



Herbivory by the biocontrol agent *Lilioceris cheni* suppresses propagule production and smothering ability of the invasive vine *Dioscorea bulbifera*



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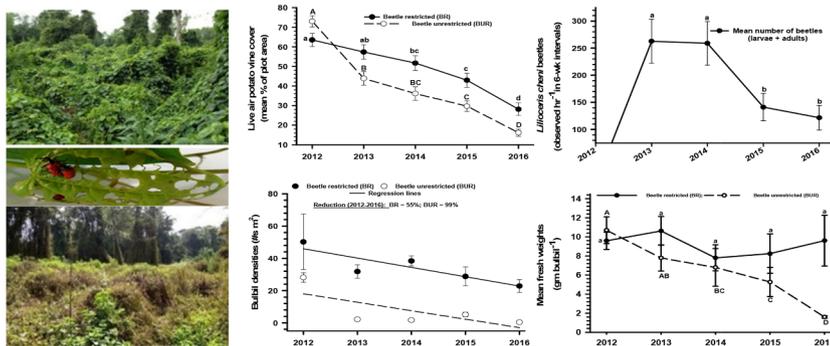
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GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:

Air potato vine
Biological control
Feeding damage
Herbivory impact
Invasive attributes
Post-release evaluation
Vegetative propagules

ABSTRACT

Expanding populations of *Dioscorea bulbifera*, a trellising invasive vine of Afro-Asian provenance, have become widely established in the southeastern United States. Its clambering habit enables the weed to grow over, smother and displace native vegetation while producing vast quantities of vegetative propagules (bulbils) and reducing biodiversity of different ecosystems in Florida. A specialized foliage-feeding beetle, *Lilioceris cheni* from Nepal and China, has been released in USA for biological control of *D. bulbifera*. To determine the beetle's potential to curb the vine's ability to overtop native vegetation and to suppress propagule production, beetle restricted (insecticide treated) and unrestricted (beetle inoculated) sites were compared at five localities in Florida. *Dioscorea bulbifera* cover, *L. cheni* population density, and herbivore damage were documented at 6-week intervals, with bulbil density and biomass measured annually, for 5 years. Results from beetle unrestricted treatment revealed that high *L. cheni* feeding damage reduced vine cover over native vegetation, and decreased bulbil density and biomass. Spillover of *L. cheni* populations from beetle unrestricted into restricted treatment areas after exhaustion of *D. bulbifera* vines resulted in coalescing effects between treatments, so the beetle restricted treatment also showed some reduction in vine cover and bulbil density but individual bulbil biomass remained unchanged. These results show that *L. cheni* has the ability to suppress the invasive attributes of *D. bulbifera* in its adventive range.

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1. Introduction

Dioscorea bulbifera L. (Dioscoreales: Dioscoreaceae), locally known as air potato, is an herbaceous perennial trellising vine of Afro-Asian origin (Martin, 1974) that was introduced into Florida in 1905 as a medicinal plant. It quickly naturalized in Florida, prompting Nehrling (1944) to recognize it as a weed of concern (Morton, 1976). By invading various habitats, it became one of the most aggressive noxious weeds in the southeastern United States (Schultz, 1993; Gordon, 1998; FLEPPC, 2009) and has spread widely and naturalized in Alabama, Georgia, Hawaii, Louisiana, Mexico, Mississippi, Puerto Rico, Texas, the West Indies, and central and northern South America (Wheeler et al., 2007; Overholt et al., 2016; EDDMaps, 2018).

Dioscorea bulbifera vigorously exploits disturbed sites, including hurricane created canopy gaps in the natural areas (Horvitz and Koop, 2001), but also in undisturbed habitats as well as urban landscapes. Like other invasive vines, it trellises up and over neighboring plants and its increased biomass smothers the vegetation beneath (Center et al., 2013; Schmitz et al., 1997). It is classified as a transformer species in Florida, capable of altering plant communities by displacing native flora, changing community structure, and disrupting ecological functions of native ecosystems (Overholt et al., 2014). Infestations occur in all 67 Florida counties and range from a few vines to large patches covering several hectares (Croxtton et al., 2011; Overholt et al., 2016).

Vegetative reproduction occurs via aerial bulbils (air potatoes) produced in leaf axils during summer through early autumn. The bulbils, often weighing over one kg, drop to the ground as the vines senesce during late autumn (Rayamajhi et al., 2016). The new shoots emerge during spring from persistent subterranean tubers or bulbils produced during the previous growing season (Overholt et al., 2014, 2016). The dioecious vines occasionally produce flowers, but only staminate forms, so seed production in North America has not been observed. Invasions of new areas occur mainly from bulbils disseminated by anthropogenic means (Schultz, 1993), hurricane strength winds (Horvitz et al., 1998), or water currents.

The phenology of *D. bulbifera* generally follows an annual cycle of growth and mortality of the aerial vines (Coursey, 1967; Center et al., 2013; Overholt et al., 2016; Rayamajhi et al., 2016). Vines from bulbils can grow as much as $25 \text{ cm} \cdot \text{d}^{-1}$ to reach lengths of up to 51 m within a growing season, and each vine can bear as many as 365 bulbils per season in southern Florida (Rayamajhi et al., 2016). This unchecked rapid growth and copious bulbil production contributes to the invasive nature of this exotic weed (Rayamajhi et al., 2016). Land managers of natural areas have traditionally used cultural, mechanical or herbicidal control methods to suppress *D. bulbifera* infestations (Overholt et al., 2014). Cultural methods include the physical removal of subterranean tubers, usually as part of annual “air potato round-ups” that remove propagules (aerial bulbils) from infested sites while increasing public awareness of *D. bulbifera* invasions (Overholt et al., 2016). These methods alone are inadequate, inefficient, and are not cost-effective for long-term suppression (Wheeler et al., 2007).

Lilioceris cheni Gressitt and Kimoto (Coleoptera: Chrysomelidae) was discovered and imported from Nepal in 2002, tested for host specificity, and found to be highly specific to *D. bulbifera* (Pemberton and Witkus, 2010). A second biotype of the beetle was imported from China in 2010, which demonstrated the same feeding habit and host-specificity (Center et al., 2013). The first release of *L. cheni* occurred in cages placed over field-grown *D. bulbifera* plants during November 2011 as an overwintering study (Center et al., 2013), followed by large-scale field releases during June 2012 (Overholt et al., 2016). Releases of as few as 10 adults per site successfully established populations, and both Nepalese and Chinese biotypes of *L. cheni* appeared to have a wide tolerance to latitudinal (\approx temperature) differences in Florida (Lake et al., 2018; Smith et al., 2018). A statewide survey conducted in October 2015 showed that *L. cheni* beetles had established in 47 of the 67 Florida counties (Overholt et al., 2016).

The long-term impact of *L. cheni* on *D. bulbifera* growth and development in the field has not been tested. Therefore, the objectives of this study were to assess the impacts of *L. cheni* on *D. bulbifera* and associated plant communities using replicated field experiments as emphasized in Denslow and D'Antonio (2005) and Carson et al. (2008). Our study tested whether herbivory by *L. cheni* could suppress the vine sufficiently to reduce (1) the trellising and smothering effects of *D. bulbifera* on supporting vegetation, and (2) the density and biomass of vegetative propagules at infested sites.

2. Materials and methods

2.1. Research sites

Five *D. bulbifera* study sites were established and monitored over five years (2012–2016). Sites were selected to represent general geographical and edaphic conditions observed in areas infested by *D. bulbifera* in Florida. The sites were: Kendall Indian Hammock (KIH) (N25.696444°, W80.377639°) in Miami Dade County; Snyder Park (SNP) (N26.085778°, W80.148000°) and Pine Island Ridge (PIR) (N26.078111°, W80.275778°) in Broward County; Terra Ciega State Park (TCP) (N27.591170°, W82.549420°) in Manatee County, and the Fred Cone Park (FCP) (N29.647750°, W82.285806°) in Alachua County. *Dioscorea bulbifera* infestations in the sites were estimated (based on Google maps) to range from 27 to 236 ha. All sites consisted of well-drained, predominantly sandy soils except FCP, which had predominantly organic, moderately drained soils and a high degree of short-term saturation after heavy rains. As per our observations, all sites experienced 25–50% of lower- and mid-canopy (up to 10 m) coverage by *D. bulbifera* vines, with limited (ca. 5%) coverage in the upper tree canopies (above 10 m).

2.2. Experimental design

Control and treatment blocks were set up at each study site. Control areas were *L. cheni* restricted (hereafter BR) blocks that received a combination of systemic and contact insecticide applications (soil treatment) of 270 g/plot of Aloft GC G (granular form, containing 0.25% clothianidin active ingredient and 0.125% bifenthrin by weight) at ca 4-mo intervals. Beetle unrestricted blocks (referred to as BUR) consisted of areas where no insecticide exclusion was used. Two blocks of *D. bulbifera* infested areas, each ranging from one to five ha, were delineated at each of the five sites and the two blocks were separated from each other by 300 m or more. Each block contained three 5×3 m plots representing one of the two treatments. Treatment plot locations within block were spatially randomized and separated from each other by 5–100 m. Particular attention was placed on ensuring that the plots represented similar *D. bulbifera* coverage (horizontal and trellising) on shrubs and trees within the site. Distances between blocks were also maximized to limit possible movement of insecticides from BR to BUR areas and also to delay the spread of released beetles from BUR to BR. A total of 30 plots (5 sites \times 3 plots \times 2 treatments) were used in this study.

Each plot in unrestricted blocks received 700 and 800 *L. cheni* beetles during 2012 and 2013, respectively. Complete exclusion of beetles in the insecticide-treated plots was not expected; only reduced herbivory was anticipated due to dispersive abilities of the beetles and the amount of feeding required to ingest a lethal insecticide dose.

2.3. Data collection

Dependent variables measured at the onset of the experiment included: *D. bulbifera* vine cover (percentage of the total plot area), vine damage (percentage of overall green leaf area and tender growing tip damage on live vines) by herbivores, bulbil density (number of bulbils plot^{-1}) and biomass of individual bulbils. Vine cover on open ground,

trellising on other vegetation (smothering effects), and vine damage by *L. cheni* (adults and larval) populations were evaluated at the 6-wk (± 1 -wk) interval, whereas the bulbil density and individual bulbil biomass were documented at the end of each growing season after all vines had senesced and bulbils had fallen to the ground. Two or more examiners participated during each data gathering activity to provide independent estimates of coverage. The 6-wk evaluation interval captured the net influx or outflow of beetle populations due to pupal emergence or immigration from other sites and the associated vine damage. Evaluations were conducted at the plot or entire study site level as described below.

2.3.1. Plot level

Dependent variables of *D. bulbifera* measured at plot level evaluations included: 1) vine cover, 2) feeding-damage by herbivores, 3) bulbil density and 4) individual bulbil biomass. Data on vine cover and damage per plot were used to assess their relationship with the bulbil density at the end of each growing season from 2012 to 2016. The percentages of *D. bulbifera* vine cover (estimated plot area covered by *D. bulbifera* vines) and *L. cheni* (adults and larval) damaged foliage (leaf area and growing tips damaged by herbivory in the entire plot) were visually estimated by two or more examiners in ca 6-wk intervals. Data on the percentages of vine cover and damage were averaged across examiners by treatments within a given site on an annual basis for 5-year study period.

Each year, *D. bulbifera* bulbils from the ground and senesced vines in BR and BUR plots were collected during January-February. The bulbils collected in January-February of given calendar year represent the bulbil crop from the growing season of the previous calendar year and they were recorded accordingly. Bulbils were weighed individually immediately after collection, returned to their respective plots and spread randomly allowing them to sprout and grow naturally in the field. Mean bulbil density (bulbils m^2) and fresh weight (g bulbil $^{-1}$) of individual bulbil was determined by plot within treatment (BR vs BUR), site and year. The vine attributes (cover and damage percentages) were

evaluated for their relationship with the density and biomass of individual bulbils.

2.3.2. Entire site level

A census of beetle population densities was conducted at each of the five sites using a modification of the census method used by Center et al. (2000). In order to ensure a thorough census, each study site was treated as a circle and divided into three equal sections with 120° angles from the site's center (usually BUR plots). Two or more examiners within each section walked in a centrifugal manner while avoiding overlap with the other person. Each person independently estimated vine damage due to herbivory, and counted the numbers of adults and larvae for a total of 30 min (i.e., if three examiners were involved, each spent 10 min in each section; thus a total of 90 search minutes were spent per evaluation per site). The beetle (larvae and adults) counts and damage estimates were averaged across examiners by section within site and recorded accordingly as the number of beetles observed hr^{-1} as an indirect measure of beetle density at the time of evaluation.

2.4. Data analyses

Dioscorea bulbifera vine cover and damage data from the 6-wk evaluations were averaged by treatments within sites. Count data for the numbers of bulbils per plot and the individual bulbil fresh weights were subjected to the Shapiro-Wilk normality test in SigmaPlot Statistical software. Vine cover, damage data, and fresh weight data were natural-log transformed (Ln-transformed) while the count data were square root (SQRT) transformed when assumptions of normality were not met. Transformed vine cover and damage data were averaged by treatment within site for each year (to investigate general trends in vine cover and damage level due to treatment differences) but bulbil density and individual biomass data from each treatment was averaged by plot within site for each year. Both data sets were subjected to multivariate repeated measures analyses of variance (ANOVA) to determine the effect of sites and treatments over the 5-year study period

Table 1

Repeated measure analyses of variance, testing the effect of *Lilioceris cheni* on *Dioscorea bulbifera* vine cover and vine damage in five sites as documented during the 5-yr study period.

Source	DF	Type III SS	Mean square	F Value	Pr > F	H-F
Vine cover						
Between subjects						
Sites	4	6.46	1.62	1.18	0.3506	–
Treatments	1	12.89	955.20	2.76	0.1722	–
Error	4	1386.50	346.63	–	–	–
Within subject						
Years	4	14834.06	3708.52	95.80	< 0.0001	< 0.0001
Years*Sites	16	1623.71	101.48	2.62	0.0312	0.0550
Years*Treatments	4	942.58	235.64	6.09	0.0036	0.0095
Error (Years)	16	619.37	38.71	–	–	–
Vine Damage						
Between subjects						
Sites	4	2935.37	733.84	3.90	0.1080	–
Treatments	1	3905.69	3905.69	20.75	0.0104	–
Error	4	752.86	188.22	–	–	–
Within subject						
Years	4	5516.08	1379.02	20.23	< 0.0001	< 0.0001
Years*Sites	16	2877.39	179.84	2.64	0.0304	0.0404
Years*Treatments	4	815.55	203.89	2.99	0.0508	0.0613
Error (Years)	16	1090.46	68.15	–	–	–

Ln-transformed data were used for this analysis.

(SAS Institute, 2011). Vine cover and damage variables had insufficient error degrees of freedom for multivariate tests, so the interaction term (site*treatment) was dropped from the model in the repeated measure analysis for these two variables. The Huynh-Feldt adjustment was used when the covariance matrix of the data did not meet the assumption of sphericity (von Ende, 1993; SAS Institute, 2011). Spearman's Ranked Correlation analysis was used to detect the potential correlation between transformed data on 1) vine cover and vine damage, 2) beetle density and vine cover, 3) beetle density and vine damage, and 4) vine damage and bulbil attributes (densities and biomass). Mean separations of bulbil densities and biomass were performed on transformed values by using Waller Duncan's Multiple Range Test (SAS Institute, 2011), but the data presented in the figures are based on the non-transformed values.

3. Results

3.1. Vine cover and herbivory damage

The proportion of the study plots covered by *D. bulbifera* vines and leaves was not influenced by either site or treatment (i.e., BR versus BUR); however, the years and years*treatments effects significantly influenced vine cover (Table 1). The percentage of vines damaged by herbivory was not affected by the sites, whereas it was significantly affected by treatment, year, and the year*site (Table 1). Overall, there was a strong negative correlation ($r = -0.540$; $P < 0.0001$; $N = 50$) between vine cover and the vine damage. Across five sites, vine cover within the BUR treatment decreased significantly between 2012 and 2016; BR manifested a similar trend, but at a lower scale (Fig. 1a). At the onset of the experiment in 2012, mean *D. bulbifera* vine cover at the five research sites varied from an average of 61% (lowest) at KIH to 83% (highest) at FCP. By the end of the study period in 2016, the mean vine cover across all sites (both treatments combined) decreased to 23%. The decrease was more pronounced in the BUR (from 73% in 2012 to 16% in 2016) than in the BR (from 64% in 2012 to 28% in 2016) treatment (Fig. 1a). The mean percentage of beetle damage in BUR increased remarkably from 0% to 45% by 2014, and then dropped down to 31% in 2016 whereas, in BR this damage increased from 0 to 16% and then to 20% and remained at the same level (Fig. 1b).

3.2. Beetle population dynamics

Beetles released in the plot, as well as those migrating from the adjacent areas, colonized all study sites. Mean beetle population densities (beetle counts hr^{-1}) observed during the censuses showed a sharp increase during 2013 (263 hr^{-1}) and 2014 (258 hr^{-1}), but then declined during 2015 (141 hr^{-1}) and 2016 (122 hr^{-1}) (Fig. 2). Beetle populations in the site had very strong positive correlation with the percentage of vine cover ($r = 0.965$, $P = 0.0079$, $n = 5$) and damage ($r = 0.909$, $P = 0.0325$, $n = 5$) in the research plots.

3.3. Bulbil densities

Bulbil densities were influenced by site, treatment, and a site \times treatment interaction (Table 2). Consistent with the reducing vine cover, the number of bulbils in study plots decreased over time across all sites (Fig. 3). A trend analysis demonstrated a strong positive correlation between vine cover and bulbil density ($r = 0.828$, $P = 0.000002$, $n = 50$) and a negative correlation between herbivore damaged leaves and bulbil density ($r = -0.523$, $P = 0.000112$,

$n = 50$). However, the influence of time (year) was variable as evidenced by strong interactions with both site and treatment factors (Table 2). The site \times treatment and year \times treatment interactions was at least partially attributable to human interference (e.g., people were observed collecting bulbils from BR (BR) plots at SNP site during 2016, and an illegal trash dumping buried vines in one-third of a plot at PIR site early in the growing season during 2015). During the first year after beetle releases, a marked decline in bulbil density was observed at FCP (60% in BR vs. 95% in BUR), KIH (37% in BR vs. 92% in BUR), TCP (36% in BR vs. 81% in BUR) and SNP (36% in BR vs. 66% in BUR). In contrast, bulbil density decline was relatively less in PIR (61% in BR vs. 29% in BUR) (Fig. 3). By the end of the study in 2016, overall bulbil density across all sites was < 1 bulbil per m^2 in BUR and 1–5 bulbils per m^2 in BR, whereas, it was 20–42 bulbils m^2 in BUR and 12–56 per m^2 in BR treatments at the onset of the study in 2012 (Fig. 3).

3.4. Bulbil biomass

Individual bulbil biomass did not vary among sites, but restricted herbivory by *L. cheni* resulted in greater bulbil biomass as compared to the unrestricted herbivory treatment (Table 3, Fig. 4). Therefore, the factor “site” was dropped from further ad-hoc analyses and the mean separations were performed and presented by year for both treatments (Fig. 4a). Despite some fluctuations, mean bulbil biomass estimates were not different (with an increase of $< 1.0\%$) for BR treatments over time; in contrast, bulbil biomass decreased (by 85%) markedly over

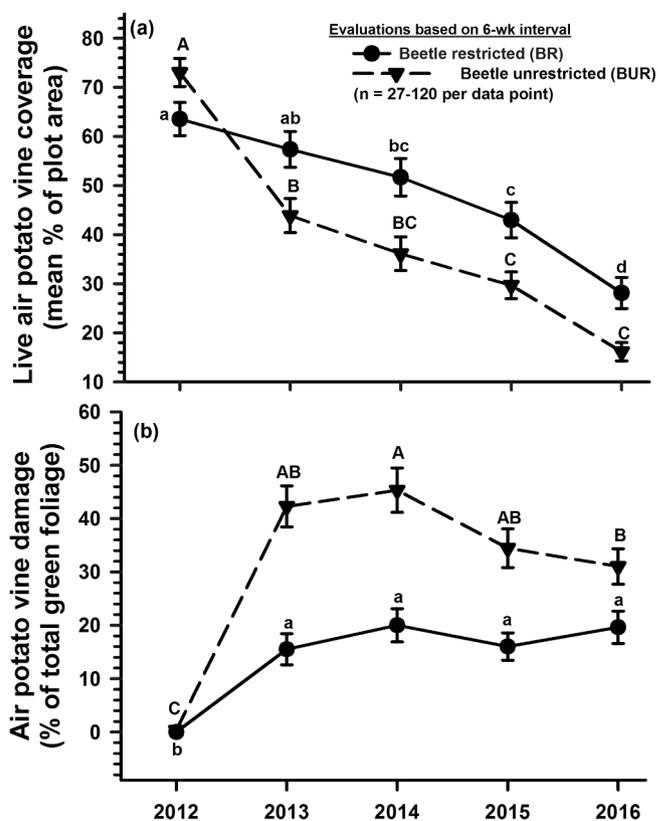


Fig. 1. Mean (\pm SE) *D. bulbifera* (air potato) live vine cover and level of foliage feeding damage (percentage of the total green foliage on vines) by *L. cheni* beetles during 5-year study period as determined at 6-wk interval during each growing season.

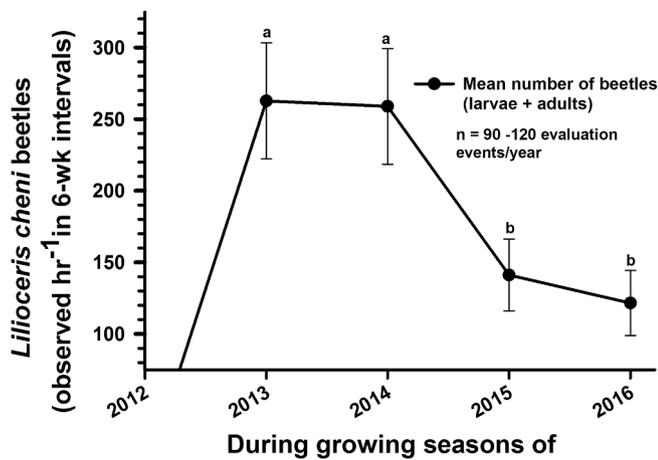


Fig. 2. *Lilioceris cheni* densities (mean number of adult and larval life stages observed hr^{-1} in 6-wk intervals) during growing seasons across five research sites. Mean (\pm SE) denoted by different letters within the beetle restricted (BR) or beetle unrestricted (BUR) treatments are significantly different from one year to another at $P < 0.05$.

Table 2

Repeated measure analyses of variance, testing the effects of *Lilioceris cheni* treatments on *Dioscorea bulbifera* bulbil production (measured as the number of bulbils m^2 of plot area) cross five sites as documented during the 5-yr study period.

Source	DF	Type III SS	Mean square	F Value	Pr > F	H-F
Between subjects						
Sites	4	70.68	17.67	10.78	< 0.0001	–
Treatments	1	78.39	78.39	47.85	< 0.0001	–
Sites*Treatments	4	83.01	20.75	12.67	0.0001	–
Error	20	32.77	1.64	–	–	–
Within subject						
Years	4	266.14	66.54	188.20	< 0.0001	< 0.0001
Years* Sites	16	29.53	1.85	5.22	< 0.0001	< 0.0001
Years*Treatments	4	8.42	2.11	5.95	0.0003	0.0024
Years* Sites *Treatments	16	16.03	1.01	2.83	0.0011	0.0067
Error (Years)	80	28.28	0.35	–	–	–

Square root-transformed bulbil count data were used for this analysis.

time in BUR plots (Fig. 4a). Mean biomass of individual bulbils between BR vs. BUR treatments was not significantly different at the onset of the experiment (2012) but significant (by 85%) reduction was noted in BUR by the end of the study period (Fig. 4b). The steady reduction in mean bulbil biomass in BUR showed very strong positive correlation ($r = 0.955$, $P = 0.0113$, $n = 5$) with the level (percentage) of *L. cheni* mediated feeding damages on *D. bulbifera* vines.

4. Discussion

The rapid growth rate of *D. bulbifera*, which is second only to another invasive alien kudzu vine *Pueraria montana* (Lour.) Merr. lobata (Willd.) with a remarkable growth rate, and its ability to produce numerous vegetative propagules (bulbils) are important biological attributes that contribute to its invasive nature (Nehrling, 1933, 1944; Rayamajhi et al., 2016). Herein, we investigated the influence of herbivory by *L. cheni* on invasive attributes of *D. bulbifera*. Overall vine cover decreased in both BR and BUR treatments. The decreasing trend

in vine cover coincided with the outbreak of the *L. cheni* populations during the 2013 and 2014 growing seasons. High levels of *D. bulbifera* cover during early phases of the study represented greater carrying capacity to support out-breaking populations of *L. cheni*, but beetle populations declined concomitantly with decreases in vine cover beginning in 2015. These observations provide evidence of a mutual regulatory relationship between *D. bulbifera* and *L. cheni*. A similar phenomenon between the invasive plant *Solidago altissima* L. and specialist herbivore *Microrhopala vittata* (F.) has been noted, in which the herbivore resulted in a drastic (reaching 63%) reduction of the weed's biomass (Carson and Root, 2000). Insect and disease outbreaks have been historically more pronounced (causing up to 100% damage to the plant foliage) in monotypic stands, due possibly to resource concentration and apparency of host plants (Feeny, 1976; Schowalter et al., 1986; Stieha et al., 2016).

Feeding damage from *L. cheni*, with no other dietary options, resulted in significant top-down impacts on *D. bulbifera* vines. Thus, our efforts to chemically exclude this herbivore during outbreak conditions met with variable success. This was due in part to the need for herbivores to test feed on "protected" plants before avoidance or toxicity responses take effect. This type of interaction has also been noticed in other invasive plant-biocontrol systems (Tipping et al., 2008; Rayamajhi et al., 2010). The systemic insecticide used in our study was unable to completely deter adult beetles from feeding on vines in chemically restricted treatment plots; beetle populations readily migrated, fed voraciously, and caused significant damage to *D. bulbifera* vines prior to death. Mortality of adult *L. cheni* beetles usually occurred within 48 h of first feeding activities (Rayamajhi, unpublished data). Herbivory on vines in BR treatments occurred primarily late in the growing season, when healthy *D. bulbifera* foliage in BUR treatments as well as surrounding host plants at the site were exhausted due to extensive beetle feeding. Overall, there is strong evidence that herbivory by *L. cheni* reduces vine cover and, as a result, helps suppress the smothering effects of *D. bulbifera* on other vegetation. Similar impacts from insect herbivory have also been reported for other invasive plants: *Hypericum perforatum* L. (Campbell and McCaffrey 1991), *Melaleuca quinquenervia* (Cav.) S.T. Blake (Franks et al., 2006; Tipping et al., 2008; Rayamajhi et al., 2010) and *Cirsium vulgare* (Savi) Ten. (Schultz et al., 2017).

Vegetative propagules are the sole source of *D. bulbifera* proliferation in Florida (Overholt et al., 2016). Rayamajhi et al. (2016) have reported a strong positive correlation between the linear length of *D. bulbifera* vines and the quantity of bulbils produced. Therefore, the authors predicted that any management approach that results in the reduction of vine growth would also reduce bulbil production, given that vegetative propagules are produced at the leaf axils along the length of vines. Consistent with the aforementioned report, results from the present study demonstrated that *L. cheni* may have been responsible for disrupting photosynthetic capacity of *D. bulbifera* through chronic feeding damage to the green foliage. This assumption is supported by similar reports on invasive plant *Parthenium hysterophorus* L. and herbivore *Zygogramma bicolorata* Pallister system in which herbivore damage caused 36% reduction in photosynthetic capacity of the invasive plant (Cowie et al., 2018). Therefore, it is likely that *D. bulbifera* may be reallocating resources towards replacement of the damaged foliage at the cost of vegetative propagules. It has been demonstrated in other weed-herbivore systems that invasive plants, when challenged by herbivores, compensate for the effects of herbivory by allocating more nutrient resources to foliage production at the expense of propagules (Zhang et al., 2018; Center et al., 2005; Pratt et al., 2005; Tipping et al., 2015)

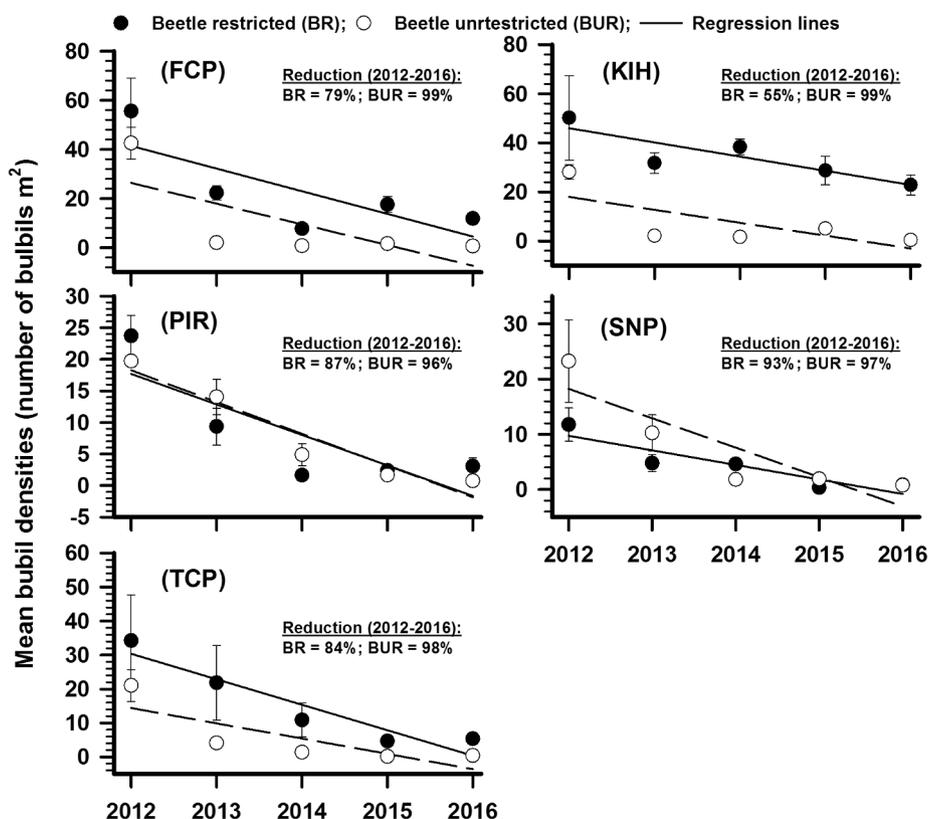


Fig. 3. Mean *D. bulbifera* (air potato) bulbil (vegetative propagule) densities (produced each year and fallen on the ground) at the five *L. cheni* impact study sites at the end of growing seasons in Florida.

Table 3

Repeated measure analyses of variance, testing the effect of *Lilioceris cheni* treatments on *Dioscorea bulbifera* bulbil biomass (g fresh weight bulbil⁻¹) across five sites as documented during the 5-yr study period.

Source	DF	Type III SS	Mean square	F Value	Pr > F	H-F
Between subjects						
Sites	4	6.46	1.62	1.18	0.3506	–
Treatments	1	12.89	12.89	9.40	0.0061	–
Sites*Treatments	4	15.00	3.75	2.73	0.0579	–
Error	20	27.43	1.37	–	–	–
Within subject						
Years	4	38.57	9.64	19.00	< 0.0001	< 0.0001
Years*Sites	16	23.89	1.49	2.94	0.0008	0.0100
Years*Treatments	4	13.67	3.42	6.73	0.0001	0.0027
Years*Sites*Treatments	16	21.51	1.34	2.65	0.0022	0.0182
Error (Years)	80	40.60	0.51	–	–	–

Ln-transformed bulbil fresh biomass data were used for this analysis.

Herbivore-mediated reduction in propagule production among invasive perennial plants is not uncommon. For example, a 54–99% reduction in seed production due to insect herbivory of foliage has been reported for the invasive tree *M. quinquenervia* in Florida (Pratt et al., 2005; Tipping et al., 2008). In addition, specialist herbivores reduced horse nettle (*Solanum carolinense* L.) fruit production by more than 75% (Wise and Sacchi, 1996), vegetative propagules of *Solanum dulcamara* L. by 64% (Hare, 1980), and *Mimosa pigra* L. seed production by 90% (Paynter, 2005). Densities of the vegetative propagules (bulbils) of *D. bulbifera* in this study were reduced in both BR and BUR treatments

(75% vs 98% declines, respectively), although the reduction was more pronounced in the later treatment. In one of the instances, the impact of *L. cheni* in areas like Gainesville, Florida, (near the FCP site) have caused landscape level depletion of bulbil production in *D. bulbifera* to such an extent that annual bulbil collections or “bulbil round up” events have ceased (Jester, 2015).

In addition to reduced bulbil densities, herbivory by *L. cheni* resulted in a 95% reduction in reproductive biomass of *D. bulbifera* in Florida. The physiological mechanism responsible for observed herbivore-mediated reductions in bulbil biomass likely includes compensation as described above but may also be related to *L. cheni* feeding behavior. Three notable reasons for this drastic *L. cheni*-mediated reduction in bulbil biomass may include: 1) vine mortality prior to bulbil maturation during the growing season, 2) sub-lethal feeding damage to vines that forced plants to divert more resources to compensate for foliage loss to herbivory instead of new bulbil production and growth of the existing bulbils, or 3) feeding damage to young, developing bulbils especially late in the growing season following the near-total exploitation of foliage. Smith and Hough-Goldstein (2014) reported a similar phenomenon with mile-a-minute weed wherein weevil herbivory caused a significant reduction in propagule biomass.

As evidenced by our findings, herbivory from *L. cheni* is capable of causing pronounced foliar damage of *D. bulbifera* – often prior to, or during early stages of bulbil development. This resulted in 1) unseasonal vine mortality and release of native vegetation from smothering effects of *D. bulbifera*; 2) significant reduction in the production of vegetative propagules as measured by decreases in both bulbil density and biomass. Hence, there is a growing body of evidence to indicate that *L. cheni* will provide a sustainable, ecologically safe and presumably financially effective alternative to traditional *B. bulbifera* management in its adventive range in southeastern United States.

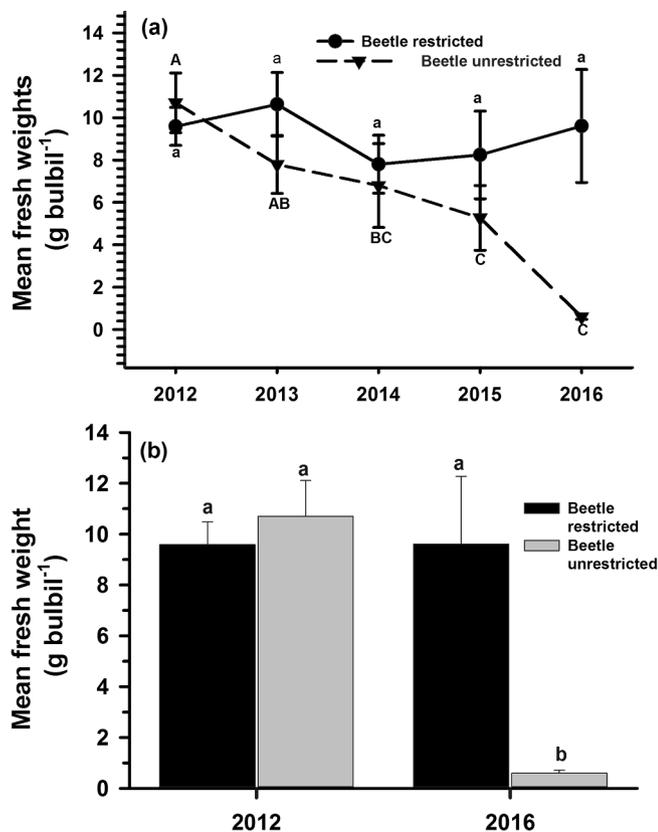


Fig. 4. Overall mean fresh biomass of individual bulbs (g bulbil^{-1}) of *D. bulbifera* produced each year across five *L. cheni*-impact study sites and fallen on the ground at the end of each growing season: a) mean (\pm SE) denoted by different letters within beetle restricted (BR) and beetle unrestricted (BUR) treatments are significantly different ($P < 0.05$) from one year to another; b) mean (\pm SE) shown in vertical bars representing two BR and BUR treatments, at the onset (2012) and the end (2016) of the study period, denoted by different letters are significantly different ($P < 0.05$) from each other.

CRediT authorship contribution statement

Min B. Rayamajhi: Conceptualization, Project administration, Methodology, Formal analysis, Writing - original draft. **Eric Rohrig:** Investigation, Methodology, Resources. **Jorge Leidi:** Methodology, Data curation. **Christopher Kerr:** Investigation, Methodology, Resources. **Eduardo Salcedo:** Methodology, Resources. **Ryan Poffenberger:** Investigation, Methodology, Resources. **Melissa Smith:** Resources, Investigation, Writing - review & editing. **Ellen Lake:** Resources, Investigation, Writing - review & editing. **F. Allen Dray:** Resources, Investigation, Validation, Writing - review & editing. **Paul Pratt:** Conceptualization, Resources, Investigation, Writing - review & editing. **Philip Tipping:** Resources, Validation, Investigation, Writing - review & editing. **Ted Center:** Conceptualization, Methodology, Resources, Investigation, Writing - review & editing.

Acknowledgments

We thank county parks (Alachua, Broward and Miami Dade), Terra Ciega State Park (Manatee County), and City Parks System (City of Fort Lauderdale) for providing research sites. Thanks are due to Reynaldo Moscat, Jorge Cedeno, Molly Clark, Ken Hibbard, Beth Curry and Raynier Mendez for help in monitoring research sites. Mention of the trade names or commercial products in this article is solely for the purpose of providing information and does not imply any recommendation or endorsement by the U.S. Department of Agriculture. USDA

Department of Agriculture is an equal opportunity employer. Partial support for this research was provided by the Comprehensive Everglades Restoration Project (CERP).

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