following spring growing period (Schultz 1993; Croxton et al. 2011). These invasive traits of *D. bulbifera* have been considered to be highly aggressive and dangerous by land managers (Morton 1976).

Current methods of control include mechanical removal of vines, aerial bulbils, underground tubers, prescribed burnings, and herbicidal applications (Schultz 1993; Simberloff et al. 1997; Pemberton and Witkus 2010). Herbicidal treatments reduce above ground biomass but rarely cause mortality to aerial bulbils and underground tubers (Langeland and Craddock Burks 1998). Furthermore, herbicidal applications have serious off-target effects specifically related to the native plant supporting the vines (Pemberton and Witkus 2010; Overholt et al. 2014). Native North American herbivores do not commonly feed on *D. bulbifera* and the plant grows and reproduces without any major top-down population management (Overholt et al. 2016). *Dioscorea bulbifera* was considered a suitable target for classical weed biological control because current control methods damage native flora, presence of few closely related plants in North America, and the absence of natural enemies (Wheeler et al. 2007; Pemberton and Witkus 2010).

Classical weed biological control is a management program that purposefully introduces a host specific, co-evolved natural enemy from the native range of the invasive species with the goal of suppressing the invasive species population within the introduced range. *Lilioceris cheni* Gressit & Kimoto (Coleoptera: Chrysomelidae), the air potato leaf feeding beetle, from the native range of *D. bulbifera* in Asia (China, India, Nepal, Laos, and Thailand), was found to be a suitable host specific biological control agent and approved for release in the United States in 2011 (Pemberton and Witkus 2010; Center et al. 2013; Lake et al. 2015). Releases of sexually mature adult *L. cheni* were successful: groups of as few as 10 to as many as 100 beetles were found to have an establishment rate of 50% and 85% respectively (Lake et al. 2018). A 5-year study indicated released *L. cheni* significantly reduced aerial bulbils (reproductive organ), vine cover, and biomass density (Rayamajhi et al. 2021). During these studies, *L. cheni* naturally dispersed to different field sites that did not have a previous release (Lake et al. 2018; Schaffer 2020). Furthermore, aggregations of *L. cheni* were frequently observed in the field on active feeding plants with fresh damage. The mechanism behind this may be partially explained by induction of herbivory induced plant volatile (HIPV) cues of *D. bulbifera* to *L. cheni* (Griesheimer et al. 2023). Significant attraction of *L. cheni* adults to freshly damaged adult and larval conspecific damaged plants indicated the potential of creating an attractive semiochemical lure designed to attract and aggregate *L. cheni* in field settings (Griesheimer et al. 2023). Levels of two inexpensive volatiles, β-ocimene and α-farnesene, were particularly high following herbivory damage and which suggest that these compounds encourage aggregation of *L. cheni*. An aggregation strategy that increased early season defoliation and damage impacts would significantly lower the volume of biomass and aerial bulbil production of *D. bulbifera* compared to late season damage (Schaffer 2020; Rayamajhi et al. 2021). Therefore, potentially attractive semiochemical lures were created to investigate the potential to intentionally aggregate *L. cheni* adults in field conditions.

Materials and methods

Insect rearing

Parental generation *L. cheni* beetles were collected from local *D. bulbifera* infestations around northwest Florida, USA and reared in a specialized rearing room at the United States Department of Agriculture: Agricultural Research Service (USDA: ARS) in Tallahassee, FL, USA. Parental *L cheni* beetles were housed in 0.5 m x 0.5 m x 1.5 m mesh cages (Bug Dorm, Taichung, Taiwan) with a 1 m *D. bulbifera* plant for feeding and oviposition. The rearing room was maintained at 24 °C, 65% humidity, with a day-night cycle of 14:10 L: D, as described in (Griesheimer et al. 2023).

Adult beetles were moved to new cages to oviposit on new *D. bulbifera* plants weekly. The plants remained inside the same cage until eggs hatched. When larval feeding was detected, larvae were assessed daily to ensure proper feeding and growth. If the larvae needed more plant material, the old plant was removed from the cage and a new plant was placed into the cage. Larvae were transferred using a fine bristle paint brush.

Larvae in the fourth instar stage were removed and placed into an 18 L plastic rearing box (Weathertight IRIS, Pleasant Prairie, WI, USA) with plant material. Tops of the rearing boxes were fitted with mesh openings for proper

air circulation with 3 cm of autoclaved vermiculite (Specialty Vermiculite Crop, Enoree, SC, USA) in the bottom of the box as a pupation substrate. Boxes were housed in a Percival (Model: I36VL) growth chamber (Percival Scientific Inc, Perry, IA, USA) maintained at 22–25 °C with a 14:10 L: D cycle (Griesheimer et al. 2023).

Plant rearing

Plants for feeding and field experimentation were grown and housed at the USDA: ARS Tallahassee, FL, USA greenhouse facilities. Aerial bulbils were collected from greenhouse and screenhouse reared plants in late October through the end of November of the previous growing season. Bulbils were placed outside in plastic bins (113.5 L) (ULINE, Pleasant Prairie, WI, USA), under a pole barn to allow exposure to a chilling period before normal sprouting occurred. Three bulbils (total weight approximately 250 g) were placed in an 8.5 L pot (Nursery Supplies, Chambersburg, PA, USA) filled with Promix BX general purpose soil (Quakertown, Quebec, CA) in March. These plants served as feeding and rearing plants. Plants used in the field trials were grown from previously dormant, mature plants in 8.5 L pots using Promix BX general purpose soil with bamboo hoops (1 m tall) to support the vines. Seven grams of Osmocote Smart Release® Indoor Outdoor plant food (Marysville, OH, USA) was distributed to each pot and Miracle Grow All Purpose Plant Food (Marysville, OH, USA) was applied once every two weeks for feeding and experimental plants.

Chemicals

Beta-ocimene and α-farnesene were previously characterized as being produced by *D. bulbifera* in response to feeding by *L. cheni* (Griesheimer et al. 2023). Synthetic ocimene (90%, with isomer) and farnesene (racemic mixture) were each diluted with dichloromethane to a concentration of 100 ng μL^{-1}. All chemicals were purchased from Sigma-Aldrich, St. Louis, MO, USA.

Electrophysiology of *L. cheni*

Electrophysiology of the beetles was conducted to ensure the adults were able to detect the synthetic ocimene and farnesene racemic mixtures prior to field deployment. Beetles reared in laboratory settings were used to assess antennal responses of adult male and female *L. cheni* stimulation of ocimene or farnesene. Negative control treatment consisted of 10 µL of dichloromethane and semiochemical treatments consisted of 10 µL of 100 ng μL^{-1} ocimene in dichloromethane, or 10 µL of 100 ng μL^{-1} farnesene in dichloromethane, were tested (Gaffke et al. 2020). Inclusion of the negative control dichloromethane showed responses to the solvent and quantified mechanical stimulation of the antennae by moving air. Treatments were applied in the following sequence: solvent control, ocimene or farnesene, solvent control, ocimene or farnesene, and solvent control, with approximately 30 s between each treatment and replicated 5 times per beetle sex.

Electroantennography (EAG) was performed by removing both antennae at the base from a live beetle and the tips of the antenna were removed using a scalpel. The pair of antennae were immediately attached with electrode gel (Signa Gel, Fairfield, NJ, USA) onto two metal electrodes. Treatments used were prepared by adding 10 µL of solution to a 14.5 cm Pasteur pipet (Fisher Scientific, Waltham, MA, USA). The solvent was given 60 s to evaporate from the Pasteur pipet before the tip of the Pasteur pipet was placed about 3 mm inside a small hole through the wall of a metal tube (13 cm x 1 cm) (Yang et al. 2019). The metal tube was positioned in the direction of the antennal preparation with a continuous flow rate (1 L/min) of humidified, charcoal-filtered air from the stimulus controller (Syntech, CS-55) with a stimulus duration of 1 s. A 90 s interval between stimulations was given to allow for the antennae to recover (Ceballos et al. 2015). EAG signals were stored and analyzed using EAD (version 2.5) software (Syntech, Hilversum, Netherlands). After each EAG assay, the beetle was sexed based on dissected genitalia.

Attractiveness in field trials

Field trials were conducted in summer 2023 at the USDA: ARS facilities in Tallahassee, FL, USA. A field plot of 120 m x 50 m was created to support 50 potted 1 m tall *D. bulbifera* plants. Prior to placing plants in the field, metal posts (1.5 m tall) (Everbilt, Atlanta, GA, USA) were placed to allow the vine to grow in a natural fashion, and TopHats™ (Better Bilt Products, Addison, IL, USA) were placed in the field to hold the pots upright during inclement weather. Plants were placed into the TopHats™ while still in their pot. Each plant was spaced 10 m from another plant in an alternating pattern of a buffer (no treatment) and experimental plant (control or treatment).

Based on previous volatile analyses, ocimene and farnesene mixtures were used singularly and in combination as experimental lures (Griesheimer et al. 2023). Field lures were created by inserting a 2 cm cotton wick inside a microcentrifuge tube (1.5 mL) (Fisher Scientific, Waltham, MA, USA) with 1 mL of mineral oil and 4 µL of one of the different treatments. Treatments evaluated were (1) ocimene; (2) farnesene; (3) combination of ocimene (2 µL) and farnesene (2 µL); (4) control (mineral oil only). The lures were

covered in aluminum foil to prevent solar degradation of the semiochemicals, then attached to the metal posts using a 15 cm long wire. Lures were replaced weekly after data were collected and experimental trials lasted 4 weeks.

Data were collected on seven metrics: (1) visual count of adult *L. cheni*; (2) observed number of cupped leaves on terminal vines indicating oviposition occurrence (Center et al. 2013; Diaz et al. 2013; Kraus et al. 2022); (3) number of un-cupped leaves on terminal vines; (4) larval damage to terminal vines; (5) count of undamaged leaves; (6) count of adult damaged leaves; and (7) count of larval damaged leaves. A cupped leaf is defined as a leaf that has been deformed by adult *L. cheni* to form a "cupping" shape around oviposition sites (Fig. 1A). The visual count of adults was conducted during a 1 min duration where the observer moved around the plant to count the adult *L. cheni* on the plant. Differentiation of adult and larval *L. cheni* damage was completed based on the different characteristics presented on the herbivore damaged leaves (Fig. 1B). Adult *L. cheni* herbivore damage had a pocked appearance on the leaves while larval *L. cheni* herbivore damage skeletonized the leaf causing it to turn brown and desiccate (Fig. 1B). To randomize the observation on each plant, a ribbon was tossed onto the plant with the end of it being used as the center of a metal hoop (0.3 m diameter) used to subsample leaves for *L. cheni* herbivore damage on the observed plant. Every leaf within the hoop (even partially) were categorized either as undamaged, adult damaged, or larvae damaged. If a leaf was both adult and larvae damaged, the dominant type of damage was considered. Two subsamples were taken with the metal hoop for each individual plant. The total percent of counted leaves damaged was calculated by adding counted larval and adult damaged leaves together then dividing it by the total number of leaves counted. Terminal vines are defined as the newly grown vines that produce tender new leaves preferred by *L. cheni* females to "cup" for use as ovipositional sites (Center et al. 2013; Diaz et al. 2013; Kraus et al. 2022). A terminal vine was measured to 3 m and the leaves on the measured portion of the vine were assessed for cupped or un-cupped leaves as well as larval damage to the terminal vine.

For each field trial, each treatment was replicated six times with a total of six replicates for each sampling date. Data for the first field trial were collected weekly between 13 July 2023 to 3 August 2023 and collected weekly between 5 September 2023 to 26 September 2023 for the second field trial. One week prior to the first weekly data collection, 200 adult beetles were divided into 3 groups and released in the field at 40 m intervals within the plot (Lake et al. 2018). Initial lures were also deployed at this time.

Statistical analyses

Unless stated otherwise, data were analyzed using the statistical software R (4.2.2). Electrophysiology data were analyzed using a paired t-test. Data collected from the field trial were natural log plus one transformed to better fit the

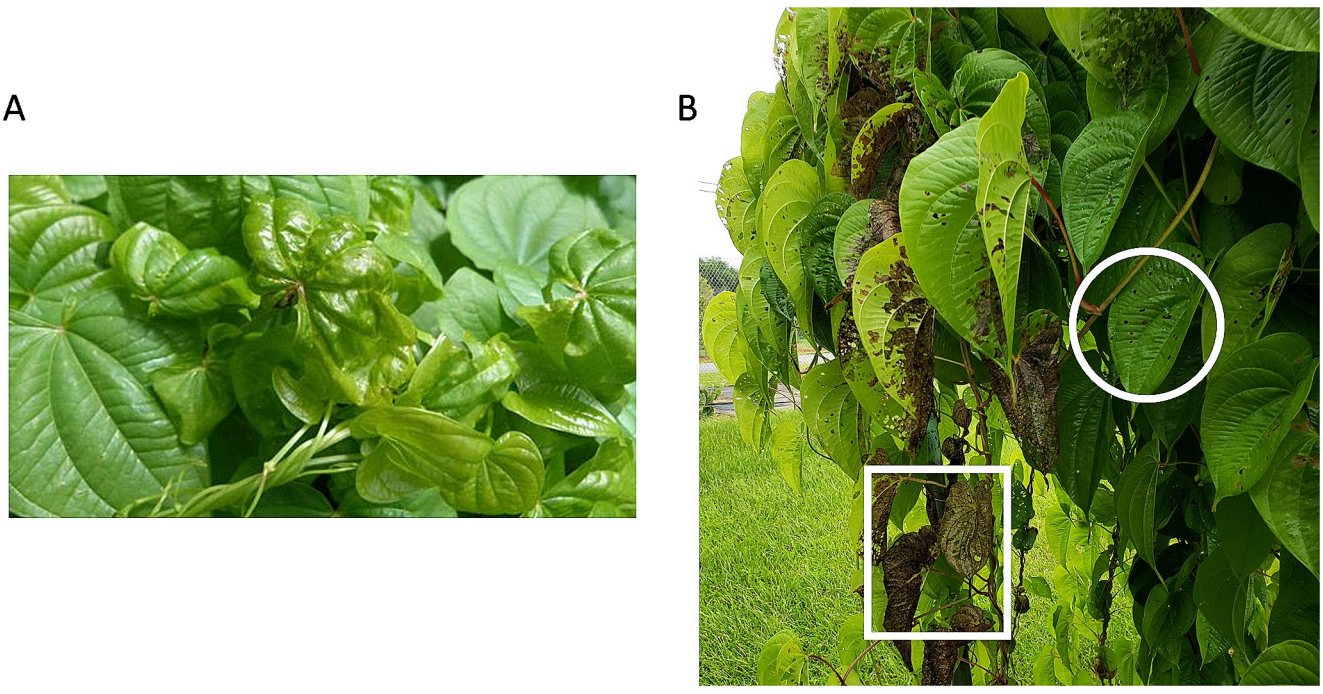

Fig. 1 Images of *D. bulbifera* for damage identification **A** Cupped *Dioscorea bulbifera* leaves indicating oviposition has occurred Photo Credit: Ted D. Center, USDA/ARS Invasive Plant Research Laboratory, Fort Lauderdale, FL **B** Herbivore damage to *D. bulbifera* from *Lilioceris cheni* adults (circle) and larvae (rectangle)

assumptions of normality. Multiple models were compared with an ANOVA testing to determine the model with the best fit. The model with the best fit was analyzed with a one-way ANOVA and further with a post hoc Tukey HSD. Results were considered significant if $P < 0.05$.

Results

Electrophysiology of *L. cheni*

Significant depolarization from antennae were induced in EAG experiments using a pair of antennae from *L. cheni* puffed with 1,000 ng ocimene or farnesene from a Pasteur pipet (Fig. 2). Female beetles had greater depolarization from stimulation by ocimene (0.24 ± 0.05 mV, $P = 0.01$, $t = -4.32$, $n = 5$) or farnesene (0.21 ± 0.05 mV, $P = 0.01$, $t = -4.13$, $n = 5$) compared to the dichloromethane control. Male beetles also had greater depolarization from stimulation by ocimene (0.22 ± 0.03 mV, $P = 0.01$, $t = -4.37$, $n = 5$) or farnesene (0.25 ± 0.04 mV, $P = 0.01$, $t = -7.03$, $n = 5$) compared to the dichloromethane control. Males and females did not respond differently to ocimene ($P = 0.77$, $t = 0.59$, $n = 5$) or farnesene ($P = 0.71$, $t = 0.73$, $n = 5$).

Field Study

Environmental conditions during the first field trial consisted of an average maximum temperature of 25 °C, an average minimum temperature of 18 °C, and an overall average temperature of 20 °C. The average maximum rainfall was 11.78 cm with an overall average of 1.27 cm. Environmental conditions during the second field trial consisted of an average maximum temperature of 35 °C, an average minimum temperature of 25 °C, and an overall average temperature of 28 °C. The average maximum rainfall was 5.05 cm with an overall average of 0.43 cm. Model analyses determined no significant differences between field trials, therefore the field trials were pooled for analysis. Pooled data indicated significant differences for visual count of adult beetles ($P = 0.02$, df = 3, F = 3.22). Combination lured plants had the most adult beetles visually observed (1.46 ± 0.38) (Fig. 3). There was a significant difference for the un-cupped ($P = 0.01$, df = 3, F = 3.57) and cupped ($P = 0.02$, df = 3, F = 3.19) leaves. Combination lured plants had the most observed cupped leaves (1.91 ± 0.32), while control plants had the least observed cupped leaves (0.79 ± 0.24) (Fig. 4A). Control lured plants had the most observed un-cupped leaves (8.11 ± 0.41) (Fig. 4B). Plants observed to have a higher density of adult beetles were also found to be correlated with more cupped leaves ($P = 0.03$, df = 3, F = 3.17), specifically combination lured plants ($P = 0.01$) compared to control lured plants. Count of undamaged leaves ($P = 0.01$, df = 3. F = 4.00), count of adult damaged leaves ($P = 0.0004$, df = 3, F = 6.43), count of larval damaged leaves ($P = 0.002$, df = 3, F = 5.14), and total percent of counted leaves damaged ($P = 0.0001$, df = 3, F = 7.36). Plants lured with any of the chemically enhanced lures, ocimene (51% ± 4.97), farnesene (45% ± 4.94), or combination (41% ± 4.71), were found to have more herbivore damaged leaves compared to control plants (22% ± 4.02) (Fig. 5).

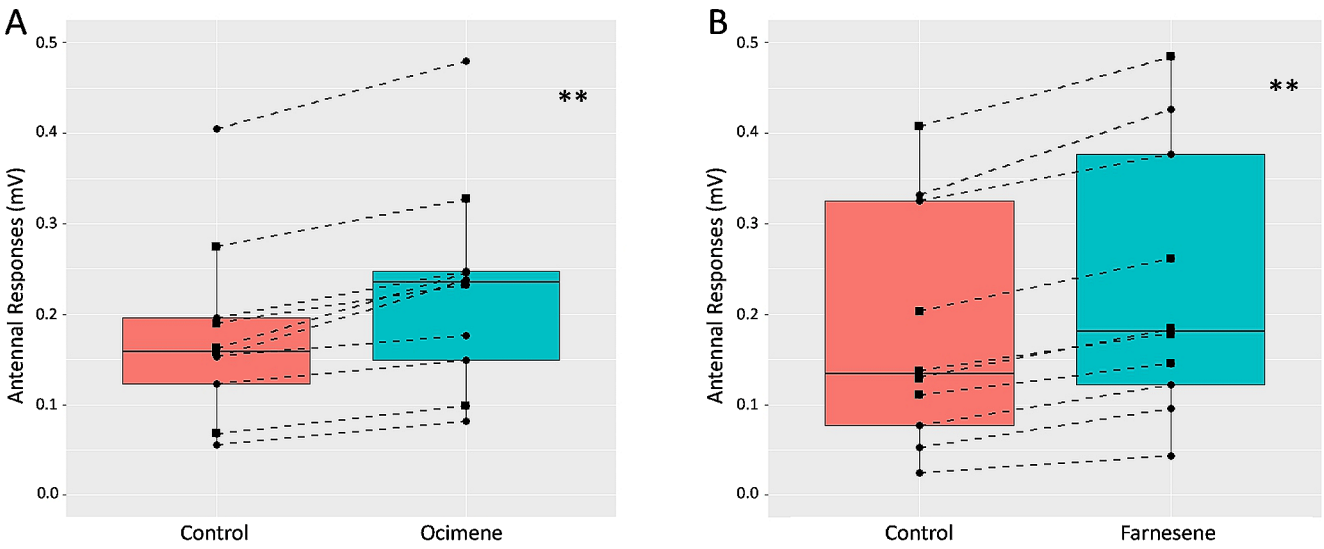

Fig. 2 Electrophysiology evaluation of *Lilioceris cheni*.**A** Electroantennogram (EAG) responses of *L. cheni* to negative control (dichloromethane) or 1,000 ng of ocimene **B** EAG responses of *L. cheni* to dichloromethane or 1,000 ng of farnesene. Squares indicate male responses and circles indicate female responses. Within each tested volatile (ocimene or farnesene), asterisks indicated statistical difference between the treatments ($P < 0.01$)

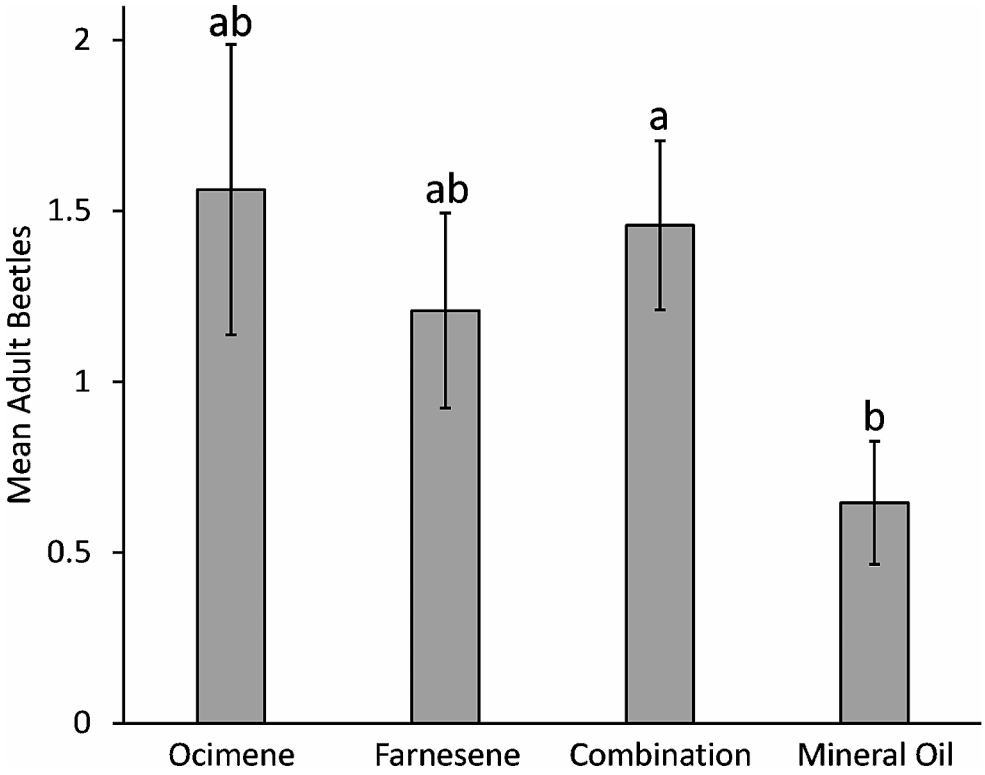

Fig. 3 Mean visual count of adult *Lilioceris cheni* on field *Dioscorea bulbifera* plants. Different letters indicated significant differences between the treatments ($P < 0.05$)

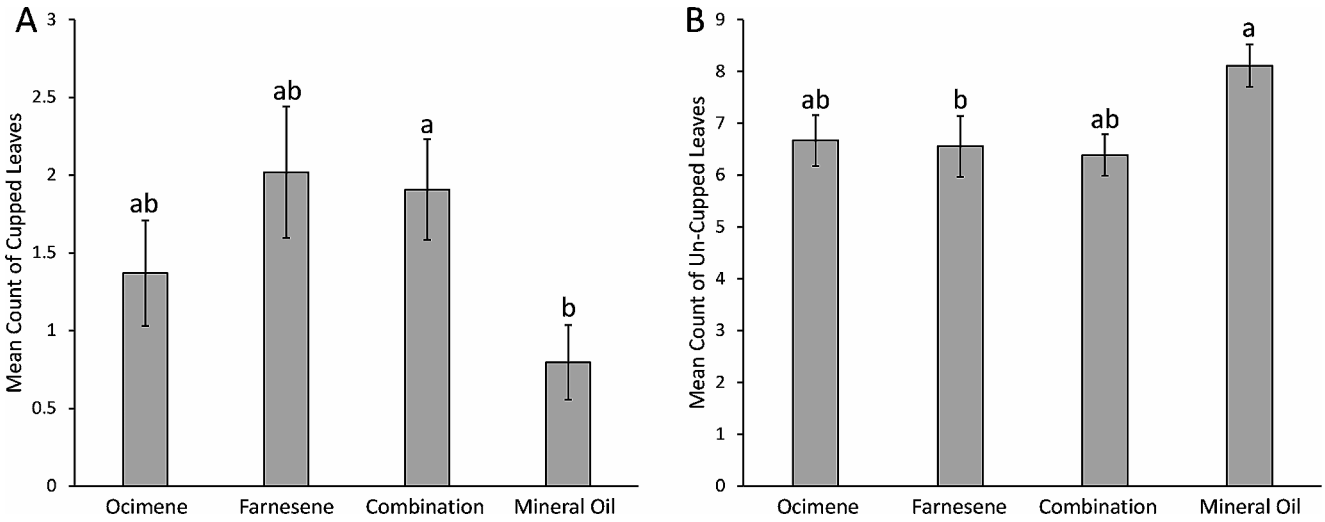

Fig. 4 Comparison of leaf cupping for lure types. **A** Mean count of cupped leaves observed on terminal vines of *Dioscorea bulbifera* plants depending on lure treatments during the two field trials. **B** Mean count of UN-cupped leaves observed on terminal vines of *Dioscorea bulbifera* plants depending on lure treatments during the two field trials. Different letters indicated significant differences between the treatments ($P < 0.05$)

Discussion

Successful biological control of invasive plants can be ephemeral when natural distribution of released biological control agents is sporadic, leading to less herbivore reproduction and damage. Use of semiochemicals to enhance biological control of invasive plants through purposeful herbivore aggregation has been shown in one other plant-herbivore system to be beneficial in monitoring the biological control agent, increasing damage to the target plant, and increasing the population of the biological control agent (Cossé et al. 2006; Gaffke et al. 2018, 2019, 2021). Our study added to this knowledge and further indicated the potential of integrating attractive semiochemicals into current and future invasive plant biological control programs. The antennal response

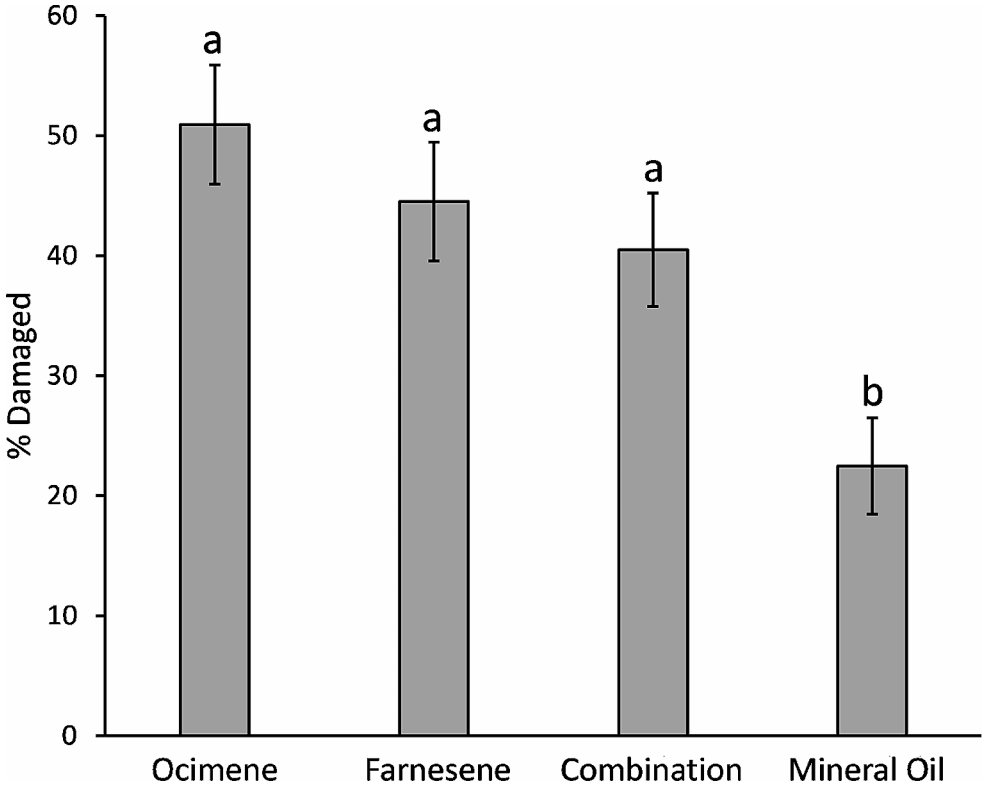

Fig. 5 Percent of total counted leaves damaged depending on lure treatments during the two field trials. Different letters indicated significant differences between the treatments ($P < 0.05$)

of adult *L. cheni* to two previously uncharacterized semiochemicals, ocimene and farnesene, and the behavioral field response of adult *L. cheni* to these semiochemicals was investigated. Specifically, the purpose of this study was to demonstrate the enhancement of the classical biological control program of *D. bulbifera* using attractive semiochemicals and broadly to demonstrate the potential use by land managers to purposefully aggregate biological control agents to specific targeted pest populations.

Our research validated the ability of *L. cheni* to detect ocimene and farnesene through EAG. Results from our field application indicated these semiochemicals attracted adult *L. cheni* to *D. bulbifera* plants, increasing two-fold the herbivory damage compared to the control plants. Combination lured plants were found to have higher densities of adult *L. cheni* which was correlated with an increased number of cupped leaves on terminal vines, indicative of oviposition and future larval development. The blend may be important due to the inclusion of farnesene which may attract sexually mature female *L. cheni* to *D. bulbifera* plants prior to oviposition. A blend of alpha and β-farnesene has been found to be attractive to *Tetropium fuscum* Fabricius (Coleoptera: Cerambycidae) females, particularly in short-range host location and α-farnesene was found as an oviposition stimulant for *Cydia pomonella* L. (Lepidoptera: Tortricidae) (Sutherland et al. 1977; Silk et al. 2010). While we obtained good results with synthetic and racemic blends of ocimene and farnesene, respectively, it is possible that more purified blends of β-ocimene and α-farnesene may increase the attraction of *L. cheni.* Plants in the control group had a significant reduction in observed numbers of cupped leaves compared to combination lured plants, indicating less oviposition and suggested less larval *L. cheni* herbivore damage in the future.

Our results indicated the potential of semiochemicals to be used to attract and aggregate beneficial herbivores to the target plants, increasing the defoliation and reducing the biomass of the weed to enhance pest management (Cossé et al. 2006; Gaffke et al. 2018, 2019). Studies of semiochemicals to enhance insect-insect control, specifically biological control of pest or invasive species, are abundant (Simpson et al. 2011; Kelly et al. 2014; Martini et al. 2014; Sharma et al. 2019; Zhao et al. 2020; Abd El-Ghany 2023) but are lacking for biological control programs related to invasive plants (Cossé et al. 2006; Wheeler and Schaffner 2013; Gaffke et al. 2018, 2019, 2021; Griesheimer et al. 2023). Prior to field release, a candidate biological control agent is required to have rigorous and specific evaluations to determine host specificity and potential impact to non-target plants such as agriculturally significant or native plants (Hinz et al. 2020). Host specificity of herbivorous insects have intricate physiological and ecological relationships, partially effected by semiochemicals, which can broaden the understanding between a host plant and a co-evolved herbivore (Wheeler and Schaffner 2013; Gaffke et al. 2021).

A possible challenge for chemical ecology research in invasive plant biological control programs is acquiring additional funding for extra research, as these programs are highly expensive to start. However, these extra funds are a good investment into these programs as they have a consistently high cost:benefit ratio over time (van Wilgen et al. 2004; Culliney 2005). Another possible challenge to continued integration of chemical ecology into biological control of invasive plants is the availability and cost of the semiochemicals as well as deployment of the semiochemicals by land managers and biological control practitioners (Gaffke et al. 2021). This challenge can be mitigated using cooperative relationships with companies to deliver a semiochemical lure for field deployment. This was demonstrated by the *Tamarix* spp. biological control program by using SPLAT® infused with *Diorhabda* pheromones that had an acceptable semiochemical release rate for 30 days in field conditions (Gaffke et al. 2018). Therefore, ocimene and farnesene were chosen as the potentially attractive semiochemicals to align with these challenges faced by land managers and biological control practitioners.

Deployment of attractive semiochemical lures by land managers to direct movement of *L. cheni* would be useful in management of areas that are not adequately controlled. Management and dieback of uncontrolled *D. bulbifera* infestations has been highly successful in Florida (Amenyo 2021; Rayamajhi et al. 2021). Other states, such as Louisiana, have had irregular control management and defoliation in comparison (Schaffer 2020; Amenyo 2021). Timing of beetle attack with the early growth of *D. bulbifera* rather than late-stage growth is critical to proper management of the invasive plant (Schaffer 2020). During spring when the beetles naturally end their diapause and are released by biological control practitioners, they begin to defoliate early growth of *D. bulbifera* but do not naturally disperse as well as beetles released in Florida (Schaffer 2020; Amenyo 2021). Therefore, the use of attractant lures to increase *L. cheni* populations into areas where control is limited may aid in beetle retention and increase management efficacy in areas with irregular or low defoliation.

Further research needs to be conducted on *L. cheni* and *D. bulbifera* to identify optimized concentrations, placement, deployment substrates, and ratios of semiochemicals that increase and maximize herbivore damage. Limitations to the use of an attractive lure to purposefully aggregate a population of insects will largely depend on the biology of the insect. It is important to know their survivability in the habitat prior to deployment of the attractant lure to ensure successful aggregation and defoliation of the target plant. Future studies evaluating the efficacy of reapplication times of the lures in combination with initial colonizing density of adult *L. cheni* damage attractance should be conducted to further optimize the lure and improve the success of biological control.

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Author contributions All authors contributed to the studies conception and design. Data collection and material preparation were performed by Jessica L. Griesheimer. Analysis of data was performed by Jessica Griesheimer, Alexander Gaffke, and Xavier Martini. The first draft of the manuscript was written by Jessica Griesheimer, Alexander Gaffke, and Xavier Martini and all authors commented on previous versions of the manuscript. All authors read and approved of the final manuscript.

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Declarations

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