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RESEARCH

# Attraction of the Air Potato Leaf Beetle, *Lilioceris Cheni*, (Coleoptera: Chrysomelidae) to leaf Volatiles of the Air Potato, *Dioscorea bulbifera*

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## Abstract

Air potato, *Dioscorea bulbifera* L., is an invasive vine found in the southeastern United States and is native to Asia and Africa. The air potato leaf beetle *Lilioceris cheni* (Coleoptera: Chrysomelidae), is a host specific biological control agent introduced for *D. bulbifera* control. In this study, odor cues that control the attraction of *L. cheni* to *D. bulbifera* were investigated. The first experiment investigated the response of *L. cheni* to *D. bulbifera* leaves versus no leaves in the presence or absence of air flow. The experiment showed a significant response of *L. cheni* to *D. bulbifera* leaves in the presence of air flow with leaves placed upwind. When air flow and/or leaves were absent, *L. cheni* dispersed randomly between the upwind and downwind targets, indicating *L. cheni* uses volatiles from *D. bulbifera* in host selection. The second experiment investigated *L. cheni* response to undamaged, larval-damaged, and adult-damaged plants. *Lilioceris cheni* showed preference to move towards conspecific damaged plants compared to undamaged plants but did not discriminate between larvae-damaged or adult-damaged plants. The third experiment investigated volatile profiles of damaged *D. bulbifera* plants using gas chromatography coupled with mass spectroscopy. We found significant differences in volatile profiles between adult and larval damaged plants compared to mechanically damaged and undamaged plants, with increases in 11 volatile compounds. However, larval and adult-damaged volatile profiles did not differ. The information acquired during this study could be used to develop strategies to monitor for *L. cheni* and improve its biological control program.

**Keywords** Biological control · Weeds · Invasive · Herbivory induced volatiles · Aggregation

## Introduction

Biological control of invasive plants consists of using specialized herbivores or pathogens to manage the population of the target plant pest. While there is an increase of strategies to enhance biological control using predators or parasitoids with semiochemicals (Sharma et al. 2019; Simpson et al. 2011), biological control of plants rarely integrates those tools. This is despite that integration of semiochemicals into weed biological control programs has resulted in increased monitoring efficiencies, establishment, and damage to the target plant (Gaffke et al. 2018, 2019, 2021).

*Dioscorea bulbifera*, commonly referred to as air potato, is an invasive dioecious, perennial vine found in the southern region of the United States. Infestations of this invasive plant primarily occur in Florida, Hawai'i, Georgia, Alabama, Mississippi, Louisiana, Texas, and Puerto Rico (Schultz 1993; EDDMapS 2021). Populations of *D. bulbifera* native to tropical Asia were initially introduced to Florida in 1905 (Croxton et al. 2011; Lake et al. 2015). Once introduced,

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*D. bulbifera* rapidly escaped cultivation and invaded natural areas, displacing native species and disrupting habitat. *Dioscorea bulbifera* is well documented to disrupt forest floor habitats through shading and smothering underbrush communities and seedlings. When the vines reach the forest canopy they create dense mats, referred to as "vine blankets" obscuring sunlight to native flora (Simberloff et al. 1997; Horvitz and Koop 2001). Areas which become infested with *D. bulbifera*, especially areas highly disturbed by natural disasters such as hurricanes, have decreased growth of native flora and decreased the native plant diversity (Schultz 1993; Horvitz and Koop 2001; Odom et al. 2008). *Dioscorea bulbifera* is extremely problematic once it establishes due to asexual reproduction as it propagates primarily from aerial bulbils that drop to the ground in winter and sprout into new vines the following spring (Schultz 1993; Croxton et al. 2011). Additionally this perennial vine can re-sprout year after year from underground tubers (Duxbury et al. 2003; Pemberton and Witkus 2010; Center et al. 2013; Overholt et al. 2014). Due to these invasive traits, *D. bulbifera* is considered to be a highly aggressive and dangerous by land managers (Morton 1976).

Current control methods for *D. bulbifera* include herbicidal sprays, mechanical removal of the vines and underground tubers, and removal of aerial bulbils present in an area (Simberloff et al. 1997; Pemberton and Witkus 2010). In some instances, prescribed burning has been administered to control the spread of *D. bulbifera* (Schultz 1993). Herbicide treatments can kill the above ground biomass; however, the treatments rarely cause mortality of the belowground tubers and the aerial bulbils, allowing for the plant to sprout and regrow (Langeland and Craddock Burks 1998). Additionally, herbicide treatments result in significant concerns for non-target effects, specifically damage to the underlying plants supporting the vines of *D. bulbifera* (Pemberton and Witkus 2010; Overholt et al. 2014). Native herbivores in North America rarely feed on *D. bulbifera* allowing the plant to be freed from any top-down population regulation (Overholt et al. 2016). Due to control methods damaging native flora, limited closely related plants within North America, and the absence of natural predators, *D. bulbifera* was considered a suitable target for classical weed biological control (Wheeler et al. 2007; Pemberton and Witkus 2010).

Classical weed biological control is a management program that utilizes the introduction and establishment of a host specific, coevolved natural enemy from the native range of the invasive plant with the goal of providing permanent suppression of the invasive plant in the introduced range. The air potato beetle, *Lilioceris cheni* Gressit & Kimoto (Coleoptera: Chrysomelidae), originating from within the natural range of *D. bulbifera* in Asia (China, India, Nepal, Laos, and Thailand), was selected as a potential biological control candidate. Host-specificity testing conducted in laboratory and open-field settings verified *L. cheni* to be highly specific to *D. bulbifera.* Host-specificity tests were conducted on *L. cheni* using 41 plant species, 15 of which were members of Dioscoreaceae, and no oviposition, or larval development was observed in any other species than *D. bulbifera* (Pemberton and Witkus 2010). *Lilioceris cheni* was approved for release to control *D. bulbifera* in the United States in 2011 (Pemberton and Witkus 2010; Center et al. 2013; Lake et al. 2015).

*Lilioceris cheni* populations released in the United States were collected in Nepal and China in 2002 and 2011, respectively, and represent two distinct biotypes, Nepalese and Chinese. The Chinese biotype was released in Florida in 2011 and the Nepalese biotype was released in Louisiana in 2016 (Center et al. 2013; Schaffer 2020). Results from *L. cheni* releases were highly positive. Sexually mature adults in a group of as few as 10 to as many as 100 have a 50% and 85% rate of establishment respectively (Lake et al. 2018). A 5-year study revealed that *L. cheni* significantly reduced vine cover, and decreased bulbil density and biomass (Rayamajhi et al. 2019; Lake et al. 2018) noted that *L. cheni* tended to stay within the patch of *D. bulbifera* where initially released and only dispersed after the foliage had been consumed. This dispersal pattern was also noted at field sites where *L. cheni* had naturally dispersed (Schaffer 2020). Additionally, aggregations of *L. cheni* were frequently observed on the freshly damaged plants with active feeding, with lower densities on fully consumed and undamaged plants. However, the mechanism of this aggregation has never been elucidated.

Host specific insects such as *L. cheni* have complex relationships with their host plant often using signaling chemicals, known as semiochemicals, to locate each other and their host plant (Gaffke et al. 2021). This phenomenon has been demonstrated with another host specific weed biological control agent, *Diorhabda elongata*, for the management of *Tamarix* spp., commonly called saltcedar (Cossé et al. 2006). We hypothesize that herbivorous-induced volatiles may trigger *L. cheni* attraction to damaged plant, hence leading to the observed aggregation. These unknown cues that potentially attract *L. cheni* to freshly damaged plants need to be further investigated as it may uncover new management strategies to optimize the biological control of *D. bulbifera.*

The ability to purposefully aggregate populations of *L. cheni* would be highly advantageous to land managers, as early season damage and defoliation impacts the canopy volume and bulbil production of *D. bulbifera* significantly more than late season damage (Schaffer 2020; Rayamajhi et al. 2021). Therefore, experiments were undertaken to investigate the attraction of *L. cheni* to *D. bulbifera* volatile cues.

## Materials and methods

### Insect Rearing

Parental generation *L. cheni* beetles, Chinese biotype, were obtained from Florida Department of Agriculture and Consumer Services-Department of Plant Industry in Gainesville, FL, USA and reared inside rearing rooms at the United States Department of Agriculture: Agricultural Research Service (USDA: ARS) in Tallahassee, FL, USA. Parental beetles were split into groups of 20 to 25 haphazardly selected individuals, housed in 0.5 m x 0.5 m x 1.5 m mesh cages (Bug Dorm, Taiwan) with a 1 m tall, potted *D. bulbifera* plant inside for oviposition. The rearing room was maintained at 24 °C, 65% humidity, with a day-night cycle of 14:10 L:D.

All adult beetles were moved every 5–7 days to new cages to oviposit on new plants. The undersides of the leaves were inspected for oviposition at this time, and if oviposition was detected, would remain inside the cage till hatching. Once larval feeding was observed, larvae were checked daily to ensure proper growth and feeding behaviors. If the larvae needed more plant material, the old plant was removed and a new plant was placed inside the mesh cage.

As the larvae entered the fourth instar, they were removed from the plant, counted, and placed into an 18 L plastic rearing box with plant material (Weathertight IRIS, Pleasant Prairie, WI, USA) fitted with mesh openings for air circulation. Rearing boxes were filled with 3 cm of autoclaved vermiculite to function as a pupation substance (Specialty Vermiculite Crop, Enoree, SC, USA). Pupation boxes were checked daily, and leaves and dead larvae were removed as needed. Pupation boxes were housed in a Percival growth chamber maintained at 22–25 °C with a 14:10 L:D cycle. Adult beetles emerged 17 to 20 days after pupation and were transferred and maintained in a mesh cage with a plant till sexual maturity, after which these adults were used for experimentation. Due to insects being housed together, and mating was visually observed, it is assumed that they were mated at the time of experimentation.

### Plant Rearing

Aerial bulbils were collected from sites in northwest Florida infestations in late October through the end of November. *Dioscorea bulbifera* vines for feeding, rearing, and experimentation were grown at the USDA: ARS, Tallahassee, FL greenhouse facilities. All new bulbils were potted in March of the following season. Plants used to rear the *L. cheni* colony were grown from newly planted bulbils in 8.5 L pots (Nursery Supplies, Chambersburg, PA, USA) using Promix BX general purpose soil (Quakertown, Quebec, CA) with bamboo hoop to support the vines. Experimental plants for use in the wind tunnel and volatile collections were grown from three bulbils, roughly 80 to 85 g total, planted together in a 0.75 L pots (Nursery Supplies, Chambersburg, PA, USA). Three 15 mL of Osmocote Smart Release® plant food (Marysville, OH, USA) were administered to the pots in April.

### Experiment 1: Attraction of Lilioceris cheni to Host Plant Volatiles and Visual Cues

The experiment was conducted in a 0.6 m x 0.6 m x 2.1 m low-speed wind tunnel (USDA-ARS, Gainesville, FL, USA) at the North Florida Research and Education Center, in Quincy, FL. Air was pulled through the tunnel at 0.1 m/sec with the use of a tubular duct fan and was exhausted outside the room. A charcoal infused fabric cleaned the air at the tunnel entrance. Room temperature in the wind-tunnel facility was $22.5 \pm 2$ °C and humidity around 55%. Two plastic vials filled with distilled water were placed at the upwind and downwind positions of the wind tunnel. Treatments evaluated were: (1) no air flow, no leaves; (2) air flow, no leaves; (3) air flow with leaves in both vials; (4) no air flow but leaves in both vials. Treatment 1 acted as a negative control to ensure the absence of positional bias in our experiment; treatment 2 tested if the presence of air flow will induce a response of the beetles in the absence of odor cues; treatment 3 tested the response of the beetles in response to odors from its host plant with air flow; treatment 4 tested the response of the beetles in response to odors from its host plant without air flow. A single, sexually mature beetle was released in the middle of the wind tunnel. Unsexed beetles were used as males and females are morphologically indifferent. Beetles were given 1 h to make a choice which was then recorded. If the beetle was within 5 cm of a leaf target, it was considered a choice. Replication took place at intervals of at least 2 days with different leaves and beetles. Each treatment was replicated 20 times. Beetles were between 7 and 21 days old and were deprived of food 24 h prior to testing in the wind tunnel. *Lilioceris cheni* are long-lived insects with life spans up to 3 months without food and in a growth chamber with food, up to 6 months with normal behavior detected (Pemberton and Witkus 2010).

### Experiment 2: Lilioceris cheni Attraction to Conspecific Damaged Plants

Experiment 2 was conducted in the low-speed wind tunnel described above with similar conditions as Experiment 1. Three treatments were applied to plants, adult feeding damage, larval damage, and undamaged which acted at the control. Prior to experimentation, 1 to 2 month-old plants were

placed in a 0.3 m x 0.3 m mesh cages (Bug Dorm, Taiwan) to receive the treatments. Ten adults or larvae were added to the cages and allowed to feed for 24 h. The control plants did not receive any insects but were kept in the mesh cage for 24 h. After the 24 h period, the adults and larvae were removed from the plants. The plants were then immediately placed at the upwind section of the wind tunnel with a space of 20 cm between the plants in the following treatment combinations: (1) adult damaged plant versus undamaged plant; (2) larval damaged plant versus undamaged plant, (3) larval damaged plant versus adult damaged plant. Ten adult beetles were released at the downwind side of the wind tunnel and observed after 4 h. The response variable measured for this experiment is the total number of adults on the plants at the 4 h observation point. After adults were counted, the beetles and plants were removed, and the wind tunnel was cleaned with warm soapy water and re-set up for additional replications. This experiment was replicated 12 times.

### Experiment 3: Volatile Collection & Analysis

Volatile collections were conducted at the USDA ARS Tallahassee, Florida facilities. Potted vines were collected from USDA ARS Tallahassee, FL greenhouses. Mesh cages (0.3 m x 0.3 m) were used to enclose a single plant to receive the experimental treatment. Four treatments were evaluated: (1) adult damage; (2) larval damage; (3) mechanical damage; (4) undamaged, which acted as the control. There were 16 samples of each treatment collected and evaluated. Plants were placed inside the mesh cage and exposed to the different treatments and placed in a Percival incubation chamber (Percival Scientific, Perry, Iowa, USA) at 22–25 °C with a 14:10 L:D cycle for 24 h. Plants exposed to insect damage had either 10 unsexed adults or 10 larvae added to the cage. Mechanically damaged plants were damaged with a 1 cm diameter paper hole punch (Fiskars, Helsinki, Finland) 10 times. Plants were removed from the cages and conspecifics were removed prior to placement in glass collection jars. Collection jars were created from 7.5 L, glass jars (Anchor Hocking, Lancaster, OH, USA) with 2 inflow ports drilled through the lid. Volatiles were collected for 6 h using a volatile collection system (Volatile Assay Systems/Vassays PVAS22, Rensselaer, NY, USA). Due to the short collection period, we were not concerned with breakthrough of the volatiles (Cossé et al. 2006). External air was purified by charcoal-filter then pushed into the jar at 1 L/min and pulled at 1 L/min through a HayeSepQ filter (Volatile Assay Systems/Vassays, Rensselaer, NY, USA) connected to PTFE tubing. Volatile filters were cleaned prior to use with 500 µL of dichloromethane (Sigma-Aldrich, St. Louis, MO, USA).

After sampling, filters were eluted with 200 µL of dichloromethane (Sigma-Aldrich, St. Louis, MO, USA) into 2 mL glass vials with a 250 µL glass insert, with 32.4 ng of nonyl acetate as an internal standard (TCI America, Portland, OR, USA). Vials were stored in a -20 °C freezer until they were analyzed by gas chromatography (Agilent GC 8890 GC System, Santa Clara, CA, USA) coupled with mass spectrometry (Agilent 5977B MSD, Santa Clara, CA, USA) (GC-MS). One µL of each sample was injected into the GC-MS using an autosampler (Agilent 7693 A, Santa Clara, CA, USA). Helium was used as the carrier gas with a pressure of 8.5 psi and velocity of 1.1 mL/min. All samples were analyzed by a HP-5MS ultra-inert capillary column (Agilent, Santa Clara, CA, USA), 30 m long with 0.25 mm diameter. The column temperature was maintained at 40 °C for 4 min and increased at a rate of 10 °C/min to a final temperature of 300 °C for 5 min. The inlet temperature was set to 250 °C and the source temperature was set to 230 °C. Mass spectrometry was performed using electron impact at 70 eV. Chromatograms were integrated using the auto integration function from MassHunter Qualitative Analysis (Agilent Version 10.0). Compound identification was based on authentic standards when available and tentative identification through the NIST 2020 spectral library.

### Statistical Analysis

Unless stated otherwise, statistical analyzes were conducted with the statistical software R (4.2.2). Experiment 1 data was analyzed using $\chi^2$ test, while attraction of *L. cheni* to conspecific damaged versus undamaged plants and adult versus larval damaged plants were analyzed with a Welch two sample t-test. Amounts of compounds collected from the GC-MS were square root transformed to better fits the assumptions of normality, then analyzed with multivariate ANOVA and further with post hoc Tukey HSD. Results were considered significant if $P < 0.05$. Volatile data were also visualized using principal component analysis (PCA) conducted in JPM Pro.

## Results

### Experiment 1: Attraction of Lilioceris cheni to Host Plant Volatiles

In the absence of air flow and plant material, beetles randomly distributed within the wind tunnel ($\chi^2 = 0.6667$, $P = 0.4142$, df = 1) with 4% nonresponse. When presented with airflow and upwind and downwind leaves, the beetles demonstrated a preference to move upwind towards the upwind leaves versus moving downwind ($P = 0.0396$, $\chi^2 = 4.2353$, df = 1) with 13% nonresponse (Fig. 1). When presented with leaves and no airflow, the beetles were equally distributed to both

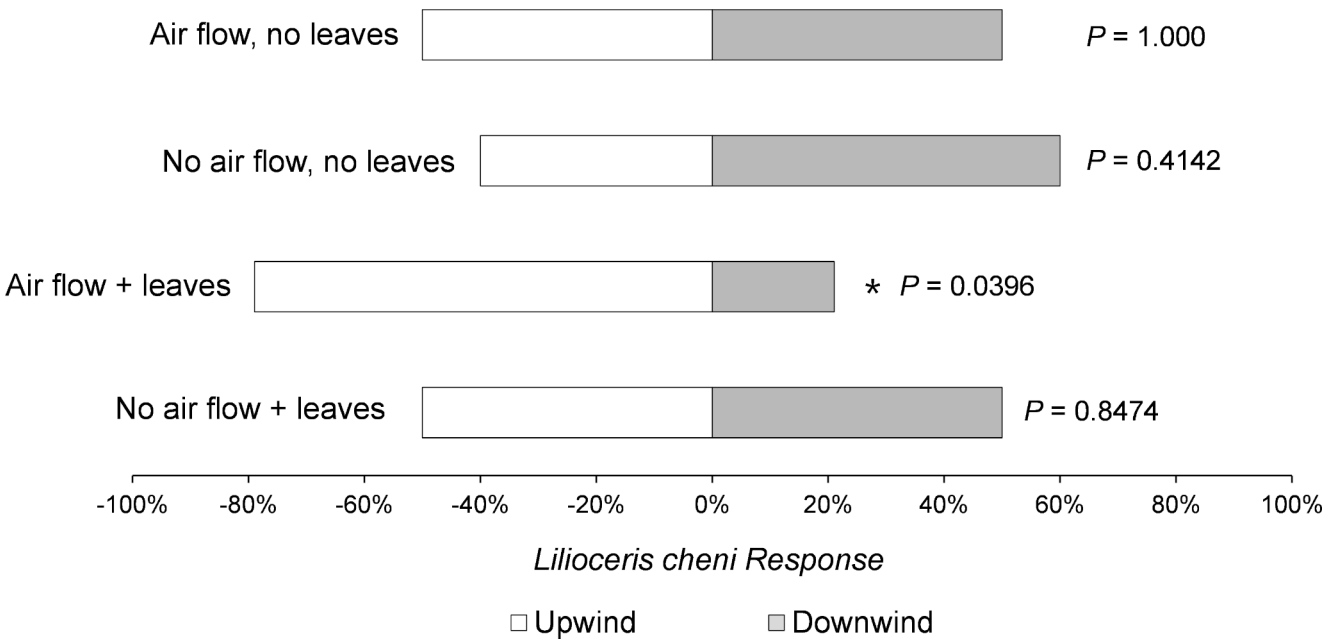


**Fig. 1** Response of *Lilioceris cheni* in the wind tunnel in the presence or absence of airflow, and in the presence or absence of leaves. Asterisk indicates significant difference ($P < 0.05$)

sets of leaves ($\chi^2 = 0.0370$, $P = 0.8474$, df = 1) with 16% nonresponse. When the beetles were presented with airflow and no leaves, the beetles equally dispersed upwind and downwind in the wind tunnel ($\chi^2 = 0.000$, $P = 1.000$, df = 1) with 6% nonresponse (Fig. 1).

## Experiment 2: Lilioceris cheni Attraction to Conspecific Damaged Plants

Beetles demonstrated a preference to move towards the adult and larval damaged plants as compared to undamaged counterparts. Specifically, 65% and 74% of tested *L. cheni* were found on adult and larval damaged plants compared to 35% and 25% on the undamaged plant, respectively (adult damage: $P = 0.0006$, df = 35, t = 3.7841; larval damage: $P < 0.0001$, df = 34, t = 5.30). When adults were presented with a choice of larval versus adult damaged plants, the beetles did not demonstrate a significant preference for either treatment ($P = 0.7201$, df = 20.8, t = 0.3632) (Fig. 2).

## Experiment 3: Volatile Collection & Analysis

A total of 27 volatile peaks were detected through GC-MS analyses (Table 1). Eleven volatile compounds were induced through conspecific herbivory damage: (*Z*)-hex-3-en-1-ol ($P < 0.001$, df = 3); (NZ)-N-(2-methylbutylamine)hydroxylamine (NMBH) ($P < 0.001$, df = 3); (1 *S*,3*R*)-1-methyl-3-propan-2-ylcyclohexane (MPYH) ($P = 0.004$, df = 3); (Z)-3-hexen-1-yl acetate ($P < 0.001$, df = 3); β-ocimene ($P < 0.001$, df = 3); 2,6,10-trimethyldodecane ($P = 0.007$, df = 3); E-4,8-Dimethylnona-1,3,7-triene (DMNT) ($P < 0.001$, df = 3); Benzyl nitrile ($P < 0.001$, df = 3); Indole ($P < 0.001$, df = 3); Methyl anthranilate ($P = 0.013$, df = 3); and α-Farnesene ($P < 0.001$, df = 3). In mechanically damaged or undamaged plants, many of these volatiles were not detected by the GC-MS or were emitted at significantly lower levels (Table 1).

The eleven induced compounds were further analyzed by Tukey HSD to determine where the treatment differences occurred. For (*Z*)-hex-3-en-1-ol; NMBH; (Z)-3-hexen-1-yl acetate; DMNT; Benzyl nitrile; and Indole, there was no difference between undamaged and mechanical damaged plants, while all other treatments were different from each other. For MPYH, there was no difference between larval and adult damage or undamaged and mechanically damaged plants, while all other treatments were different from one another. For β-ocimene there was no difference between adult and larval damaged plants or undamaged and mechanically damaged plants, while all other treatments were different from one another. For 2,6,10-trimethyldodecane and methyl anthranilate, there was significant differences between mechanically damaged and larval damaged plants and undamaged and larval damaged plants, while all other treatments were not different from one another. For α-farnesene there was no difference between larval and adult damaged plants or mechanically damaged and undamaged plants, while all other treatments were different from one another (Table 1).

The PCA indicate that 40.8% and 26.4% of the variation was explained by principal component 1 (PC1), and

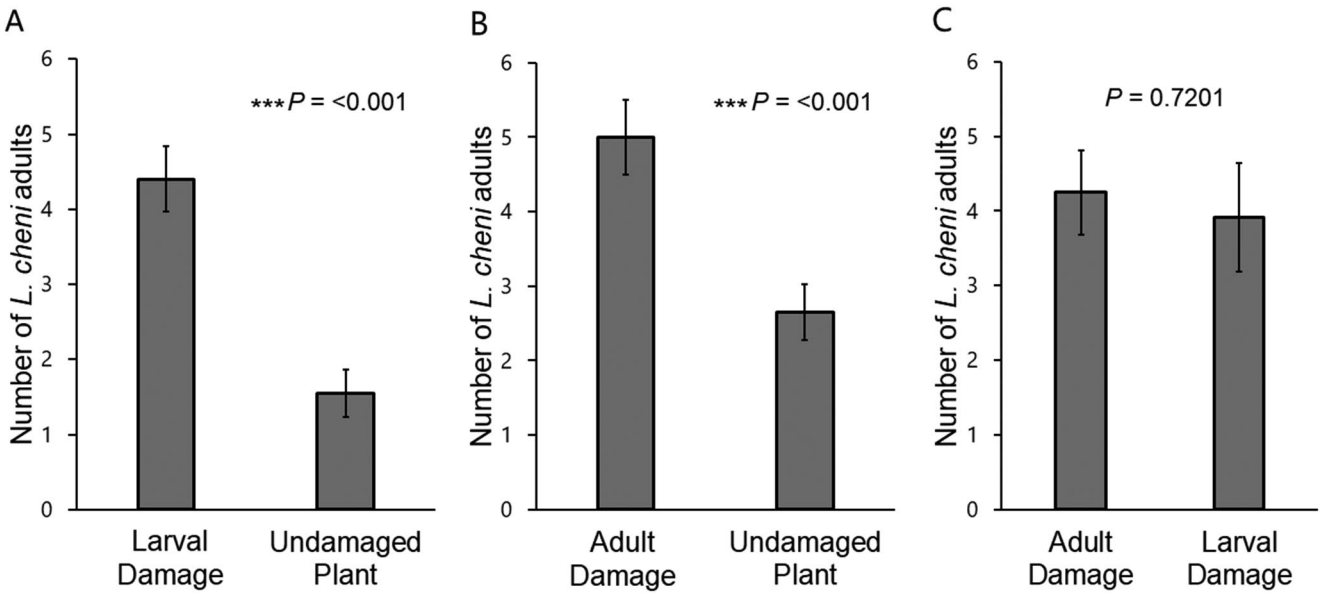


**Fig. 2** **A** Response of *Lilioceris cheni* to conspecific larval herbivory damaged versus undamaged leaf volatiles in the wind tunnel. **B** Response of *Lilioceris cheni* to conspecific adult herbivory damaged versus undamaged leaf volatiles in the wind tunnel. **C** Response of *Lilioceris cheni* to adult versus larval damaged conspecific leaf volatiles in wind tunnel in the presence of airflow. The three asterisks indicate significant difference ($P < 0.001$)

principal component 2 (PC2), respectively. PC1 ($P < 0.001$, df = 3) and PC2 ($P < 0.001$, df = 3) increased significantly for larval and adult-damaged volatile profiles. There was a significant difference between larval damaged plants compared to undamaged and mechanically damaged plants with 95% confidence ellipses separated (Fig. 3A). Adult damaged plant volatiles were heavily overlapped with larval damaged but had only minimal overlap with undamaged and mechanically damaged plant volatiles (Fig. 3A). There is greater spread of the points along the PC2 axis compared to PC1 which indicates PC2 may be the cause of separation between the different treatments (Fig. 3A). The vectors parallel to PC2 on the PCA biplot indicated PC2 is correlated with multiple defensive, nitrogenous compounds and green leaf volatiles (Fig. 3B).

## Discussion

In this study we investigated volatiles produced by *D. bulbifera* in response to mechanical and herbivore damage using GC-MS and assessed the attraction of these different damage types to *L. cheni*. To our knowledge, this is the very first chemical description of the induced response of *D. bulbifera* by herbivory damage of *L. cheni*. *Lilioceris cheni* displayed positive chemotaxis toward upwind leaf targets with airflow in the wind tunnel, suggesting *L. cheni* was primarily attracted to volatiles of *D. bulbifera* rather than visual cues from the plant material in the wind tunnel. These results were further built upon in experiment two which assayed the response of adults to volatiles produced by larval, adult, and undamaged plants. *Lilioceris cheni* was significantly attracted toward conspecific-damaged leaf volatiles. This attraction towards freshly damaged plants is an important result for the field of biological control, as it could allow for the development of lures to increasing monitoring efficacy for the *L. cheni* in the field and could be used to purposefully aggregate populations to target locations in the field.

Analysis of the volatile profiles produced by these damage plants provides evidence of the chemical cues that could be driving this increased attraction from the beetle. Clear differences between herbivory-damaged and undamaged or mechanically damaged plants could be detected. Results showed (*Z*)-hex-3-en-1-ol; NMBH; MPYH; (Z)-3-hexen-1-yl acetate; β-ocimene; 2,6,10-trimethyldodecane; DMNT; Benzyl nitrile; Indole; Methyl anthranilate; and α-Farnesene had significantly higher emissions in herbivory-damaged plants. Many of these compounds were undetectable or were produced in very small amounts in undamaged or mechanically damaged plants. Certain volatiles, 2,6,10-trimethyldodecane and Methyl anthranilate, were emitted in higher amounts in larval damaged plants which is likely due to their higher levels of feeding compared to adults.

A result in this study that we were not expecting is the heavy induction of nitrogenous compounds from the insect feeding (butyl aldoxime, benzyl nitrile, indole, and methyl anthranilate). The different nitrogenous compounds, along with induced green leaf volatiles, were found to be correlated with PC2 based on PCA biplot data (Fig. 3B). In general,

**Table 1** Mean and standard error concentration (ng/h) of volatile compounds detected through GC-MS analyses identified or tentatively identified from leaves of *Dioscorea bulbifera* that experienced damage from adult or larval feeding by *Liliocerис cheni*, mechanical damage, or undamaged. Compounds in bold font were induced by *L. cheni* herbivory (both adult and larval feeding), and mean separation comparisons were assessed with Tukey's HSD between larval damage and both mechanical and undamaged

| NO. | Compound | RT[1] | Amount of Volatile Compounds According to Damage Type (ng/h) | | | | F | P |
|---|---|---|---|---|---|---|---|---|
| | | | Adult | Larval | Mechanical | Undamaged | | |
| 1 | 2,3,5-trimethylhexane | 5.54 | 1.52 ± 0.47 | 1.38 ± 0.47 | 1.54 ± 0.47 | 1.169 ± 0.45 | 0.21 | 1.00 |
| 2 | 2,4-dimethylheptane [θ] | 5.73 | 6.94 ± 1.80 | 7.14 ± 1.90 | 7.07 ± 1.78 | 6.88 ± 1.76 | 0.02 | 1.00 |
| 3 | 2,4-dimethylhept-1-ene | 6.24 | 2.51 ± 0.67 | 2.60 ± 0.75 | 2.62 ± 0.67 | 2.51 ± 0.68 | 0.02 | 1.00 |
| 4 | 2,3,5-trimethylheptane | 6.59 | 0.59 ± 0.21 | 6.24 ± 2.48 | 0.64 ± 0.21 | 0.59 ± 0.20 | 4.53 | 0.89 |
| 5 | (*Z*)-hex-3-en-1-ol [θ] | 6.68 | 2.57 ± 0.50 | 13.45 ± 2.46 | 0.11 ± 0.21 | 0.11 ± 0.11 | 24.17 | < 0.001 |
| 6 | NMBH [2] | 6.72 | 2.40 ± 0.73 | 17.73 ± 2.26 | 0.061 ± 0.06 | 0.213 ± 0.21 | 89.10 | < 0.001 |
| 7 | 1,2,3,4,5-pentamethylcyclopentane | 8.05 | 3.81 ± 0.41 | 3.88 ± 0.45 | 4.25 ± 0.44 | 4.40 ± 0.39 | 0.63 | 1.00 |
| 8 | MPYH [3] | 8.65 | 1.08 ± 0.26 | 0.79 ± 0.27 | 1.72 ± 0.15 | 1.59 ± 0.18 | 4.80 | 0.005 |
| 9 | Unknown Methylated Alkane 1 | 9.21 | 1.40 ± 0.46 | 1.61 ± 0.51 | 1.46 ± 0.41 | 1.40 ± 0.40 | 0.02 | 1.00 |
| 10 | (Z)-3-hexen-1-yl acetate [θ] | 9.84 | 3.47 ± 0.93 | 18.66 ± 2.77 | 1.21 ± 0.29 | 1.09 ± 0.25 | 42.95 | < 0.001 |
| 11 | Unknown Methylated Alkane 2 | 10.22 | 1.524 ± 0.42 | 1.93 ± 0.46 | 1.44 ± 0.31 | 1.44 ± 0.31 | 0.18 | 1.00 |
| 12 | β-ocimene [θ] | 10.58 | 129.92 ± 25.74 | 195.61 ± 19.36 | 3.87 ± 0.78 | 3.72 ± 1.72 | 92.15 | < 0.001 |
| 13 | Unknown Methylated Alkane 3 | 10.83 | 12.98 ± 3.40 | 12.76 ± 3.28 | 13.40 ± 3.41 | 12.92 ± 3.40 | 0.03 | 1.00 |
| 14 | 2-methylundecane-2-thiol | 11.18 | 1.71 ± 0.49 | 1.74 ± 0.47 | 1.52 ± 0.46 | 1.74 ± 0.47 | 0.07 | 1.00 |
| 15 | 2,3-Dimethyldecane | 11.38 | 1.77 ± 0.51 | 1.96 ± 0.49 | 1.70 ± 0.47 | 1.84 ± 0.48 | 0.11 | 1.00 |
| 16 | 2,6,10-trimethyldodecane | 11.58 | 18.32 ± 3.30 | 26.30 ± 3.74 | 11.78 ± 2.88 | 12.29 ± 2.95 | 4.46 | 0.007 |
| 17 | DMNT [4θ] | 11.74 | 24.39 ± 4.57 | 48.34 ± 8.95 | 4.37 ± 0.79 | 5.62 ± 1.24 | 35.49 | < 0.001 |
| 18 | Benzyl nitrile [θ] | 12.13 | 37.94 ± 8.62 | 118.71 ± 18.61 | 0.25 ± 0.22 | 1.25 ± 1.15 | 78.02 | < 0.001 |
| 19 | Benzothiazole [θ] | 13.49 | 0.79 ± 0.24 | 1.32 ± 0.41 | 0.98 ± 0.19 | 0.99 ± 0.18 | 0.56 | 0.65 |
| 20 | Indole [θ] | 14.46 | 40.80 ± 5.90 | 101.83 ± 12.21 | 3.42 ± 1.37 | 3.15 ± 0.89 | 102.28 | < 0.001 |
| 21 | Methyl anthranilate | 15.14 | 1.59 ± 0.43 | 3.45 ± 0.66 | 0.94 ± 0.35 | 0.95 ± 0.33 | 3.88 | 0.01 |
| 22 | Unknown Carboxylic Acid | 15.29 | 0.77 ± 0.25 | 1.16 ± 0.34 | 1.01 ± 0.22 | 0.77 ± 0.18 | 0.33 | 0.80 |
| 23 | 2,6-di*tert*-butyl-4-hydroxy-4-methylcyclohexa-2,5-dien-1-one | 16.8 | 2.00 ± 0.44 | 2.60 ± 0.25 | 1.81 ± 0.26 | 2.09 ± 0.24 | 1.97 | 0.13 |
| 24 | α-Farnesene [θ] | 17.09 | 41.12 ± 9.87 | 71.95 ± 12.85 | 0.85 ± 0.51 | 2.74 ± 2.43 | 26.80 | < 0.001 |
| 25 | Butylated Hydroxytoluene [θ] | 17.35 | 11.85 ± 0.44 | 14.23 ± 0.25 | 10.47 ± 0.26 | 12.29 ± 0.24 | 0.07 | 0.97 |
| 26 | [2,2,4-trimethyl-3-(2-methylpropanoyloxy)pentyl] 2-methylpropanoate | 18.34 | 3.89 ± 1.05 | 7.30 ± 1.03 | 4.08 ± 0.87 | 3.17 ± 0.80 | 2.01 | 0.12 |
| 27 | Benzophenone [θ] | 18.78 | 1.13 ± 0.44 | 1.29 ± 0.68 | 2.92 ± 1.28 | 1.41 ± 0.34 | 1.42 | 0.24 |

[1] RT = Retention time; [2] NMBH: (NZ)-N-(2-methylbutylamine)hydroxylamine; [3]MPYH: (1 S,3R)-1-methyl-3-propan-2-ylcyclohexane; [4] DMNT: E-4,8-Dimethylnona-1,3,7-triene. [θ] = compounds identification verified using analytical standards, all other compounds tentatively verified using NIST

nitrogenous compounds are emitted in minor amounts from herbivore damaged plants (Clavijo Mccormick et al. 2014; Aljbory and Chen 2018). For plants damaged by the larval *L. cheni*, on average they released 698 ng/h of volatile organic compounds. Of this total, 238 ng or 34%, were from a nitrogenous compound. The emission rate of these compounds was significantly higher in the insect damage plants compared to the mechanically damaged plants, suggesting they were specifically induced in response to the insect feeding and not the mechanical damage of the plant. The increase production of volatile organic compounds, including the nitrogenous compounds, for the insect damaged plants likely represents a significant metabolic cost to the plant. This result may explain a common observation in weed biological control, that mechanical removal rarely impacts the ability of plants to regrow and reproduce, while insect damage plants can exhibit significant reductions in growth and vigor (Delaney et al. 2008).

Mechanical damage did not induce systemic changes in the plant chemistry, while damage from this insect can cause the allocation of carbon and nitrogen away from primary metabolism. The larval and adult damaged plants produced similar volatile profiles suggesting either stage induces similar responses from the plant or will likely exert similar levels of stress. In this study, adult and mechanically damaged plants volatile profiles did not fully separate in the PCA, while the larval damaged plants were fully separated from mechanically damaged plants. We hypothesize that this result is likely due to the higher amount of feeding done by

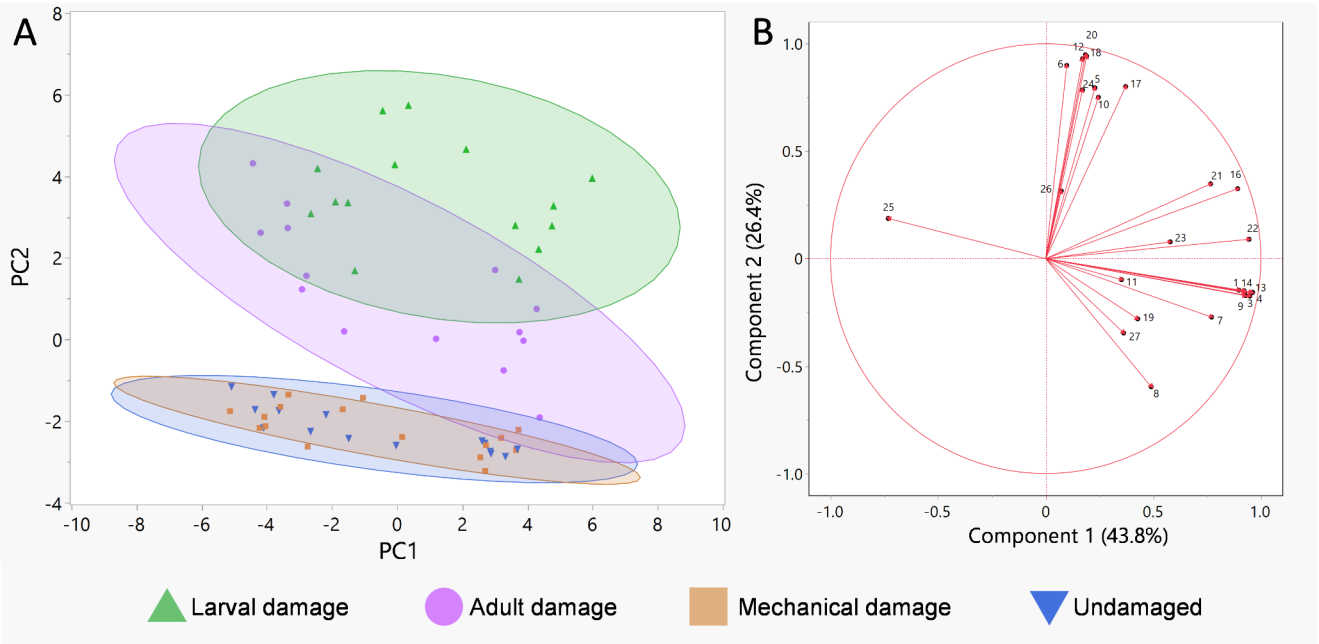


**Fig. 3** **A** Principal component analysis for the volatile components of *Dioscorea bulbifera* depending on different damage treatments: undamaged (orange), mechanical damaged (blue), larvae-damaged (green), and adult-damaged (purple). **B** PCA biplot for *Dioscorea bulbifera* volatile profiles. Component 1 explains 40.8% and Component 2 explains 26.4% of the variance. Ellipses indicate 95% confidence interval for each treatment. Numbers refer to the compounds listed in Table 1

10 larvae, which are still developing, compared to mature adults.

Reduced density and management of an invasive weed is the goal of classical biological control. In this study *L. cheni* behaviors toward volatile cues of its host plant were discovered which could be used to manipulate their behavior in the field using attractant lures. Attractant lures can be easily deployed in the field and would be valuable for land managers to aid in monitoring for this biocontrol agent as well as to help them target patches of *D. bulbifera* growing in sensitive areas, such as a high value, mature trees or ecosystems with protected species where mechanical or chemical control are not appropriate. The results of this study have indicated an attractant lure could be created to help attract beetles to and maintain an aggregation at a *D. bulbifera* infestation site which can lead to increased herbivory damage as found in previous weed biological control efforts (Gaffke et al. 2018, 2019). However, further tests will need to be conducted to determine which compounds cause the attraction and aggregation of the beetles on damaged plants. Combinations of these volatiles, based on the data found through PCA, should be investigated too as it is likely that the beetle is responding to a blend of compounds rather than a single component (Bruce and Pickett 2011). An attractant lure would also be ideal in situations where the infestation site is small or separated from much larger, and more attractive, sites. Broadly, the results of this study and others indicate the behavior of not only *L. cheni*, but other weed biological control agents might be manipulated to increase efficacy and aggregation of the biological control agent, and also achieve greater management and control of an invasive plant by integrating semiochemials into weed biological control (Gaffke 2021).

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**Data Availability** Data are available on the Institutional Repository at the University of Florida (IR@UF), and available directly from the authors upon request.

## Declarations

**Competing Interests** The authors declare that they have no conflict of interest.

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