Minimum threshold for establishment and dispersal of Lilioceris cheni (Coleoptera: Chrysomelidae): a biological control agent of Dioscorea bulbifera

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Minimum threshold for establishment and dispersal of *Lilioceris cheni* (Coleoptera: Chrysomelidae): a biological control agent of *Dioscorea bulbifera*

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### ABSTRACT

The successful establishment or failure of a new population is often attributed to propagule pressure, the combination of the number of independent introduction events, and the number of individuals released at each event. The design of optimal release strategies for biological control agents benefits from an understanding of the impact of propagule pressure on the species being released. The dispersal rate of individuals from nascent population foci can also affect establishment success. We assessed the minimum threshold for establishment and measured dispersal of *Lilioceris cheni* (Coleoptera: Chrysomelidae), a biological control agent for *Dioscorea bulbifera* (Dioscoreales), air potato. Replicated releases of 10, 50, and 100 adults of *L. cheni* were conducted on the east and west coasts of south Florida. Dispersal was measured from 19 of these sites plus 19 additional release locations in south and central Florida. *Lilioceris cheni* established populations from all three release sizes with no apparent influence of site location. Releases of 10, 50, and 100 adults resulted in 50%, 67%, and 85% establishment, respectively. Beetles dispersed an average of 1.41 ± 0.515 km/yr. Dispersal distance was significantly affected by the time since release but not the number of individuals released. Our results suggest that future releases of 100 individuals could be spaced several kilometres apart on the landscape to facilitate rapid colonisation of *D. bulbifera* infestations.

### Introduction

The role of propagule pressure in the success or failure of biological invasions has been evaluated in numerous systems and contexts, such as colonisation of invading species, biological control, and conservation biology (Fauvergue, Vercken, Malauasa, & Hufbauer, 2012 and references therein). Propagule pressure is a combination of two factors: propagule size, the number of individuals in each release event, and propagule number, the number of different introduction events (Fauvergue et al., 2012; Hufbauer, Rutschmann, Serrate, Vermeil de Conchard, & Facon, 2013; Lockwood, Cassey, & Blackburn, 2009). For individual populations, propagule pressure is the most important factor in determining establishment success (Lockwood et al., 2009).
Demographic and genetic factors play a role in the effect of propagule pressure on the success or failure of accidental and intentional introductions (Hufbauer et al., 2013). Demographic stochasticity, random variation in mean demographic rates, is a threat to the establishment and persistence of small populations (McEvoy, Grevstad, & Schooler, 2012). In contrast, environmental stochasticity decreases the chance of a given population establishing regardless of release size (Grevstad, 1999a). If environmental stochasticity reduces the size of a population, it may become susceptible to demographic stochasticity (McEvoy et al., 2012). Additionally, small populations may be vulnerable to Allee effects, reductions in population growth rates that can occur at low densities (Grevstad, 1999b; Hopper & Roush, 1993; McEvoy et al., 2012).

Releases of biological control agents are intentional introductions that are subject to demographic and environmental stochasticity. Julien (1989) estimated that only 63% of arthropods released for weed biological control before 1980 successfully established. However, increased efforts to release and redistribute agents have resulted in substantial improvements in establishment rates, including 94% success in New Zealand (Syrett, Briese, & Hoffmann, 2000 and references therein). The design of an optimal release strategy for a biological control agent includes two main components: the minimum number of individuals needed to establish a population and the dispersal rate, which is important for determining how far apart to space releases. Biological control practitioners often have a limited number of individuals available for release, particularly at the beginning of a biological control programme, when information about the biology and behaviour of the agent may be limited (Dray, Center, & Wheeler, 2001; Memmott, Fowler, Harman, & Hayes, 1996; Shea & Possingham, 2000). In a meta-analysis of 74 species of biological control agents released in Oregon, Grevstad, Coombs, and McEvoy (2011) concluded that when an agent first becomes available, there are often too few releases made to maximise the overall chances of establishment. Conducting more releases and utilising a range of release sizes early in a programme will increase the chances of establishment and help determine the optimal approach for a given species (Grevstad et al., 2011; Shea & Possingham, 2000). We applied this recommended strategy of multiple releases of a variety of sizes to a newly available biological control agent.

*Dioscorea bulbifera* L. (Dioscoreaceae), air potato, is an herbaceous, perennial vine from Asia and Africa that has become invasive in the southeastern U.S. It was first reported in Florida in 1899 (USDA, 1900). Genetic analysis concluded that the Florida populations originated from Asia (Croxton, Andreu, Williams, Overholt, & Smith, 2011; Morton, 1976). *Dioscorea bulbifera* has since been reported in 60 of the 67 counties in Florida, and its distribution extends north to Savannah, GA, with additional unverified reports in the southeastern U.S.; it has also invaded Texas (EDDMaps, 2017). *Dioscorea bulbifera* seldom flowers in the U.S. and reproduces primarily through potato-like aerial bulbils that form in the leaf axils, hence the common name air potato (Overholt et al., 2014). The plant is deciduous, even in frost-free areas, and the bulbils dehisce when the plants die back in the winter (Langeland & Craddock Burks, 1998). In the spring, new vines sprout from bulbils and persistent subterranean tubers. Vines often reach 20 m long, climb into trees, and outcompete native vegetation (Overholt et al., 2014). Air potato invades a variety of habitat types and is very challenging and expensive to manage using chemical and physical control techniques (Overholt et al., 2014; Wheeler, Pemberton, & Raz, 2007).
The host specificity of *Lilioceris cheni* Gressitt and Kimoto (Coleoptera: Chrysomelidae) was verified through quarantine host-range testing and a permit for its release as a biological control agent of *D. bulbifera* was issued by the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) in 2011 (Center et al., 2013; Pemberton & Witkus, 2010). The beetles were initially released in cages in the fall of 2011, and open-field releases began in Florida the following spring (Center et al., 2013). Ecological host-range testing in Florida with *D. bulbifera* and native and non-native congeners further confirmed the host specificity of *L. cheni* (Lake, Smith, Dray, & Pratt, 2015). Adults and larvae of *L. cheni* consume large quantities of *D. bulbifera* foliage, which decreases plant growth and reproduction (Overholt et al., 2016).

*Dioscorea bulbifera* is abundant in natural areas and neighbourhoods in many areas of Florida (EDDMaps, 2017; Wheeler et al., 2007). Public lands and conservation areas were initially prioritised for *L. cheni* releases (Overholt et al., 2016). The prevalence of this weed and media coverage of the potential for *L. cheni* to be an effective biological control agent have resulted in substantial public pressure to make beetles available for release on private property (CBSMiami.com Team, 2012; Overholt et al., 2016). Several entities are now mass-rearing the beetles for release on public and private lands. Demand remains high and beetles can be requested through an online form (UF/IFAS Solutions for Your Life, 2017). Thus, determining how many beetles are needed to establish a new population and the rate at which beetles disperse are critical to maximising the efficacy of the ongoing rearing and release effort. The objectives of this study were to determine the minimum number of adults needed to establish viable populations in the field in south Florida and to quantify dispersal rates from early release sites located in south and central Florida.

**Methods**

**Establishment release size**

Twenty-four sites invaded by air potato were selected on the east and west coasts of south Florida. The locations ranged from 26.663662–27.139741 north latitudes on the east coast and 25.99354–26.737193 north latitudes on the west coast (Table 1(A and B) and Figure 1). *Lilioceris cheni* was not present at any of the selected sites, which were located a minimum of 1 km from each other and prior release sites. Each site was randomly assigned a release size of 10, 50, or 100 adults for a total of four replicates per release size per coast (Table 1(A and B)). The Chinese biotype of *L. cheni* was used for all releases; a Nepalese biotype of this species has since become available for release (Center et al., 2013; Manrique et al., 2017). The beetles were reared at the U.S. Department of Agriculture Agricultural Research Service Invasive Plant Research Laboratory in Fort Lauderdale, FL. Beetles were reared in cages on whole plants in outdoor screen houses or indoors on cut plant material in plastic cages. Late instar larvae were provided with moist vermiculite as a substrate for pupation. When possible, the releases of 10 adults consisted of 5 mating pairs. The beetles released ranged in age from 2 to 21 d old. All releases were conducted between 19 June and 23 July 2013.

*Lilioceris cheni* activity was monitored monthly from the time of release until 31 October 2013, prior to leaf dehiscence. The sites were also monitored between 29 July and 15 August 2014. Each survey consisted of a total of 20 min of search time by two observers. The presence or absence of *L. cheni* eggs and larvae and the
number of adults were recorded. *Lilioceris cheni* activity and feeding damage at the sites was assessed using the following scale: 0, no *L. cheni* or damage; 1, no *L. cheni* but old feeding damage; 2, a few *L. cheni* and feeding damage at the point of release; 3, *L. cheni* and damage present in three or fewer discrete patches; 4, *L. cheni* and damage common throughout the site; 5, *L. cheni* and damage abundant throughout the site, with dieback of vines visible. If no score is provided, a + indicates that *L. cheni* activity was present and a – indicates activity was absent. NS designates that a site was not sampled. Several west coast sites experienced significant flooding, feral hog damage, or other disturbance in 2013, and this is noted with an asterisk (*) after the site name.

### Table 1. East (A) and West (B) coast field sites used in the establishment release study.

<table>
<thead>
<tr>
<th>Site name</th>
<th>Dioscorea bulbifera percentage cover</th>
<th>Number of beetles released</th>
<th>Number of beetles in October 2013</th>
<th>Feeding score in October 2013</th>
<th>Number of beetles in Summer 2014</th>
<th>Feeding score in Summer 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. East coast release sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pine Jog Environmental Education Center</td>
<td>30</td>
<td>10</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Robbins Park</td>
<td>80</td>
<td>10</td>
<td>16</td>
<td>4.5</td>
<td>128</td>
<td>5</td>
</tr>
<tr>
<td>Seabrook Park</td>
<td>40</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>104</td>
<td>4</td>
</tr>
<tr>
<td>Winston Park neighbourhood</td>
<td>80</td>
<td>10</td>
<td>37</td>
<td>3</td>
<td>58</td>
<td>5</td>
</tr>
<tr>
<td>Delaplaine</td>
<td>80</td>
<td>50</td>
<td>18</td>
<td>4.5</td>
<td>104</td>
<td>4</td>
</tr>
<tr>
<td>Eastern County Park</td>
<td>80</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mills Pond Park</td>
<td>80</td>
<td>50</td>
<td>11</td>
<td>4</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Sailboat Bend Park</td>
<td>80</td>
<td>50</td>
<td>17</td>
<td>5</td>
<td>37</td>
<td>4</td>
</tr>
<tr>
<td>Hugh Taylor Birch State Park</td>
<td>50</td>
<td>100</td>
<td>0</td>
<td>4</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Warbler Wetland Natural Area</td>
<td>80</td>
<td>100</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>John U. Lloyd State Park</td>
<td>80</td>
<td>100</td>
<td>9</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phipps Park</td>
<td>80</td>
<td>100</td>
<td>9</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. West coast release sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>River’s Road Preserve*</td>
<td>80</td>
<td>10</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>Matanzas Pass</td>
<td>50</td>
<td>10</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>–</td>
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<tr>
<td>Sugden Regional Park*</td>
<td>80</td>
<td>10</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>Veteran’s Park*</td>
<td>50</td>
<td>10</td>
<td>0</td>
<td>3</td>
<td>3</td>
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</tr>
<tr>
<td>Buckingham Road</td>
<td>90</td>
<td>50</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>North Shores Nature Trail</td>
<td>60</td>
<td>50</td>
<td>0</td>
<td>3.5</td>
<td>3.5</td>
<td>+</td>
</tr>
<tr>
<td>Persimmon Ridge</td>
<td>40</td>
<td>50</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>Sanibel–Blue Skies Preserve</td>
<td>40</td>
<td>50</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>Collier–Seminole State Park</td>
<td>80</td>
<td>100</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>+</td>
</tr>
<tr>
<td>Rookery Bay*</td>
<td>80</td>
<td>100</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>+</td>
</tr>
<tr>
<td>Sanibel–Markay Preserve</td>
<td>80</td>
<td>100</td>
<td>0</td>
<td>3.5</td>
<td>3.5</td>
<td>–</td>
</tr>
<tr>
<td>Sanibel–SCCF Vinca Way</td>
<td>60</td>
<td>100</td>
<td>0</td>
<td>3.5</td>
<td>3.5</td>
<td>+</td>
</tr>
</tbody>
</table>

Notes: The following scale was used to assess *Lilioceris cheni* activity: 0, no *L. cheni* or damage; 1, no *L. cheni* but old feeding damage; 2, a few *L. cheni* and feeding damage at the point of release; 3, *L. cheni* and damage present in three or fewer discrete patches; 4, *L. cheni* and damage common throughout the site; 5, *L. cheni* and damage abundant throughout the site, with dieback of vines visible. If no score is provided, a + indicates that *L. cheni* activity was present and a – indicates activity was absent. NS designates that a site was not sampled. Several west coast sites experienced significant flooding, feral hog damage, or other disturbance in 2013, and this is noted with an asterisk (*) after the site name.
and damage present in three or fewer discrete patches; 4, *L. cheni* and damage common throughout the site; 5, *L. cheni* and damage abundant throughout the site, with dieback of vines visible.

**Figure 1.** *Lilioceris cheni* release sites in south and central Florida used for the establishment release size experiment and dispersal measurements.
Dispersal

Beetle dispersal was measured in October 2013 at 38 sites: 19 of the release size experimental sites and 19 additional sites located in south and central Florida (Figure 1). The number of individuals released at the additional sites ranged from 100 to 49,305 individuals, and these releases consisted of adults only, larvae only, or a mixture of the two life stages. The 38 release sites were categorized by region (east, west, or central Florida), beetle life stage released, and whether or not released individuals had overwintered at the site. At each site, a GPS point was recorded at the release point and at the furthest points of L. cheni damage in the four cardinal directions, when possible. The distance from each cardinal direction dispersal point to the release point was calculated in ArcGIS (ESRI, 2014).

Statistical analysis

Linear regression was used to assess the relationship between beetle counts and damage scores (PROC REG, SAS Institute, 2011). The influence of cardinal direction on dispersal distance was analysed with a one-way ANOVA (PROC GLM, SAS Institute, 2011). The mean dispersal distance was calculated for each site, and an ANOVA was used to determine if beetle development stage, region, or if the beetles had overwintered at the site influenced the mean dispersal distance (PROC GLM, SAS Institute, 2011). Multiple linear regression was used to determine the influence of release size and time since release on dispersal distance (PROC REG, SAS Institute, 2011).

Results

Establishment release size

Beetle activity was observed at 10 of the 11 sampled east coast sites and all 11 sampled west coast sites in October 2013, 3–4 months post-release. In October 2013, the mean beetle count and activity score were 10.5 ± 3.4 and 3.5 ± 0.4 for the east coast sites and 0.4 ± 0.4 and 3.6 ± 0.2 for the west coast sites, respectively. There was no relationship between beetle counts and activity scores in October 2013 (P = .4679, R² = 0.0266). By summer 2014, 3 of the 10 sampled east coast sites had been destroyed. The remaining sites all had adults present, with an average of 49.29 ± 19.0 beetles and a damage score of 3.6 ± 0.6 (Table 1(A and B)). Multiple west coast sites experienced flooding, feral hog damage, or other disturbance in 2013. Despite this extensive disturbance, beetle activity was observed at 7 of the 12 west coast sites in summer 2014. Beetles established from all three release sizes on both coasts. When the results from the two coasts were combined, 50% of releases of 10 individuals established, compared to 67% and 85% of releases of 50 and 100 individuals, respectively.

Dispersal

Cardinal direction had no effect on dispersal distance (F₃, ⁹₄ = 0.32, P = .8109). Thus, these measurements were averaged to determine the mean dispersal distance for each of the 38 sites; mean dispersal distance was used in subsequent analyses. The dispersal distance was higher if beetles had overwintered at a site (F₁, ³₂ = 7.79, P = .0088) but was not affected by
the stage of insect released ($F_{2, 32} = 0.56, P = .5754$). The region of release influenced dispersal distance when all 38 sites were included in the model ($F_{2, 32} = 3.36, P = .0472$), with the greatest dispersal measured from release sites in central Florida ($N = 4$). However, when the establishment release size experiment sites were analysed separately, there was no difference in the dispersal distance from east and west coast sites ($F_{1, 16} = 0.65, P = .1296$). When all 38 sites were included in the analysis, dispersal distance was greatly affected by time since release ($P = .0001, R^2 = 0.4227$) but not the number of individuals released ($P = .7456$). Beetles dispersed at an average rate of $1.41 ± 0.515$ km/yr, and this ranged from 0.03 to 15.05 km/yr.

Discussion

Releases of the biological control agent *L. cheni* were conducted in 24 air potato patches in south Florida. We considered a population to be established if beetle activity was detected in a field site one year post-release; this required successful overwintering of adult beetles while their host plant was dormant. The three release sizes used, 10, 50, or 100 individuals, resulted in 50%, 67%, and 85% establishment, respectively. In south Florida, very limited beetle activity is regularly observed in the early spring, despite several metres of growth on new *D. bulbifera* vines (Lake, Smith, Rayamajhi, personal observation). These observations prompted a study examining the overwintering survival of beetles in field cages located at different latitudes throughout Florida. Both the Chinese and Nepalese biotypes had little or no overwintering survival at the most southern site, Homestead, Florida, over multiple years (Smith et al., 2018). This low overwintering survival is likely due to a combination of differential fat storage by the two biotypes and annual variability in winter temperatures. It is possible that beetles in south Florida are too active during the winter and metabolise their fat reserves before air potato is available in the spring (Smith et al., 2018). Many of our experimental releases established in spite of low initial release numbers and a tendency for poor overwintering survival in south Florida.

Despite the bright red colour of their elytra, it can be difficult to accurately count the number of *L. cheni* in the field. Air potato vines climb 20 m into trees and adults prefer to oviposit on the fresh growth at the top of the vines and often feed on the underside of leaves (Overholt et al., 2014; Lake, Smith, Rayamajhi, personal observation). Additionally, beetle activity may vary with the time of day or weather conditions (Overholt et al., 2016). We scored a site as beetles present or absent based on several metrics, so a site was considered occupied if characteristic feeding damage was present even if no individuals were observed, to avoid false negatives. There was no relationship between our beetle counts and the activity score in the sites in 2013. Overholt et al. (2016) surveyed 113 random air potato patches throughout Florida and the number of adults observed was positively correlated with their damage rating, which estimated the percentage of leaves damaged in a site. The more coarse scale of damage used in our study may have been better suited for confirming the presence of beetles in a site than assessing their number.

The adult beetles used in the establishment release size experiment ranged in age from 2 to 21 d old. A study conducted after the experimental releases determined that the Chinese biotype of *L. cheni* has a pre-ovipositional period of 10.1 ± 0.6 days (Manrique et al., 2017). Additionally, there appears to be a pre-mating period of several days (Lake and Smith, personal observation). Some releases conducted for this experiment likely consisted of pre-
ovipositional and potentially unmated individuals. Thus, particularly with the releases of 10 individuals, we may have exacerbated the potential impact of Allee effects by releasing unmated beetles that may have dispersed before finding a mate (Hopper & Roush, 1993). Additionally, the sex ratio of the 10 individual releases may have been skewed if the beetles were not collected as mating pairs. This is the only reliable method to sex live beetles due to similar body length between males and females and lack of other external distinguishing characters (Manrique et al., 2017). When possible, the later releases of 10 individuals consisted of 5 mating pairs to ameliorate these issues. Regardless of these potential limitations, 50% of the small releases established populations. Small releases of other biological control agents have successfully established populations. For example, Grevstad (1999b) documented the establishment of a population of *Galerucella calmiensis* L. (Coleoptera: Chrysomelidae), a biological control agent of *Lythrum salicaria* L., purple loosestrife (Lythraceae), from the release of one gravid female.

*Lilioceris cheni* has a negative impact on *D. bulbifera* growth and reproduction despite the late onset of population development each year (Overholt et al., 2016). Chinese biotype females produced an average of 1907 ± 341 eggs over the course of their lifetime, with a net reproductive rate of 700.67 and an intrinsic rate of increase of 0.46 in a laboratory study (Manrique et al., 2017). However, calculating these parameters in the laboratory eliminated the impact of predators and parasitoids and environmental stochasticity (Pratt, Slone, Rayamajhi, Van, & Center, 2003). *Lilioceris cheni* adults are long-lived and field populations consist of multiple overlapping generations (Lake et al., 2015; Manrique et al., 2017). The combination of these life history traits and the potential for rapid population growth bodes well for population establishment and suggests that if disturbances are short-term and occur after some post-release reproduction and population growth has occurred, they may not prevent establishment.

Paynter and Bellgard (2011) reviewed dispersal data for 66 arthropod biological control agents and concluded that an arthropod's dispersal rate was not an indication of its effectiveness as a biological control agent. However, a high rate of dispersal may limit population establishment, and a low rate of dispersal may necessitate an intensive redistribution effort (Heimpel & Asplen, 2011). Resource availability can affect both the probability of dispersing and the distance moved (Dwyer & Morris, 2006; Herzig, 1995). *Lilioceris cheni* tend to stay within a patch when initially released, but will disperse after heavily damaging air potato vines in search of higher quality foliage (Rayamajhi & Dray, personal observation). The 24 establishment experiment releases were conducted in large patches of air potato with an average percentage cover greater than 65% in the 5 m² area surrounding the release point. The density of air potato in these patches was likely sufficient to prevent immediate depletion of food resources that could potentially trigger widespread dispersal from the patch and limit establishment.

*Lilioceris cheni* dispersed at an average rate of 1.41 ± 0.515 km/yr in this study, and the dispersal distance was highly correlated with time since release. Overholt et al. (2016) estimated a dispersal rate of 8.2 km/yr, but acknowledged that human-assisted dispersal may have occurred. Measurements of dispersal rates can change over time and may be driven by changing resource availability (Andow, Kareiva, Levin, & Okubo, 1993; Dwyer & Morris, 2006; Paynter & Bellgard, 2011). We did not assess factors that cued *L. cheni* dispersal or colonisation of new air potato patches in this study. In other experiments with chrysomelids, dispersal behaviour and colonisation of new host populations was
influenced by beetle density or plant damage (Herzig, 1995), the presence or absence of conspecifics (Grevstad & Herzig, 1997), distance between host patches (Grevstad & Herzig, 1997), or the habitat matrix (Dávalos & Blossey, 2011).

Understanding the dispersal behaviour of biological control agents can have important implications for effective weed control because alternate management techniques should be applied in habitats unlikely to be colonised by dispersing agents (Dávalos & Blossey, 2011; Hough-Goldstein, Lake, D’Amico, & Berg, 2012). Although the habitat preference of *L. cheni* has not yet been directly tested, field surveys suggest it may be challenging for the beetles to locate *D. bulbifera* populations within urban areas (Overholt et al., 2016). If this is due to the urban matrix, and not because of mortality due to the use of insecticidal sprays for mosquito control, which is currently under investigation, a more intense release strategy in urban locations could be beneficial (Overholt et al., 2016).

Rearing methods have been optimised for *L. cheni*, and both the Chinese and Nepalese biotypes are currently being mass reared and released in Florida, partially through the Comprehensive Everglades Restoration Plan (CERP). Additional releases are now being conducted in the southeastern U.S. (Winston et al., 2017; C. Kerr, personal communication, January 19, 2018). *Lilioceris cheni* is widely distributed in Florida but additional releases, particularly in urban areas, would benefit efforts to control air potato. Our results suggest that releases of 100 adults in unoccupied patches would be sufficient to establish new populations of this biological control agent.

**Acknowledgements**

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**Disclosure statement**

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