



Biocontrol Science and Technology

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/cbst20>

Host range validation, molecular identification and release and establishment of a Chinese biotype of the Asian leaf beetle *Lilioceris cheni* (Coleoptera: Chrysomelidae: Criocerinae) for control of *Dioscorea bulbifera* L. in the southern United States

Ted D. Center^a, Min Rayamajhi^a, F. Allen Dray^a, Paul M. Madeira^a, Gloria Witkus^a, Eric Rohrig^b, Elizabeth Mattison^a, Ellen Lake^a, Melissa Smith^a, Jialing Zhang^c, Matthew Purcell^d, Alex Konstantinov^e & Don Schmitz^f

^a USDA-ARS, Invasive Plant Research Laboratory, Fort Lauderdale, FL, USA

^b Florida Department of Agriculture and Consumer Services, Division of Plant Industries, Gainesville, FL, USA

^c Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Gardens, Chinese Academy of Sciences, Wuhan, Hubei, China

^d USDA-ARS, Australian Biological Control Laboratory and CSIRO, Division of Entomology, Brisbane, Australia

^e USDA-ARS, Systematic Entomology Laboratory, Washington, DC, USA

^f Florida Fish and Wildlife Conservation Commission, Tallahassee, FL, USA

Accepted author version posted online: 22 Apr 2013. Published online: 17 Jul 2013.

To cite this article: Ted D. Center, Min Rayamajhi, F. Allen Dray, Paul M. Madeira, Gloria Witkus, Eric Rohrig, Elizabeth Mattison, Ellen Lake, Melissa Smith, Jialing Zhang, Matthew Purcell, Alex Konstantinov & Don Schmitz (2013) Host range validation, molecular identification and release and establishment of a Chinese biotype of the Asian leaf beetle *Lilioceris cheni* (Coleoptera: Chrysomelidae: Criocerinae) for control of *Dioscorea bulbifera*

L. in the southern United States, *Biocontrol Science and Technology*, 23:7, 735-755, DOI:
[10.1080/09583157.2013.790931](https://doi.org/10.1080/09583157.2013.790931)

To link to this article: <http://dx.doi.org/10.1080/09583157.2013.790931>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at <http://www.tandfonline.com/page/terms-and-conditions>

RESEARCH ARTICLE

Host range validation, molecular identification and release and establishment of a Chinese biotype of the Asian leaf beetle *Lilioceris cheni* (Coleoptera: Chrysomelidae: Criocerinae) for control of *Dioscorea bulbifera* L. in the southern United States

Ted D. Center^a, Min Rayamajhi^{a*}, F. Allen Dray^a, Paul M. Madeira^a,
Gloria Witkus^a, Eric Rohrig^b, Elizabeth Mattison^a, Ellen Lake^a, Melissa Smith^a,
Jialing Zhang^c, Matthew Purcell^d, Alex Konstantinov^e and Don Schmitz^f

^aUSDA-ARS, Invasive Plant Research Laboratory, Fort Lauderdale, FL, USA; ^bFlorida Department of Agriculture and Consumer Services, Division of Plant Industries, Gainesville, FL, USA; ^cLaboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Gardens, Chinese Academy of Sciences, Wuhan, Hubei, China; ^dUSDA-ARS, Australian Biological Control Laboratory and CSIRO, Division of Entomology, Brisbane, Australia; ^eUSDA-ARS, Systematic Entomology Laboratory, Washington, DC, USA; ^fFlorida Fish and Wildlife Conservation Commission, Tallahassee, FL, USA

(Received 26 December 2012; returned 9 January 2013; accepted 26 March 2013)

Dioscorea bulbifera, an Asian vine, is invasive in the southeastern USA. It rarely flowers but propagates from potato-like bulbils formed in leaf axils, which persist into the subsequent growing season. *Lilioceris cheni* Gressitt and Kimoto, a foliage-feeding beetle (Coleoptera: Chrysomelidae: Criocerinae) from Nepal, had been tested, proven to be a specialist and approved for release as a biological control agent. Regulatory delays, however, resulted in the demise of quarantine-held colonies, and acquisition of new Nepalese stock proved untenable. Searches then undertaken in southern China resulted in the collection of over 300 similar beetles. Two Chinese *Lilioceris* species were identified: one confirmed to be *L. cheni* and the other identified as *Lilioceris egena* (Weise). Mitochondrial analysis revealed an exact DNA match between some Chinese and one of the two Nepalese *c* oxidase subunit I haplotypes and all Chinese *L. cheni* haplotypes clustered as a single species but the comingling of the two species aroused concerns over possible hybridisation. These concerns were allayed by nuclear D2 analysis showing the absence of dual parental sequences. Nonetheless, diligence was exercised to ensure that the Chinese strains were safe to release. Abridged host testing using critical test species verified specificity. Caged releases during autumn 2011 documented the ability of adult beetles to overwinter in south Florida despite a prolonged lack of foliage. Open releases the following year produced vigorous populations that caused extensive defoliation. Preliminary observations indicate that *L. cheni* now contributes to the control of *D. bulbifera* and the bulbil-feeding *L. egena* should complement these effects if its host range proves appropriate.

Keywords: weed biological control; invasive species; habitat restoration; insect–plant interactions; air potato; Dioscoreaceae

*Corresponding author. Email: Min.Rayamajhi@ars.usda.gov

This Article is a collaborative work. The contributions of Ted D. Center, Min Rayamajhi, Allen Dray, Paul M. Madeira, Gloria Witkus, Eric Rohrig, Elizabeth Mattison, Ellen Lake, Melissa Smith, Matthew Purcell, Alex Konstantinov and Don Schmitz were conducted as part of these persons' official duties as employees of the United States Government and is therefore a work of the United States Government. In accordance with 17 U.S.C. 105 no copyright protection is available for such works under U.S. law. Jialing Zhang waives his own assertion of copyright but not his status as co-author

1. Introduction

Dioscorea bulbifera (air potato or air yam), an herbaceous, perennial twining vine, envelops understory vegetation and clammers over trees, often attaining lengths of 20 m or more (e.g., Gordon, Gann, Carter, & Thomas, 1999; Langeland & Craddock Burks, 1998; Schmitz, Simberloff, Hofstetter, Haller, & Sutton, 1997). Although Overholt et al. (2003) suggested an African provenance, later genetic analysis determined that Florida material originated in Asia (Croxtton, Andreu, Williams, Overholt, & Smith, 2011). Introduced to Florida as a medicinal plant in 1905 by Henry Nehrling (Morton, 1976), it has since become 'one of the most aggressive weeds ever introduced' (Hammer, 1998). By the 1980s, air potato vines infested many parts of south and central Florida (Bell & Taylor, 1982). Now regarded as a transformer species, *D. bulbifera* alters plant communities by displacing native flora, changing community structure and disrupting ecological functions (FLEPPC, 2003). It propagates by way of aerial bulbils ('air potatoes') formed in leaf axils during late summer. These bulbils, which weigh up to 1 kg, fall to the ground as the vines senesce during late autumn. Shoots emerge during spring from the bulbils and from persistent subterranean tubers (Overholt et al., 2008). Seed production is rare in Florida with spread mainly through anthropogenic dissemination of the bulbils (Schultz, 1993).

Air potato is a scourge in natural areas, having been found in 15% of 315 conservation areas in south Florida and 25% of 48 habitats surveyed (Gann, Bradley, & Woodmansee, 2001). Gulf Coast states, Puerto Rico and Hawaii also report extensive populations (Overholt et al., 2008). Despite climate matching models that predicted the spread of *D. bulbifera* along the Atlantic Coast north to South Carolina (Overholt et al., 2008), it may already occur as far north as Kentucky and Virginia and as far west as western Oklahoma (EDDMapS, 2012). It invades a multitude of tropical and subtropical habitats (Schultz, 1993) and vigorously exploits disturbed sites, such as hurricane-damaged forest canopies, thereby pre-empting recovery of native species (Gordon et al., 1999).

We initiated a biological control programme against air potato following the 2002 discovery of a chrysomelid beetle (initially identified as *Lilioceris* sp. near *impressa*) damaging *D. bulbifera* bulbils and vines in the Kathmandu Valley, Nepal (Pemberton & Witkus, 2010). Chrysomelid beetles are often host-specific and effective biological control agents (Van Driesche, Hoddle, & Center, 2008), so we elected to investigate this beetle, ultimately identified as *Lilioceris cheni* Gressitt and Kimoto (Tishechkin, Konstantinov, Bista, Pemberton, & Center, 2011) for biological control of *D. bulbifera*. *L. cheni* was extensively studied and found to be essentially monophagous with an extremely narrow host range limited to the genus *Dioscorea* (Pemberton & Witkus, 2010). Permission for release was then requested from the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS). A permit (P526P-11-01258) was granted in October 2009. Unfortunately, due to the prolonged regulatory review, quarantine colonies had perished and attempts during 2010 to resupply stocks from Nepal were unsuccessful. However, the species was originally described from China with populations also occurring in Taiwan and Southeast Asia (Kimoto & Gressitt, 1979) where we conduct ongoing collaborative projects. It was subsequently discovered in southern China during the course of surveys on other weed targets. However, concern about the risk of introducing a

biotype from a different region that might differ from the original stock in terms of its host range (Paynter et al., 2008) prompted us to implement several precautionary steps, such as host range validation, confirmation of the identity by the specialist from USDA, Agricultural Research Service (ARS) Systematic Entomology Laboratory (SEL) entomologists and molecular analyses of the individuals from the colonies of Chinese biotype with the originally tested Nepalese biotype.

L. cheni is a large (8–9 mm) brown to red–orange Asian leaf beetle (Coleoptera: Chrysomelidae) in the subfamily Criocerinae (Tishechkin et al., 2011). The following description of the life cycle is based on studies done in Nepal as described by Tishechkin et al. (2011), with our added observations: The life cycle (from egg to adult) is completed in about 28 days. Females deposit, on average, more than 1200 eggs during their lifetime. The pale white, oblong eggs are deposited in loosely aggregated clusters on the undersides of young, expanding leaves. The ovipositing female appears to nip tissue in the expanding leaf, which causes the edges to curl abaxially into a cup-like shape. The ‘cupped’ leaf partially envelopes the egg clusters, perhaps modifying the microenvironment and providing protection. Females sometimes oviposit in more exposed locations on older leaves when young foliage is sparse or unavailable. The eggs become yellowish as the embryo develops with dark reddish eye spots apparent mid-way through the incubation period. Embryonic development requires about four days. Neonate larvae are yellowish but later instars turn greyish to reddish with black legs, head capsules and prothoracic shields. They exude a glutinous secretion, possibly containing compounds sequestered from the host plant. Faecal matter adheres to this coating, perhaps functioning as a deterrent to generalist predators (Schaffner & Müller, 2001). Larvae feed gregariously on the underside of the leaves, preferring young tender foliage although larger larvae also consume older, tougher leaves. Development of the four larval instars requires about eight days. Larvae and adults will also feed on young bulbils.

Larvae descend from the host plant when fully grown to enter the substratum. They produce a whitish exudate that hardens into a foam-like cocoon (Tishechkin et al., 2011) thought to be produced by the mesenteron (Venturi 1949 as cited in Schmitt, 1988). Adults emerge after about 12–16 days, begin mating about 10 days later, and initiate oviposition about five days after mating. The adults live five months or more and can survive several weeks without food (Center et al. unpublished data).

Both adults and larvae consume leaf tissue. Larvae often aggregate on the growing tips, which may be completely devoured (Center et al. unpublished data). This damage potentially inhibits vine elongation, altering plant architecture and possibly reducing its ability to grow vertically (Hough-Goldstein, Schiff, Lake, & Butterworth, 2008; Irwin & Aarssen, 1996).

The host plant drops its leaves and vines senesce during late autumn forcing adult beetles to survive several months without foliage, although they are able to feed on the persistent bulbils. They reportedly overwinter in Nepal on the ground beneath debris such as leaf litter or in the soil. The overwintered adults emerge during spring and lay about 90 eggs/day during an initial 13-day period of ovipositional activity.

The host range validation, field releases, population establishment and initial impact of the Chinese biotypes of *L. cheni* have not been reported for *D. bulbifera* populations from Florida. Therefore, the specific objectives of this study were (1) to validate taxonomic identity of the Chinese biotype of *L. cheni*, (2) conduct field

releases at various *D. bulbifera* infestations in Florida and (3) assess and document their initial establishment in release sites.

2. Methods and materials

2.1. Acquisition of beetles from China

Chinese and Australian collaborators searched for leaf beetles on *D. bulbifera* while working in southern China. Ten specimens found in Yunnan Province during August 2010 were submitted to the SEL for identification. Although very similar, these proved to be *Lilioceris egena* Weise, rather than *L. cheni*, the species of interest known from Nepal. Later collections during October 2010 produced 32 individuals that proved to be *L. cheni*. Unfortunately, none survived transit to the USA. More extensive collecting ensued in southern Yunnan Province, China, during May 2011 by US and Chinese scientists (Figure 1). We focused search efforts in the southern subtropical portion of Yunnan where plants and insects were most likely to have begun emerging based on our knowledge of the seasonal phenology of *D. bulbifera* and experience from previous surveys. Beetles were collected by hand; those on the younger portions of the plants often as mating pairs, those on the larger leaves usually as single individuals. They immediately dropped from the plants when disturbed, so small nets placed under the stems helped prevent escape. Containers were labelled with site identifications and cross-referenced to field notes including

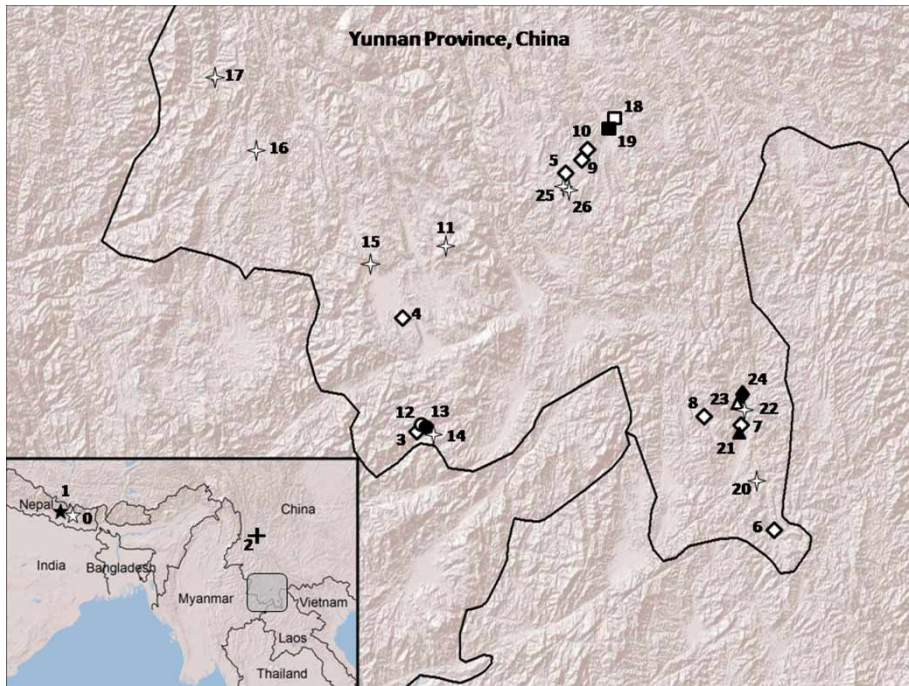


Figure 1. A map of southern Yunnan Province in China showing the locations where *L. cheni* was collected. The insect also shows source of original collections in Nepal. Symbols correspond with those in Figure 2.

collection data and global positioning system (GPS) coordinates. The live beetles were ultimately hand-carried to the USDA-ARS Invasive Plant Research Laboratory (IPRL) quarantine facility in Fort Lauderdale, Florida where they were sorted to species based on morphological characters. Breeding lines were established based on collection localities and preliminary species determinations. They were held in isolation to eliminate parasitoids and pathogens pending emergence of the F1 generation and confirmation of identifications. None of the parental stock was ever removed from the isolation area while alive. Beetles were sacrificed periodically for microscopic examination of tissue squashes to check for microsporidia and other internal parasites.

2.2. Confirmation of species identity

When the 2011 Chinese individuals were acquired, the *L. egena* arrived intermingled with *L. cheni* but they were identified by behavioural (bulbil feeding and oviposition behaviour) and morphological (see Tishechkin et al., 2011) criteria followed by molecular testing. Species identifications were verified in two ways. Voucher specimens were collected from each breeding line and forwarded to USDA-ARS SEL entomologists who confirmed the presence of two species, *L. cheni* and *L. egena*, in our colonies. Those identifications were based on morphological characters, primarily the structure of male genitalia. We also conducted genomic analyses.

2.2.1. Voucher specimens

Shifting the focus of the project from Nepal to China risked the possibility of encountering biotypes or cryptic species with different host preferences. We also could not discount the possibility of *L. egena* × *L. cheni* hybrids. All adult beetles used to develop breeding lines were therefore preserved as vouchers, a portion of which was sent to SEL for confirmation of identity. Tissue samples of each specimen sent to SEL were preserved for molecular analysis.

2.2.2. Molecular analyses

We compared genetic variation between Chinese populations (2011), Nepalese populations (2001, 2006–2007) and from two diverse survey shipments (2010). Genetic variation was examined using differences in the maternally inherited mitochondrial cytochrome *c* oxidase subunit I (COI) sequence. Potential hybridisation events between putative biotypes and/or species were examined using the biparentally inherited nuclear D2 expansion domain of the 28S rRNA sequence. We, therefore, elected to genetically identify the 2011 samples by creating breeding pairs and confirming identity in the F1 generation by both COI and D2 analysis before incorporating them into a general interbreeding colony. We removed individual pairs of beetles observed *in copulo* and isolated each pair in a plastic 9.5 oz Glad® ‘Small Snack’ container. A 4 cm × 8 cm rectangular hole in the lid was covered with 500-µm mesh screen to provide ventilation. Each container housing a mated pair was provisioned with a newly expanded leaf of *D. bulbifera*, the petiole of which had been inserted into an Aquatic® Aquatube #54. Each resultant lineage was maintained separately.

DNA was extracted using the Charge Switch gDNA kit (Invitrogen). PreCR Repair Mix (NEB) was used to help repair nicks in samples, especially those not preserved properly. PCR amplifications for COI were conducted using the primers LCO1490 and HCO2198 (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994), and an annealing temperature of 49°C. COI reactions utilised Terra™ (ClonTech) PCR Direct Polymerase Mix (2.5 mM Mg, 400 μM dnTPs, 0.28 μM primers, 0.025 U/μL polymerase). Amplifications for D2 were conducted using the D2F and D2R primers (De Barro, Driver, Naumann, Clarke, & Curran, 2000) and (Bulldog Bio) BioReady™ rTaq DNA Polymerase (10 × buffer, 2.0 mM Mg, 0.5 mM Betaine, 0.001% BSA, 270 μM dNTPs, 0.45 μM each primer and 0.06 U/μl). PCR products were purified from gel slices using the Zymoclean Gel DNA Recovery kit which also eliminated the occasional extraneous band. PCR concentrations were measured, adjusted, a sequencing primer added, and samples sent to Eurofins MWG Operon Technologies for sequencing. The sequencing reaction used BigDye® Terminator v3.1 (ABI) chemistry and a 3730 ×1 DNA Analyzer (ABI) along with the same primers used in the PCR reactions.

Sequences were initially aligned and reviewed using Sequencher (Gene Codes) then the COI sequences were exported as Fasta files. Analysis of D2 sequences was conducted within Sequencher. The COI Fasta files were imported into MEGA 5 (Tamura et al., 2011) which was used to conduct further analysis. The COI sequences were aligned using the MUSCLE routine producing an unambiguous alignment. Sequence Data Explorer (Select Genetic Code) was set to ‘Invertebrate Mitochondrial’ and each sequence examined for stop codons, which were absent. Maximum Likelihood best fits were run for 24 different nucleotide substitution models yielding the lowest Bayesian Information Criterion (BIC = 3970) for the Tamura 3-parameter model plus gamma [T92 + G] where gamma = 0.07 (Tamura, 1992). This model was developed to estimate nucleotide substitutions where there are strong transition–transversion and G + C-content biases such as for *Drosophila* mitochondrial DNA. Discrete gamma distribution categories were tested, from 2 to 6, by generating Maximum Likelihood Trees using T92 + G, complete deletion and Close-Neighbor-Interchange. When set at five categories the resulting gamma also equalled 0.07 so this setting was used for the final tree. Next, 1000 bootstraps were conducted using the same criteria.

2.3. Host range validation

Although molecular analyses and morphological determinations verified species identifications, the possibility of biotypes remained. We therefore conducted abridged host range trials with Chinese *L. cheni*. The trials focused only on critical test species, *Dioscorea* congeners and phylogenetic relatives, to substantiate host specificity: *D. bulbifera*, *D. floridana* Barlett, *D. villosa* L., *D. alata* L., *D. rotundata* Poir., *D. pilosiuscula* Bertero ex Spreng, *D. batatas* Decne [= *D. oppositifolia* L.], *D. sansibarensis* Pax, *D. altissima* Lam., and *Rajania cordata* L.). Inclusion of more distantly related plant species was deemed unnecessary inasmuch as all previous studies clearly indicated that the beetle was restricted to the genus *Dioscorea*. Experiments were similar to the *L. cheni* ‘No choice larval development tests – egg transferral’ described by Pemberton and Witkus (2010), but with smaller cages constructed from acrylic tubes 10 cm diameter × 15 cm length. Two 4-cm-diameter

holes cut into either side of each tube were covered with 500- μm mesh screen. Each tube was placed over a single vine of each plant. A sponge bung was forced into the bottom of the tube encircling the vine and the tube was secured to a 60-cm bamboo stake. Eggs removed from *D. bulbifera* were transferred onto the underside of recently expanded leaves of each test plant and a *D. bulbifera* control. A second foam bung encircling the vine pushed into the top of the tube prevented escape of the larvae.

Enclosed larvae were allowed to develop, with amount of feeding and survival with full development to pupation as test criteria. The number of plants used in each test was limited by availability, so some had to be reused (e.g., *D. pilosiuscula*) but always with different insects in order to achieve replication. Availability of beetles also precluded simultaneous testing of all species, so we replicated experiments from 9 August 2012 to 11 October 2012, with varying combinations of test plant species in each series. A *D. bulbifera* control was included each time we conducted a new trial. Numbers of eggs used ranged from 8 to 12 per test species based on the variable size of the egg masses, with even numbers distributed among the plants in the test series. Tests terminated when active larvae were no longer present, having either died or pupated. Area of feeding was measured and larval development time to pupation was documented.

2.4. Release and establishment

2.4.1. Overwintering study

Two 1.8 \times 1.8 \times 1.8 m Lumite[®] mesh cages (7.87 \times 7.87 mesh/cm, 965- μm opening; BioQuip[®] Outdoor Cage #1406B) were placed over hummocks of *D. bulbifera* at Long Key Natural Area, located in Davie, Broward Co., FL (26° 4.407' N, 80° 19.462' W). A full-length vertical zipper in the middle of a side panel provided access to the enclosed area. The bottom was open and the bottom edges of the side panels were buried to inhibit escape of the beetles. The soil had a natural covering of leaf litter. Bulbils were added to the cages as a supplemental food source. Seventy-five *L. cheni* adults were released directly onto the foliage in each cage on 4 November 2011. The cages were checked weekly at first then at irregular intervals through the winter. Two temperature–humidity data loggers (NexSens[®] micro-T model DS1923) were placed in each cage on 4 January 2012, one suspended above ground in the foliage and the other on the soil surface beneath the leaf litter. Temperature and humidity were monitored at 30 min intervals.

Cages were removed on 1 March 2012. The screening was carefully inspected for any surviving beetles. All plant material and leaf litter were removed and spread out on a large white sheet to facilitate searches for living or dead beetles. Live and dead beetles were tallied and categorised according to their locations within the cages.

2.4.2. Development of field colonies

Laboratory colonies established from China-collected *L. cheni* were produced using methods similar to those described by Pemberton and Witkus (2010). A total of 363 beetles, all progeny of the original stock, were removed from quarantine during 2 November 2011 to 7 March 2012. We examined approximately 5% for pathogens. These were then used to develop laboratory colonies to support future field releases.

The first beetles released were from the overwintering study described above. The 16 beetles recovered at the conclusion of this study were released at the Long Key site on 1 March 2012. Additional releases were then made at local parks during summer 2012 (Table 2).

It became apparent that the beetles were reproducing at some sites, so we began to monitor abundance to assess population trajectories. The tenuous nature of these nascent populations precluded destructive sampling so we implemented timed counts. One or more observer surveyed each site while counting visible adults, larvae and egg clusters until a total minimum search time of 45 min was attained. Initial monitoring employed 1 m² quadrats, but this soon proved untenable as the beetles moved away from heavily defoliated areas in search of suitable foliage. Thereafter, the entire local area with evident damage was surveyed.

3. Results

3.1. Acquisition of beetles from China

The May 2011 collecting effort resulted in the acquisition of 307 adult beetles. Small differences in altitude and latitude markedly affected the seasonal progression of the plant and insect populations with more advanced growth stages of plants in the south. Conversely, at higher elevations or in more northerly latitudes developing shoots had produced few leaves. *Lilioceris* beetles were usually found at or near the apex of the shoots, although they were also common on large leaves of more advanced growth. Sixteen of the sites with *D. bulbifera*, harboured *Lilioceris* beetles. All *Lilioceris* spp. found were placed in containers provisioned with fresh *D. bulbifera* leaves. Morphological examination revealed mostly *L. cheni* (83%) along with a few *L. egena*.

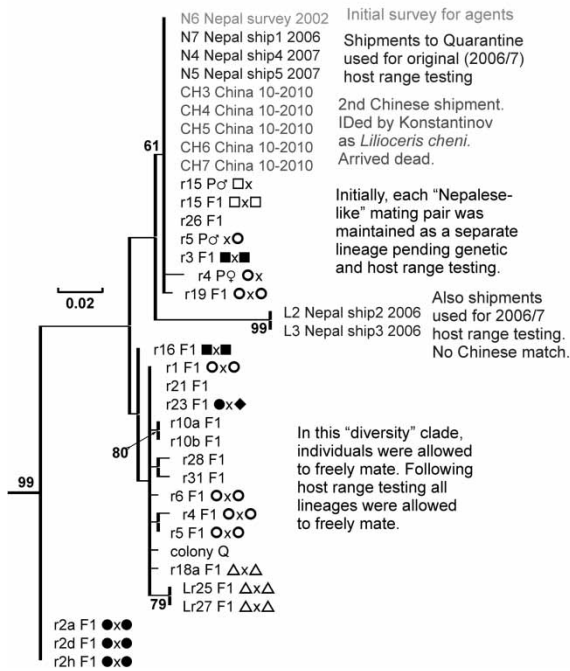
In contrast to the Nepalese beetles which harboured pupal parasitoids (Pemberton & Witku, 2010), no parasitoids were ever encountered. This was possibly due to the fact that only adults were imported. No evidence of pathogens from tissue samples was ever detected nor were debilitated or sick individuals ever observed.

3.2. Confirmation of species identity

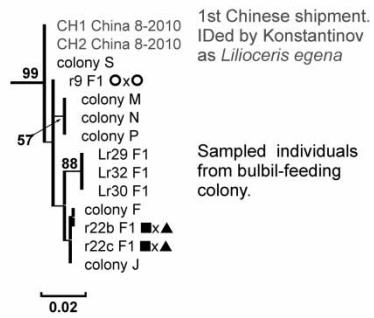
Species determinations by SEL corroborated our identifications. The initial COI analysis (see Figure 2), reflected that individuals (CH1, CH2) collected during September 2010, identified as *L. egena* by SEL were significantly different from those collected during the October 2010 survey (CH3–7), identified as *L. cheni*. These identified individuals were then used to anchor the molecular species identifications. Most of the Chinese *L. cheni* (CH3–7) were identical (COI) to some of the Nepalese insects imported for the original host trials (N4, 5, 7) as well as a specimen (N6) from the initial 2002 Nepalese survey. However, several of the 2006 *L. cheni* shipments (L2–3) from Nepal proved genetically distinct from any Chinese *L. cheni* samples.

Figure 2 displays the results of the COI analysis for mating pairs brought into quarantine. These are identified by reproductive pair numbers (#) and as a parental male (P♂), parental female (P♀) or filial generation 1 (F₁). Some samples have the parental site of origin identified by symbols such as ● × ◆ (♂ × ♀). Five mating pairs

A. *Lilioceris cheni* clade



B. *Lilioceris egena* clade



C. Between clade distance

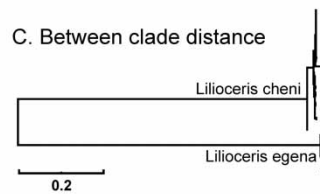


Figure 2. Maximum Likelihood Analysis using the Tamura 3-parameter plus gamma model of nucleotide substitution on mitochondrial cytochrome *c* oxidase subunit I sequences. Bootstrap percentages >50% appear in nodes (1000 ×). (A) Tree displays clade corresponding to *L. cheni*. (B) Tree displays clade corresponding to *L. egena*. (C) Complete tree displaying distance between *L. cheni* and *L. egena* clades.

Key - N# indicates Nepalese samples from original host range studies (Pemberton and Witkus 2010); CH# indicates Chinese samples shipped during surveys prior to second Host Range Testing; r#a: r# = reproductive pair number, a or b, c = egg clutch where multiple samples from same reproductive pair were sequenced; P♂ = parental male, P♀ = parental female, F₁ = filial generation 1; geographic origin: ♀ × ♂ = parental female (symbol) × parental male (symbol); (symbol): Bulangshan-1: ●; Bulangshan-2: 1; Puwan-1: □; Puwan-2: ■; Mengla: ▲; Nazhuo: ◆; Skytree-2: ★.

were identified from the ‘Nepalese-like’ clade (Figure 2A), four from F₁ individuals and a fifth from a P₁ female. A second *L. cheni* clade, termed the ‘diversity’ clade, included F₁ individuals from 14 pairs and one colony. In order to increase reproduction, all mating pairs from the ‘diversity’ clade were combined into a clear-plastic acrylic cage (41 × 41 × 41 cm) with cotton access sleeves on either side. The ‘Nepalese-like’ mating pairs were held separately until host range testing indicated that this was unnecessary. Subsequently, all individuals and their offspring that were determined to be *L. cheni* were then combined into clear acrylic cages described above.

Figure 2(B) displays a clade that includes samples (CH1, CH2) from China collected during September 2010 identified as *L. egena* by Dr. Konstantinov. Five mating pairs were identified in the *L. egena* clade by analysis of F₁ individuals.

Breeding lines were created from copulating pairs and their offspring were molecularly identified at the larval stage. After identification, all *L. egena* were pooled into a single colony.

Figure 2(C) displays an overview of the generated Maximum Likelihood tree. Note the compressed appearance of the *L. egena* and *L. cheni* clades in relation to the 'between clade' distance. The T92 + G distance between the clades exceeds 1.4 while the maximum within clade (between individuals) distance (r2a to L2, L3), found in the *L. cheni* clade, is about 0.1. If L2 and L3 are excluded (since they are not in the quarantine collection) the maximum T92 + G distance between individuals within a clade (either clade) is even less (<0.07). The large 'between clade' distance clearly represent different species.

Despite the clear speciation, we were concerned that hybridisation might occur. For this reason, the bi-parentally inherited nuclear D2 expansion domain was also analysed. All the samples were analysed within the *L. cheni* clade with no within species variation found. Similarly, all *L. egena* samples were analysed with no within species variation. However, when the species were compared over their 600 base pair sequence length there were 15 nucleotide differences (found in the alignment at bases 219, 224, 234, 271, 321, 349, 352, 373, 413, 433, 450, 464, 485, 486, 499). When the chromatograms of samples were examined at these 15 sites, there was no evidence of double peaks or other ambiguities indicating a lack of hybridisation.

3.3. Host range validation

Results of the host range determinations for the Chinese *L. cheni* were unambiguous as shown in Table 1 and agreed with the findings of Pemberton and Witkus (2010). Presentation of detailed analyses seemed unnecessary considering the unequivocal nature of the results, so replicates were pooled and means and standard errors for each test species are presented. All eggs hatched on every test plant. None of the larvae survived more than 3 days on any plant other than *D. bulbifera*. An average of 80% of the larvae pupated after feeding on *D. bulbifera*, whereas all died as first instars on the other test species (Table 1). The amount of leaf area consumed per larva on *D. bulbifera* exceeded the maximum on the test species.

3.4. Release and preliminary establishment

3.4.1. Overwintering study

Temperature within the foliage ranged from 3.5°C on 3–4 January to 35°C on 24 February and relative humidity ranged from 23% on 3 January to 46% on 29 February 2012. The minimum temperature recorded in the leaf litter was 9.0°C on 4 January and the maximum was 33.5°C on 1 March. Humidity ranged from 24% (3 January) to 50% (1 March).

D. bulbifera vines within and outside of the cages had begun to decline when the cages were examined on 14 November 2011 as evidenced by chlorosis of many leaves. Adult beetles were readily visible within the cages and had caused extensive feeding damage to the leaves, but no eggs or larvae were found. Three early instar larvae were present in one cage on 22 November 2011 but no later evidence of their presence, either as living individuals or by way of increased amounts of feeding, was found. By

Table 1. Results of host range trials with Chinese *Lilioceris cheni*.

Test species	Number of tests	Total larvae	Mean leaf tissue consumed/larva (mm ²) ($\bar{X} \pm SE$)	Maximum longevity (days)	Percentage pupating ($\bar{X} \pm SE$)
<i>D. alata</i>	3	30	0.12 ± 0.08	2	0 (0)
<i>D. altissima</i>	3	31	0.06 ± 0.03	1	0 (0)
<i>D. batatas</i>	3	31	0.00 ± 0.00	3	0 (0)
<i>D. bulbifera</i>	7	71	176.45 ± 35.20	>11	79.56 ± 2.94
<i>D. floridana</i>	5	47	0.08 ± .08	1	0 (0)
<i>D. pilosiuscula</i>	3	29	0.00 ± 0.00	1	0 (0)
<i>D. rotundata</i>	3	29	0.03 ± 0.03	2	0 (0)
<i>D. sansibarensis</i>	3	31	0.02 ± 0.01	1	0 (0)
<i>D. villosa</i>	4	40	0.00 ± 0.00	1	0 (0)
<i>R. cordata</i>	3	29	0.01 ± 0.01	1	0 (0)

late January all of the *D. bulbifera* vines had senesced, but a few live adults remained on the cage side panels. Several dead adults were also observed along the seams between the sides and tops of the cages. Fifty-two beetles were recovered when the cages were removed in March; only 16 (10.7% of those released) of which were alive. Thirteen of the live beetles were on the cage walls, three were on the dead foliage, and none were in the leaf litter. No evidence of a second generation was observed, so the surviving adults were presumed to be from the original release. Inspection of the bulbils from within the cage revealed no obvious damage or evidence of feeding aside from a few nicks.

3.4.2. Development of field colonies

Despite low overwintering survival at Long Key Natural Area (Table 2), larval feeding was abundant and conspicuous by 26 June 2012. A thriving population had established despite the small initial release. Numbers increased and were supplemented once, probably unnecessarily, during the summer (Table 2). By early August defoliation was extensive. Nearly all of the young terminal foliage was destroyed and virtually all older leaves were heavily damaged by September. Although signs of damage were apparent 10–15 m west of the release area, it was relatively slight and the population remained concentrated within the release area. Participants released 200 adults, half of which were collected in the original area, 50 m west of the original site during a public relations event on 21 September 2012. By mid-November, beetles occurred throughout the area within a 200-m radius. The population continued to grow and expand until late October (Figure 3) then became less apparent as the foliage began to senesce.

Six sequential weekly releases of 50 adults each were made at Snyder Park beginning 5 June 2012 (Table 2). Eggs and larvae were found within a 6 m radius of the release point a week after the first release of adults. Damage continued to increase as more adults were released over the following weeks. Numbers of beetles abruptly increased during mid-July suggesting emergence of a second generation. By mid-August damage was evident throughout the release area even several metres up on vines in the surrounding trees. Nearly all of the young foliage was destroyed and

Table 2. Open (uncaged) releases of *L. cheni* at sites in Florida made during 2011 and 2012.

Site	GPS coordinates	Release period	Number of releases	Adults	Larvae	Outcome
Long Key Natural Area, Broward Co., FL, USA	26.0734, -80.3244	1 March–21 September 2012	3	266	0	Established
Snyder Park, Fort Lauderdale, Broward Co., FL, USA	26.08485, -80.1496	5 June–12 July 2012	6	300	0	Established
Pine Island Ridge Natural Area, Broward Co., FL, USA	26.0858, -80.1480	6 July 2010– 18 September 2012	6	135	981	Tentatively established
	26.0827, -80.2787	28 June–13 July 2012	2	100	0	Tentatively established
	26.0786, -80.2795	3 July 2012	1	50	0	Established
Fern Forest Nature Center, Broward Co., FL, USA	26.0782, -80.2759	16 October– 23 November 2012	2	30	2625	Tentatively established
	26.2184, -80.1922	3 July 2012	1	50	0	Site destroyed
	26.2298, -80.1929	13–27 July 2012	2	125	0	Not established
Emerald Hills, Hollywood, FL, USA	26.0412, -80.1823	3 October 2012	1	0	500	Unknown
	26.0510, -80.2018	5 October 2012	1	0	750	Unknown
Kendall Indian Hammocks Park, Miami Dade Co., FL, USA	25.6965, -80.3777	14 November 2011– 7 September 2012	6	135	981	Established
Chapman Field, Miami Dade County, FL, USA	25.6447, -80.2899	28 September 2012	1	20	200	Unknown
	25.6809, -80.2761	28 September 2012	1	20	200	Unknown
Silver Palm Grove, Miami Dade County, FL, USA	25.5532, -80.4192	28 September 2012	1	20	200	Unknown

Table 2 (Continued)

Site	GPS coordinates	Release period	Number of releases	Adults	Larvae	Outcome
Ross Hammock, Miami Dade County, FL, USA	25.5584 -80.4401	28 September 2012	1	10	100	Unknown
Ross Edison Hammock, Miami Dade County, FL, USA	25.5601 -80.4451	28 September 2012	1	10	100	Unknown
Florida City (Navy Wells #23), Miami Dade County, FL, USA	25.4345 -80.4853	28 September 2012	1	20	200	Unknown
Enchanted Forest Park, City of North Miami, Miami Dade County, FL, USA	25.9021 -80.1641	3 October 2012	1	10	1500	Unknown
Labelle City Preserve, Hendry County, FL, USA	26.7540 -81.4436	9 October–8 November 2012	2	100	50	Unknown
Fred Cone Park, Alachua Co., FL, USA	29.6478, -82.2858	4 August–4 October 2012	7	1395	630	Established
Clay Co., FL, USA	30.1800, -81.7942	27 July 2012	1	15	5	Unknown
Terra Ceia State Preserve, Manatee County, FL, USA	27.5912 -82.5494	24 October–13 November 2012	2	609	450	Unknown
Ocala National Forest, Lake County, FL, USA	29.0343 -81.6260	27 September 2012	1	150	0	Unknown
	29.0359 -81.6170	27 September 2012	1	250	0	Unknown
Highland Hammock State Park, Highland County, FL, USA	27.4771 -81.5310	22 October 2012	1	200	0	Unknown
Fort Pierce, St. Lucie County, FL, USA	27.3947 -80.3277	2 October–5 November 2012	2	200	0	Unknown

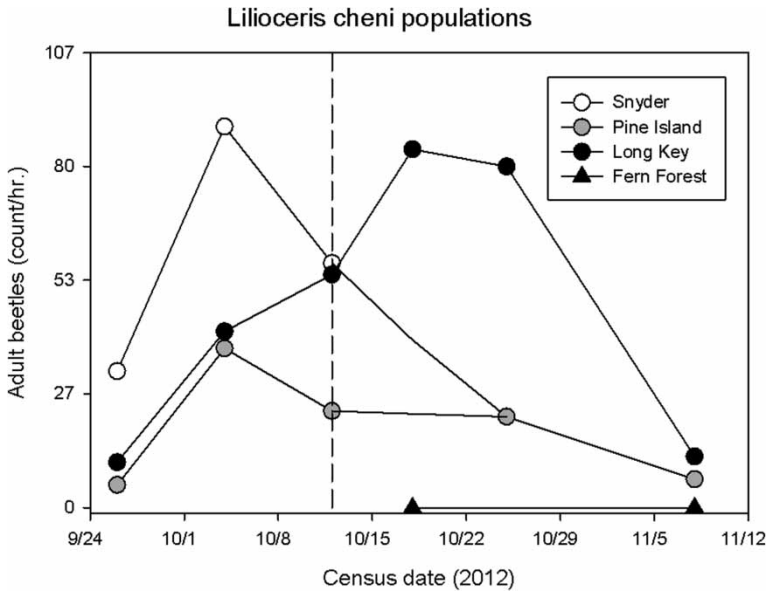


Figure 3. Fall 2012 adult *L. cheni* field populations based on timed counts at release sites. The 26 September count at Long Key does not include data from a release conducted 21 September; later counts include this new location. The dashed line represents the latest date on which eggs were observed. Low counts late in the year were due to the senescence of the vines.

numerous shoot tips consumed. Census numbers declined in early October due to the nearly complete defoliation at the initial release site (Figure 3) as the population spread into adjacent areas. A second site was established in this park in early July where both adults and larvae were released (Table 2). Plants at this location seemed to be of inferior quality, being etiolated with a wrinkled surface and a tougher texture. Here, the beetles were slow to establish but seemed to have done so by late November. Adults were found over 7 m away from the release points on 7 December 2012 when timed counts produced a mere 14 adult beetles/hour.

Individual releases were made at three sites within the live oak hammock comprising Pine Island Ridge Natural Area, although two interior releases made in close proximity are considered a single site in Table 2; one was in deep shade, the other in an open, sunny area in the same infestation. The third release was at the edge of the hardwood hammock adjacent to a road. Beetles were abundant and causing a great deal of visible damage to vines at the third site, but were not detectable in either the sunny or shaded portions of the interior site by end of summer. Two additional releases were made in the northern portion of the hammock during autumn. Conclusions regarding establishment of this and other late season releases listed in Table 2 were confounded by the seasonal senescence of the foliage and will have to await spring assessments.

Beetles were released three times at two sites during July at Fern Forest Nature Center (Table 2). The first release of 50 adults was in an area slated for removal of exotics, so we abandoned the site. Twelve beetles were recovered and moved to the second site along with additional adults to increase the total to 125. We never detected any evidence that a population had established as a result of these releases.

Kendall Indian Hammocks was another successful site. Beetles established quickly in the release areas and spread throughout the hammock by the end of September. Interestingly, nearly identical numbers were released on the same dates as at the second site in Snyder Park, which eventually succeeded, but where establishment seemed tenuous.

Additional late season releases were made in north Florida. One site in Alachua Co. (Fred Cone Park) seemed well established by end of the summer. A thriving population with thousands of beetles was present in late November so this may represent the most successful site of the first year. Although eggs were not observed after mid-October, thousands of larvae were in evidence as late as early November. Unfortunately counts were not done until 18 December 2012 after two or three periods of below-freezing weather. Nonetheless, a timed count produced 32 adult beetles/hour. The populations dispersed up to 26 m from release points and were observed vertically at least 18 m on the vines. The fates of other releases, most of which were made during autumn, are as yet undetermined and will be evaluated during 2013.

4. Discussion

Non-native lianas are often obdurate invaders, which form dense infestations overtopping or intermingled with valued native species. They can kill or damage other vegetation, alter fire behaviour and carry fire into tree canopies (FLEPPC, 2001), alter the pathway of succession (Paul & Yavitt, 2011), and change ecosystem processes (Gordon, 1998). Vines often dominate through heightened shade tolerance and growth rates (Webster, Jenkins, & Jose, 2006) assisted by an architecture that emphasises foliage over support structure. These traits enable efficient capture of light (Dillenburg, Whigham, Teramura, & Forseth, 1993) and allow for maximal allocation to growth (Lake & Leishman, 2004). This habit facilitates an opportunistic response to conditions that cause the loss of canopy cover, such as wind storms. Vines become particularly abundant at forest edges, in canopy gaps, and in early to mid-successional habitats (Dillenburg et al., 1993). Their ability to quickly exploit canopy gaps confers a competitive advantage that may pre-empt or slow recovery of disturbed habitats (Dillenburg et al., 1993; Horvitz & Koop, 2001).

The twining and climbing growth habit of many non-native vines both exacerbates the damage they cause and makes them difficult to manage (Miller, 2003). Most native vines climb by adhering to the bark of trees or other vertical structures; many non-native vines climb by twining. In doing so, they link tree canopies and the increased weight of vegetation collapses supporting plants (Gordon, 1998; Hardt, 1986). Herbicides may not translocate effectively through long vines or into belowground storage organs (Hutchinson, Langeland, MacDonald, & Querns, 2010; Miller, 2003,) and herbicide application often causes collateral damage to the native plant community (Horvitz & Koop, 2001; Miller 2003; Wheeler, Pemberton, & Raz, 2007). Thus, biological control, by its selectivity, provides a means of reducing the impact of invasive vines without further damaging the native plant community.

D. bulbifera, as a twining vine capable of overtopping and collapsing supporting vegetation, is a case in point. It translocates resources from foliage to storage organs as the vines senesce during the autumn dieback then grows rapidly in the spring by drawing on these carbohydrate reserves. Duxbury, Glasscock, and Staniszczyńska

(2003) noted that by mid-June, after 81 days of growth, vines attained lengths of 200 cm, an average growth rate of 2.5 cm/day. Repeated defoliation by phytophagous insects could conceivably interrupt this process by reducing photosynthate available for storage (Frye, Hough-Goldstein, & Kidd, 2012). Blossey and Schat (1997), for example, found that *Lythrum salicaria* L. (purple loosestrife) compensated for defoliation by the chrysomelid *Galerucella californiensis* L. by replacing foliage but at the expense of below-ground storage. Active feeding on the growing tips might also reduce apical dominance and thereby inhibit the twining ability of the vines. Alternatively, a bulbil-feeding herbivore could reduce the density of overwintering propagules and limit the ability of the plant to recolonize in the spring. The combination of the two types of agents (a defoliator and a bulbil-feeder) should therefore provide a very effective biological control system. The two *Lilioceris* species hold much promise for control of *D. bulbifera* in this regard, if both are released and establish persistent populations. The defoliating species, *L. cheni*, has been released, seems to have established, has caused extensive defoliation at release sites, and is dispersing. The bulbil-feeder, *L. egena*, is currently undergoing host range evaluation in quarantine.

The laboratory colony of *L. cheni* studied by Pemberton and Witkus (2010) originated from Nepal whereas beetles released were from southern China, a separation of at least 1300 km. Liberation of a population of organisms from such disparate regions raises the specter of 'host races' (Diehl & Bush, 1984; Drès & Mallet, 2002), in that regional 'biotypes' of plant-feeding insects might exhibit different patterns of host usage. Indeed, Volchansky, Hoffmann, and Zimmermann (1999) found that the cochineal insect *Dactylopius opuntiae* (Cockerell) introduced to Australia to control *Opuntia stricta* Haworth fared poorly on *O. ficus-indica* (L.) Miller; conversely the one introduced into South Africa to control *O. ficus-indica* fared poorly on *O. stricta*. Because of similar experiences, Zwölfer and Harris (1971) insisted that the existence of 'host races' should be investigated when selecting biological control agents. Our comparisons of both the host ranges and haplotypes of beetles from China and Nepal revealed no evidence of host races, nor any indication of hybridisation. However, we have noted variation in morphology and behaviour. The elytra of the original Nepalese beetles were predominantly brown to orange whereas those from China are consistently red. Also, Nepalese beetles seemed more likely to damage both leaves and bulbils whereas Chinese beetles seem to feed entirely on leaves, although adults will nibble on bulbils as they begin to form in leaf axils and when foliage is sparse. Nonetheless, host ranges of beetles from the two regions were identical. It might still be desirable to release the Nepalese 'type' if it can be shown that they are indeed more prone towards feeding on the bulbils.

As noted, no nuclear D2 within species variation was found for either *L. cheni* or *L. egena* samples while there were 15 nucleotide differences between species. Sonnenberg, Nolte, and Diethard (2007) found while D2 and other ribosomal genes occur in multiple copies in the genomes, there is generally little intra-individual polymorphism (<<0.1% on average) indicating that concerted evolution is very effective in most cases. This homogenisation process within evolutionarily interbreeding individuals seems to have resulted in no within species variation here as well, even though individuals show significant variation in the mitochondrial COI sequences. Sota and Volger (2001) examined the carabid subgenus *Ohomopterus* (genus *Carabus*) using two mitochondrial (mt) DNA regions and three nuclear DNA

regions. They found that mitochondrial haplotypes isolated from individuals of a single 'species' were frequently separated into distant clades. Here the mitochondrial D2 formed distinct but not distant clades with substantial genetic variation. Their three nuclear markers generally conformed better with the morphologically defined species. Here each species had a distinct single sequence. Sota and Volger (2001) hypothesised that incongruence between mitochondrial and nuclear gene trees in *Ohomopterus* may have been promoted by the complex processes of geographic isolation and hybridisation in the Japanese archipelago where there was occasional gene flow and recombination between separated entities. Similar patterns of separation and recombination, due to geographic isolation in mountain valleys, especially during times of glaciations, may have contributed to greater mitochondrial within species diversity in *L. cheni*, while concerted evolution has acted to homogenise nuclear D2 within species diversity after gene flow and recombination re-established.

The overwintering ability of *L. cheni* was of concern in that it requires the beetles to survive a prolonged period without foliage. Bulbils might provide an overwintering food source, but we saw no signs of this in the cages. However, extensive feeding by adult beetles on bulbils did occur in late autumn at the northern most release site in Gainesville, Florida, after frosts had reduced the availability of foliage. The experience in Nepal suggested that *L. cheni* adults overwinter on the ground under leaf litter. We were unable to confirm this as well. The surviving beetles remained on the sides of the cage throughout the winter in our study, but extenuating factors may have been involved. The vegetation at the site was pre-senescent when the beetles were released whereas they had previously been fed better quality foliage in laboratory culture. Thus, they may not have received the proper plant quality or photoperiod cues at appropriate points in their life cycle. Chapman (1998, and references therein), for example, noted that the stage sensitive to cues for diapause induction occurs prior to the stage that enters diapause. Hunter and McNeil (1997) demonstrated the role that plant quality plays in diapause induction wherein populations of a tortricid moth, *Choristoneura rosaceana* (Harris), entered diapause earlier when feeding on lower quality hosts. The ages of the beetles, which were unknown, could also have played a role. The placement of the cages could have been a factor as well, with inappropriate levels of shading or moisture possibly inhibiting their use of the substrate. Also, the winter was mild so colder temperatures might have initiated a different overwintering behaviour. Furthermore, the cages could have inhibited a dispersive phase or flight behaviour that might have been a prerequisite to overwintering dormancy (Johnson, 1969). Further study of the comparative phenologies of the beetles and the plant are needed to determine overwintering requirements. Nonetheless, our study demonstrated the ability of a portion of the beetle population to survive several months in the field without food during a mild, south Florida winter.

Releases made earlier in the year succeeded quickly, whereas those made in mid-summer or later in south Florida either failed to establish or established with difficulty. This may relate to some aspect of plant quality that changes when the plants are actively growing and become more reliant on acquired nutrients rather than stored reserves. Furthermore, our ability to assess establishment became more difficult due to the increased vertical structure of the infestations, which extended into the tree canopies. The increased amount of late season plant biomass also

diluted the intensity of the nascent *L. cheni* populations, which had less time (i.e., fewer generations) to increase to noticeable numbers.

The timed counts (Figure 3) were of limited usefulness in that beetles did not persist in defoliated areas. As a result, the trajectory at Snyder Park seemed to trend downwards when populations were obviously increasing, with beetles simply spreading out over larger areas and becoming more difficult to find. However, Long Key clearly showed an increasing trend attaining a maximum discovery rate of 84 adult beetles/hour. The Pine Island population seemed stable but difficult to census due to the vertical orientation of the vines.

Eggs and larvae were consistently found until mid-October, but not thereafter. This could indicate that *L. cheni* undergoes a reproductive dormancy. A variety of factors including temperature, photoperiod, host plant quantity or quality or a combination thereof can cue diapause in insects (Chapman, 1998). Day length in mid-October in south Florida was 2 hours 13 min less than peak annual day length (United States Naval Observatory, 2012). This change in photoperiod could have directly or indirectly, through plant quality cues, affected *L. cheni* reproduction. A critical day length of 12 hours 28 min in the laboratory induced diapause in *Gratiana boliviana* Spaeth (Coleoptera: Chrysomelidae), a biological control agent for tropical soda apple, *Solanum viarum* Dunal, in Florida, although this was mediated by temperature (Diaz, Overholt, Hahn, & Samayoa, 2011). Declining photoperiod also cues reproductive diapause in the chrysomelids *Leptinotarsa decemlineata* (Say), the Colorado potato beetle (de Kort, 1990), and *Diorhabda carinulata* (Desbrochers), the tamarisk leaf beetle (Dalin et al., 2010). *D. bulbifera* forms bulbils in the fall and begins to manifest symptoms of vine senescence affecting the quality of leaves as food for herbivores (Center et al., personal observation). Such changes in plant quality could contribute to changes in the reproductive status of *L. cheni*, as has been observed in the Colorado potato beetle (Voss, Ferro, & Logan, 1988). Diapause may also enable an insect to avoid increased mortality rates resulting from exposure to seasonal increases in phytochemicals (Hare, 1983).

Although these conclusions are preliminary, *L. cheni* has demonstrated an ability to overwinter in the field, develop large populations very quickly, and impact infestations of *D. bulbifera*. If significant numbers of adults survive the winter and initiate feeding on new shoots as the bulbils sprout in spring, the results could be dramatic during the ensuing years.

We have seen few signs of predation and no evidence of parasitoids, although closer examination is needed. A eulophid parasitoid (*Tetrastichus setifer* Thomson) was released in the northeastern USA to control a congeneric species, *L. lili* Scopoli, which is a pest of native and ornamental lilies (Bouchard, McNeil, & Brodeur, 2008; Tewksbury, Gold, Casagrande, & Kenis, 2005). *L. cheni* could acquire this parasitoid, and this possibility needs to be examined.

References

- Bell, C. R., & Taylor, B. J. (1982). *Florida wild flowers and roadside plants*. Chapel Hill, NC: Laurel Hill Press.
- Blossey, B., & Schat, M. (1997). Performance of *Galerucella californiensis* (Coleoptera: Chrysomelidae) on different North American populations of purple loosestrife. *Environmental Entomology*, 26, 439–445.

- Bouchard, A.- M., McNeil, J. N., & Brodeur, J. (2008). Invasion of American native lily populations by an alien beetle. *Biological Invasions*, *10*, 1365–1372.
- Chapman, R. F. (1998). *The insects structure and function* (4th ed.). Cambridge, UK: Cambridge University Press.
- Croxton, M. D., Andreu, M. A., Williams, D. A., Overholt, W. A., & Smith, J. A. (2011). Geographic origins and genetic diversity of air-potato (*Dioscorea bulbifera*) in Florida. *Invasive Plant Science and Management*, *4*, 22–30.
- Dalin, P., Bean, D. W., Dudley, T. L., Carney, V. A., Eberts, D., Gardner, K. T., ... Thompson, D. C. (2010). Seasonal adaptations to day length in ecotypes of *Diorhabda* spp. (Coleoptera: Chrysomelidae) inform selection of agents against saltcedars (*Tamarix* spp.). *Environmental Entomology*, *39*, 1666–1675. Retrieved from <http://www.bioone.org/doi/full/10.1603/EN09270>
- De Barro, P. J., Driver, F., Naumann, I. D., Schmidt, S., Clarke, G. M., & Curran, J. (2000). Descriptions of three species of *Eretmocerus Haldeman* (Hymenoptera: Aphelinidae) parasitising *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) in Australia based on morphological and molecular data. *Australian Journal of Entomology*, *39*, 259–269. doi:10.1046/j.1440-6055.2000.00194.x
- de Kort, C. (1990). Thirty-five years of diapause research with the Colorado potato beetle. *Entomologia Experimentalis et Applicata*, *56*, 1–13. doi:10.1111/j.1570-7458.1990.tb01376.x
- Diaz, R., Overholt, W. A., Hahn, D., & Samayoa, A. C. (2011). Diapause induction in *Gratiana boliviana* (Coleoptera: Chrysomelidae), a biological control agent of tropical soda apple in Florida. *Annals of the Entomological Society of America*, *104*, 1319–1326. Retrieved from <http://dx.doi.org/10.1603/AN11053>
- Diehl, S. R., & Bush, G. L. (1984). An evolutionary and applied perspective of insect biotypes. *Annual Review of Entomology*, *29*, 471–504.
- Dillenburg, L. R., Whigham, D. F., Teramura, A. H., & Forseth, I. N. (1993). Effects of below- and aboveground competition from vines *Lonicera japonica* and *Parthenocissus quinquefolia* on the growth of the tree *Liquidambar styraciflua*. *Oecologia*, *93*, 48–54. doi:10.1007/BF00321190
- Drès, M., & Mallet, J. (2002). Host races in plant-feeding insects and their importance in sympatric speciation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *357*, 471–492.
- Duxbury, C., Glasscock, S., & Staniszevska, I. (2003). Control of regrowth from air potato (*Dioscorea bulbifera* L.) bulbils. *Wildland Weed*, *6*, 14–15.
- EDDMapS (2012). Early detection & distribution mapping system. The University of Georgia - Center for Invasive Species and Ecosystem Health. Retrieved from <http://www.eddmaps.org/>
- Florida Exotic Pest Plant Council (FLEPPC). (2001). Lygodium management plan for Florida. Amy Ferriter, (Ed.). West Palm Beach, FL: South Florida Water Management District. Retrieved from http://www.fleppc.org/Manage_Plans/lymo_mgt.pdf
- Florida Exotic Pest Plant Council (FLEPPC) Plant List Committee. (2003). Florida Exotic Pest Plant Council's 2003 list of invasive species. *Wildland Weeds*, *6*(suppl.).
- Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, *3*, 294–299. Retrieved from http://www.mbari.org/staff/vrijen/PDFS/Folmer_94MMBB.pdf
- Frye, M. J., Hough-Goldstein, J., & Kidd, K. A. (2012). Response of kudzu (*Pueraria montana* var. *lobata*) to different types and levels of simulated insect herbivore damage. *Biological Control*, *61*, 71–77. Retrieved from <http://dx.doi.org/10.1016/j.biocontrol.2012.01.008>
- Gann, G. D., Bradley, K., & Woodmansee, S. W. (2001). Floristic inventory of South Florida database. Retrieved from www.regionalconservation.org.
- Gordon, D. R. (1998). Effects of invasive, non-indigenous plant species on ecosystem processes: Lessons from Florida. *Ecological Applications*, *8*, 975–989.
- Gordon, D. R., Gann, G. D., Carter, E., & Thomas, K. (1999). Post-hurricane vegetation response in South Florida Hammocks with and without *Dioscorea bulbifera* L. control. In

- D. T. Jones & B. W. Gamble (Eds.), *Florida's garden of good and evil* (pp. 309–326). West Palm Beach: South Florida Water Management District.
- Hammer, R. L. (1998). Diagnosis: *Dioscorea*. *Wildland Weeds*, 2, 8–10.
- Hardt, R. A. (1986). Japanese honeysuckle: From “one of the best”: To ruthless pest. *Arnoldia*, 46, 27–34.
- Hare, J. D. (1983). Seasonal variation in plant-insect associations: Utilization of *Solanum dulcamara* by *Leptinotarsa decemlineata*. *Ecology*, 64, 345–361.
- Horvitz, C. C., & Koop, A. (2001). Removal of nonnative vines and post-hurricane recruitment in tropical hardwood forests of Florida. *Biotropica*, 33, 268–281. doi:10.1111/j.1744-7429.2001.tb00178.x
- Hough-Goldstein, J., Schiff, M., Lake, E., & Butterworth, B. (2008). Impact of the biological control agent *Rhinoncomimus latipes* (Coleoptera: Curculionidae) on mile-a-minute weed, *Persicaria perfoliata*, in field cages. *Biological Control*, 46, 417–423. Retrieved from <http://dx.doi.org/10.1016/j.biocontrol.2008.04.001>
- Hunter, M. D., & McNeil, J. N. (1997). Host-plant quality influences diapause and voltinism in a polyphagous insect herbivore. *Ecology*, 78, 977–986.
- Hutchinson, J. T., Langeland, K. A., MacDonald, G. E., & Querns, R. (2010). Absorption and translocation of glyphosate, metsulfuron, and triclopyr in old world climbing fern (*Lygodium microphyllum*). *Weed Science*, 58, 118–125. Retrieved from <http://dx.doi.org/10.1614/WS-D-09-00046.1>
- Irwin, D. L., & Aarssen, L. W. (1996). Effects of nutrient level on cost and benefit of apical dominance in *Epilobium ciliatum*. *American Midland Naturalist*, 136, 14–28. Retrieved from <http://www.jstor.org/stable/2426628>
- Johnson, C. G. (1969). *Migration and dispersal of insects by flight*. London: Methuen & Co. Ltd.
- Kimoto, S., & Gressitt, J. L. (1979). Chrysomelidae (Coleoptera) of Thailand, Cambodia, Laos and Vietnam. I. Sagrinae, Donaciinae, Zeugophorinae, Megalopodinae and Criocerinae. *Pacific Insects*, 20, 191–256.
- Lake, J. C., & Leishman, M. R. (2004). Invasion success of exotic plants in natural ecosystems: the role of disturbance, plant attributes and freedom from herbivores. *Biological Conservation*, 117, 215–226.
- Langeland, K. A., & Craddock Burks, K. (1998). *Identification and biology of non-native plants in Florida's native areas*. Gainesville: University of Florida.
- Miller, J. H. (2003). *Nonnative invasive plants of southern forests, a field guide for identification and control*. Asheville, NC: USDA Forest Service Southern Research Station. General Technical Report SRS-62.
- Morton, J. F. (1976). Pestiferous spread of many ornamental and fruit species in south Florida. *Proceedings Florida State Horticultural Society*, 89, 348–353.
- Overholt, B., Hughes, C., Wallace, C., & Morgan, E. (2003). Origin of air potato identified. *Wildland Weeds*, 7, 9.
- Overholt, W. A., Markle, L., Meisenburg, M., Raz, L., Wheeler, G., Pemberton, R., ... Parks, G. R. (2008). *Air potato (Dioscorea bulbifera) management plan*. West Palm Beach, FL: Florida Exotic Pest Plant Council.
- Paul, G. S., & Yavitt, J. P. (2011). Tropical vine growth and the effects of forest succession: a review of the ecology and management of tropical climbing plants. *Botanical Review*, 77, 11–30. doi:10.1007/s12229-010-9059-3
- Paynter, Q., Gourlay, A. H., Oboyski, P. T., Fowler, S. V., Hill, R. L., Withers, T. M., ... Hona, S. (2008). Why did specificity testing fail to predict the field host-range of the gorse pod moth in New Zealand. *Biological Control*, 46, 453–462.
- Pemberton, R. W., & Witkus, G. L. (2010). Laboratory host range testing of *Lilioceris* sp. near *impressa* (Coleoptera: Chrysomelidae) – a potential biological control agent of air potato, *Dioscorea bulbifera* (Dioscoreaceae). *Biocontrol Science and Technology*, 20, 567–587.
- Schaffner, U., & Müller, C. (2001). Exploitation of the fecal shield of the lily leaf beetle, *Lilioceris lili* (Coleoptera: Chrysomelidae) by the specialist parasitoid *Lemophagus pulcher* (Hymenoptera: Ichneumonidae). *Journal of Insect Behavior*, 14, 739–757.

- Schmitt, M. (1988). The Criocerinae: Biology, phylogeny, and evolution. In P. Jolivet, E. Petitpierre, & T. H. Hsiao (Eds.), *Biology of Chrysomelidae* (pp. 475–495). Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Schmitz, D. C., Simberloff, D., Hofstetter, R. L., Haller, W. T., & Sutton, D. (1997). The ecological impact of nonindigenous plants. In D. Simberloff, D. C. Schmitz, & T. C. Brown (Eds.), *Strangers in paradise: Impact and management of nonindigenous species in Florida* (pp. 39–61). Washington, DC: Island Press.
- Schultz, G. E. (1993). *Element Stewardship abstract for Dioscorea bulbifera air potato*. Davis, CA: The Nature Conservancy.
- Sonnenberg, R., Nolte, A. W., & Tautz, D. (2007). An evaluation of LSU rDNA D1-D2 sequences for their use in species identification. *Frontiers in Zoology*, 4, 6.
- Sota, T., & Volger, A. P. (2001). Incongruence of mitochondrial and nuclear gene trees in the Carabid beetles Ohomopterus. *Systematic Biology*, 50, 39–59. doi:10.1093/sysbio/50.1.39
- Tamura, K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution*, 9, 678–687.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28, 2731–2739.
- Tewksbury, L., Gold, M. S., Casagrande, R. A., & Kenis, M. (2005). Establishment in North America of *Tetrastichus setifer* Thomson (Hymenoptera: Eulophidae), a parasitoid of *Lilioceris lili* (Coleoptera: Chrysomelidae). In M. S. Hoddle (Ed.), *Proceedings of the second international symposium on biological control of arthropods, 12–16 September 2005, Davos, Switzerland* (pp. 142–143). Morgantown, WV: U.S. Department of Agriculture, Forest Service Publ. FHTET-2005–08.
- Tishechkin, A. K., Konstantinov, A. S., Bista, S., Pemberton, R. W., & Center, T. D. (2011). Review of the continental Oriental species of *Lilioceris* Reitter (Coleoptera, Chrysomelidae, Criocerinae) closely related to *Lilioceris impressa* (F.). *ZooKeys*, 103, 63–83. Retrieved from <http://dx.doi.org/10.3897/zookeys.103.983>
- United States Naval Observatory, (2012). Naval meteorology and oceanography command, Stennis Space Center, MS. Retrieved from <http://www.usno.navy.mil/USNO/astronomical-applications/data-services>
- Van Driesche, R., Hoddle, M., & Center, T. (2008). *Control of pests and weeds by natural enemies* (p. 473). Malden, MA: Blackwell.
- Volchansky, C. R., Hoffmann, J. H., & Zimmermann, H. G. (1999). Host-plant affinities of two biotypes of *Dactylopius opuntiae* (Homoptera: Dactylopiidae): enhanced prospects for biological control of *Opuntia stricta* (Cactaceae) in South Africa. *Journal of Applied Ecology*, 36, 85–91.
- Voss, R. H., Ferro, D. N., & Logan, J. A. (1988). Role of reproductive diapause in the population-dynamics of the Colorado potato beetle (Coleoptera, Chrysomelidae) in western Massachusetts. *Environmental Entomology*, 17, 863–871.
- Webster, C. R., Jenkins, M. A., & Jose, S. (2006). Woody invaders and the challenges they pose to forest ecosystems in the eastern United States. *Journal of Forestry*, 104, 366–374.
- Wheeler, G. S., Pemberton, R. W., & Raz, L. (2007). A biological control feasibility study of the invasive weed-air potato, *Dioscorea bulbifera* L. (Dioscoreaceae): An effort to increase biological control transparency and safety. *Natural Areas Journal*, 27, 269–279. Retrieved from <http://www.bioone.org/doi/full/10.3375/0885-8608%282007%2927%5B269%3AABCF%5D2.0.CO%3B2>
- Zwölfer, H., & Harris, P. (1971). Host specificity determination of insects for biological control of weeds. *Annual Review of Entomology*, 16, 159–178.