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Ecological host-range of *Lilioceris cheni* (Coleoptera: Chrysomelidae), a biological control agent of *Dioscorea bulbifera*

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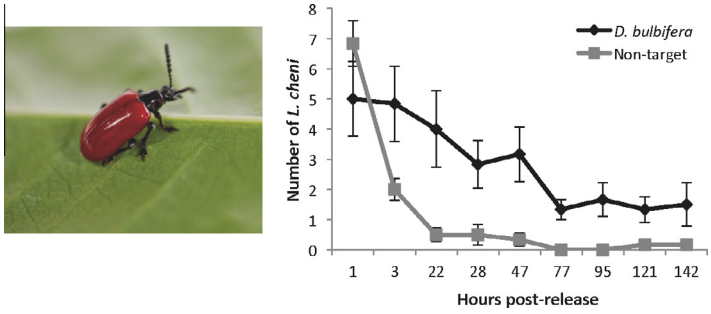
HIGHLIGHTS

- The ecological host-range of *Lilioceris cheni* was tested.
- Fed and naïve beetles preferred *Dioscorea bulbifera* to non-targets.
- A follow-up laboratory consumption and field spillover study were conducted.
- *Lilioceris cheni* consumed more *D. bulbifera* and survived longer on its host than non-targets.
- *Lilioceris cheni* does not pose a spillover risk to the native plant *D. floridana*.

GRAPHICAL ABSTRACT



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ABSTRACT

Open-field host-specificity testing assesses the host-range of a biological control agent in a setting that permits the agent to use its full complement of host-seeking behaviors. This form of testing, particularly when it includes a no-choice phase in which the target weed is killed, may provide the most accurate assessment of the ecological host-range of an agent. We conducted a two-phase field host-specificity test with experienced and naïve adults of *Lilioceris cheni* Gressitt and Kimoto (Coleoptera: Chrysomelidae), a biological control agent of *Dioscorea bulbifera* L. (Dioscoreales: Dioscoreaceae). We followed field tests with a no-choice laboratory consumption study with the congeneric plant species that received test feeding in the field, and an additional field evaluation of spillover risk. Both experienced and naïve adults strongly preferred *D. bulbifera* to non-targets in the field. Within 47 h post-release, 90% of the released beetles that remained in the plots were found on *D. bulbifera*. In the laboratory no-choice test, the beetles consumed significantly more *D. bulbifera* and survived longer on this plant than the non-targets. All naïve beetles in the *Dioscorea sansibarensis* and *Dioscorea villosa* treatments and 75% of naïve beetles on *Dioscorea floridana* died within 7 d. Potted plants of the native *D. floridana* experienced minor test feeding in the spillover experiment when surrounded by large populations of *L. cheni* in the field. At the end of this experiment, *L. cheni* eggs and/or larvae were present on 83% of *D. bulbifera* plants but none of the *D. floridana* plants. We conclude that *L. cheni* is host-specific to *D. bulbifera* and does not pose a spillover risk to the native *D. floridana*.

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1. Introduction

Open-field host-specificity testing in the introduced range is the last step in the host-range assessment process in biological control (Frye et al., 2010). Laboratory-based no-choice tests determine the physiological host-range of an arthropod; plant species on which adults readily feed and immature individuals will complete development (Sheppard et al., 2005; Van Driesche et al., 2008). In contrast, the ecological host-range of an agent encompasses the plants that an arthropod will use if given the opportunity to use all host-seeking and acceptance behaviors (Schaffner, 2001), which

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may differ slightly from the physiological host-range in an open-field choice setting (Briese et al., 2002; Clement and Cristofaro, 1995; Heard, 2000; Marohasy, 1998; Turanli and Schaffner, 2004). For example, if insects are confined to a cage, oviposition may occur on a non-target plant or the cage itself (Marohasy, 1998; Turanli and Schaffner, 2004; Van Driesche et al., 2008). Other factors, such as the amount of time that an insect is starved prior to testing and time since exposure to the preferred host, can also influence its likelihood of feeding or ovipositing on a non-target (Withers et al., 2000).

Open-field host-specificity testing may be conducted in the native range, in which case the results contribute to knowledge about the potential for non-target damage and thus to the information used to determine if a candidate is suitable for release (Clement and Cristofaro, 1995; Frye et al., 2010). When conducted in the introduced range, after release of the biocontrol agent, post-release evaluations contribute to the overall understanding of the host-range of an arthropod (Frye et al., 2010; Pratt et al., 2009; Taylor et al., 2007). Within an open-field setting, a no-choice test can also be effectively conducted by killing the target weed and examining the response of the herbivores (Briese et al., 2002; Dunn and Campobasso, 1993).

The use of open-field host-specificity testing, particularly with the incorporation of a no-choice phase, more accurately predicts the risk to non-targets (Briese et al., 2002; Clement and Cristofaro, 1995). Non-target use includes both feeding and completion of development on a plant other than the target weed and is typically restricted to plants closely related to the target weed. This type of divergence from the host plant is usually predicted during no-choice host-specificity testing (Blossey et al., 2001; Pemberton, 2000). In contrast, both related and unrelated non-target plants may experience spillover damage during population outbreaks of the biological control agent. During spillover events, large populations of the biocontrol agents reduce the quantity and quality of the target weed and the agents temporarily feed but do not complete development on non-target species (Blossey et al., 2001; Dhileepan et al., 2006). Plants in close proximity to the target weed may be the most susceptible to spillover damage (Schooler et al., 2003). In some cases, only newly emerged adults, not more experienced individuals, exhibit spillover feeding (Blossey et al., 1994, 2001). We conducted a two-phase open-field host-specificity test to assess the ecological host-range of *Liliocerис cheni* Gressitt and Kimoto (Coleoptera: Chrysomelidae), a biological control agent of *Dioscorea bulbifera* L. (Dioscoreales: Dioscoreaceae). A subsequent field test was used to assess whether *L. cheni* would spillover onto a native congener.

Dioscorea bulbifera, air potato, is an herbaceous, perennial vine native to Asia and Africa. The *D. bulbifera* present in the U.S. is of Asian origin. Its distribution extends from Florida north to Savannah, Georgia, with additional unverified reports in the southeastern U.S., plus Hawaii (Croxton et al., 2011; EDDMapS, 2014). Air potato vines grow more than 20 m, climb into trees, and outcompete other vegetation in a variety of habitat types (Overholt et al., 2008). The vines are deciduous and die back in the winter, even in areas that are frost-free (Langeland and Craddock Burks, 1998). *Dioscorea bulbifera* seldom flowers in the U.S. and reproduces primarily through aerial bulbils, vegetative propagules that form in the leaf axils and dehisce as the plant senesces in the fall. New vines sprout in the spring from bulbils and persistent subterranean tubers (Overholt et al., 2008). Herbicide and mechanical control techniques are often inadequate to control *D. bulbifera* (Wheeler et al., 2007).

Liliocerис cheni was fortuitously discovered feeding on *D. bulbifera* by USDA ARS scientists in Nepal in 2002 (Pemberton and Witkus, 2010). During no-choice host-specificity testing, adult *L. cheni* test fed on four congeners of *D. bulbifera*: *D. altissima* Lam. and *D. polystachya* Turcz., both introduced, and two native species, *D. floridana* Bartlett and *D. villosa* L. Adults oviposited on *D. bulbifera* as well as *D. floridana* and *D. villosa* but when eggs were transferred to non-target plants all first instar larvae died without feeding (Pemberton and Witkus, 2010). The USDA Animal Plant Health Inspection Service (APHIS) issued a permit for release in 2011; however, USDA ARS could not obtain additional *L. cheni* from Nepal, hereafter referred to as the Nepalese biotype (Center et al., 2013).

USDA ARS collected *L. cheni* in Yunan Province, China in 2011. The identification of the Chinese material, hereafter referred to as the Chinese biotype, was verified using taxonomic and molecular characters (Center et al., 2013; Tishechkin et al., 2011). Abbreviated host-specificity testing was conducted with larvae of the Chinese biotype; this testing focused on nine *Dioscorea* species and *Rajania cordata* L. (Dioscoreaceae) (Center et al., 2013). Eggs transferred from *D. bulbifera* hatched on all test plants. Larvae test fed on six non-target species but no larvae survived more than 3 d or beyond the first instar; 80% of the larvae on *D. bulbifera* survived to pupation (Center et al., 2013). Neither biotype of *L. cheni* completed development on any non-target plant during quarantine host-specificity testing (Center et al., 2013; Pemberton and Witkus, 2010).

Field releases of the Chinese biotype began in cages in November, 2011, and in the open in March, 2012 (Center et al., 2013). *Liliocerис cheni* development from egg to adult takes approximately 26 d at 27.5 °C (Manrique et al. unpublished data). Larvae, particularly early instars, feed gregariously on air potato leaves. Fully grown fourth instar larvae descend from the host plant to enter the substratum and produce a whitish exudate that is incorporated into a foam-like cocoon. Adults emerge, feed on air potato leaves, and after a brief pre-ovipositional period produce multiple overlapping generations in the field (Center et al., 2013; Pers. obs.). The objectives of the current research with the Chinese biotype of *L. cheni* were to evaluate the field host-specificity of the beetle; to determine if the feeding of newly emerged adults differed if they were offered their host plant prior to testing; and to determine if high densities of *L. cheni* in the field pose a spillover risk to the native congener *D. floridana*, which has a limited distribution in Florida.

2. Materials and methods

2.1. Field host-specificity experiment

Liliocerис cheni adults were collected from 15 × 32 × 53 cm plastic emergence bins (Camwear® Food Pan, Cambro USA, Huntington Beach, CA) without having fed on air potato. The beetles were held with water and a 10% honey, 90% lemon-lime Gatorade (The Gatorade Co., Chicago, IL) solution until the start of the experiment, approximately 1–18 d. These beetles are hereafter referred to as naïve adults. Prior to release the beetles were color coded with a spot of nail polish or Sharpie paint pen (Sharpie PAINT, Vietnam) on one elytron to indicate the plant species on which it was being released.

Potted plants were placed on a mowed lawn under a *Quercus virginiana* Mill. canopy at the Long Key Natural Area and Nature Center in Davie, Florida (26°4′30.06″N, 80°19′23.46″W). The plants represented a mixture of native and introduced plants in the genus *Dioscorea*, the ornamental plant *Tacca chantrieri* André (also Dioscoreaceae), plus a plastic plant that offered structure but not a food source (Frye et al., 2010; Table 1). The plants were arranged in a randomized complete block design with six blocks; individual plants were separated by 1 m within a block, blocks were separated by at least 10 m. Plants were staked to prevent them from toppling over and were watered as needed. Trap plants of *D. bulbifera* were placed approximately 30 m from the test plots at four

Table 1
List of plant species used in the field host-specificity experiment.

Accepted name[a]	Section[b]	Status (Origin[a])	Common name
Dioscorea alata L.	Enantiophyllum	Invasive (tropical and subtropical Asia)	Water yam, winged yam
Dioscorea polystachya Turcz.	Enantiophyllum	Invasive (central China to temperate east Asia)	Cinnamon vine, Chinese yam
Dioscorea floridana Bartlett	Macropoda	Native (Florida and Georgia)	Florida yam
Dioscorea villosa L.	Macropoda	Native (northern Florida to southern Ontario)	Fourleaf yam
Dioscorea bulbifera L.	Opsophyton	Invasive (tropical and subtropical Asia and Africa)	Air potato
Dioscorea sansibarensis Pax	Opsophyton	Naturalized (Madagascar and tropical Africa)	Zanzibar yam
Tacca chantrieri André	–	Introduced (Assam thru southern China to peninsular Malaysia)	Black bat flower

[a] Test list was selected based upon Raz (2002). Taxonomy and origins follow Govaerts et al. (2007).
[b] Infrageneric circumscription follows Pemberton and Witkus (2010).

approximately cardinal points. These plants were checked for beetles each time the plots were surveyed. The closest natural stand of *D. bulbifera* was approximately 175 m from the experimental area.

Naïve adults were transported to the site in a cooler and eight individuals were transferred to the soil at the base of each test plant between 1025 and 1045 on 14 August 2013. Beetles were observed flying immediately following the release. Based on the rapid dispersal from the test plots, additional beetles were released using the same methods between 1147 and 1213 on 15 August 2013. These beetles were approximately the same age as the naïve adults but had fed on air potato after emerging from the pupal bins and are hereafter referred to as fed adults. The fed adults were also painted to indicate the release plant as air potato or non-target. Five fed beetles were released at the base of each test plant. After the field releases, a subset of painted and unpainted beetles were held in containers with *D. bulbifera* to ensure painting did not cause mortality.

During each survey, the number of beetles and their color (origin) were recorded for each test plant. Naïve beetles were sampled at 3, 23, 26, 29, 47, 53, 72, 102, 120, 146, and 167 h post-release. Fed beetles were sampled at 1, 3, 22, 28, 47, 77, 95, 121, and 142 h post-release. Plants were inspected for feeding damage in each survey and were searched for eggs and larvae at the conclusion of the experiment. A two-phase approach was employed to test the response of *L. cheni* to non-targets in the absence of their preferred host (Briese et al., 2002). On 16 August 2013 at 1024, 48 h after naïve adults were released and 26 h after fed adults were released, the stems of the air potato plants were cut to initiate wilting and encourage dispersal. Beetle movement within the plots was monitored for an additional 3 d. A live air potato plant was then added to each block and checked approximately 24 and 48 h later to determine if the beetles returned to the plots.

2.2. Laboratory consumption study

Newly emerged *L. cheni* were randomly assigned to fed or naïve treatments and held as described above. *Dioscorea floridana*, *D. villosa*, and *D. sansibarensis* plants from the field experiment were numbered according to their field block and new *D. bulbifera* potted plants were selected from greenhouse stock and randomly assigned to replicates. Depending on leaf size and the amount of beetle consumption, one to three undamaged leaves of a test plant were placed in a water pick within a plastic sandwich container ($14 \times 14 \times 5$ cm). The leaves were photographed before and after exposure to the beetles and leaf area consumed was calculated using Adobe Photoshop CS4 (O'Neal et al., 2002; Adobe Systems, Inc.). Leaves were changed every other day or as needed based on beetle consumption and leaf condition.

Two beetles were randomly assigned to each container with six replicates of naïve and six replicates of fed beetles for each plant species. Beetles were added to the containers on 30 August 2013. The first 24 h was considered an acclimatization period; all beetles that died during this period were replaced. The containers were kept in an environmental chamber set at 25 ± 0.6 °C and 14:10 L:D. The position of the containers was occasionally rotated within the chamber to prevent bench effects. The beetles were checked daily and the date each beetle died was recorded. The experiment ended on 24 September 2013, when live beetles were only present in the *D. bulbifera* treatments.

2.3. Spillover experiment

Three sites in Florida with established *L. cheni* populations were selected to test if beetles would spillover onto the native plant *D. floridana*. The sites were located in Fort Lauderdale (26°4′35.31″N, 80°19′33.16″W), Fort Pierce (27°24′5.44″N, 80°26′37.94″W), and Gainesville (29°38′52.08″N, 82°17′8.88″W). Six potted *D. floridana* and six potted *D. bulbifera* plants were placed in the Fort Pierce and Fort Lauderdale sites on 21 July 2014 and in the Gainesville site on 22 July 2014. The sites were revisited 8 d after installation and plants were examined for *L. cheni* adults, eggs, and larvae. The beetle feeding damage on each plant was assessed using the following scale: 0, no damaged leaves; 1, minor test feeding on a few leaves; 2, feeding on about half the leaves; 3, feeding on most leaves; 4, extensive damage on most leaves.

2.4. Statistical analysis

Analysis of variance using the mixed procedure with the repeated measures statement was used to compare beetle residency amongst the non-targets only, and on *D. bulbifera* compared to the non-targets as a group in the field experiment (PROC MIXED, REPEATED statement, SAS Institute, 2011). Plant, block, and hour were considered fixed effects (Littell et al., 2002). The Kenward–Roger (KR) correction was applied and the covariance structure ANTE(1) was used whenever possible; the structure AR(1) was used if the model did not converge. The AR(1) covariance structure is utilized when observations are equally spaced and the correlation structure is consistent over time, while the ANTE(1) structure permits more uneven spacing and change over time. Both models account for adjacent observations being more correlated than observations recorded over a longer period of time (Littell et al., 2002). Fed and naïve beetle treatments were analyzed separately.

The total leaf area consumed in the no-choice laboratory consumption study was divided by the total number of days live beetles were present to determine the leaf area consumed per beetle per day for each replicate of plant species and feeding treatment. These data were analyzed using a two-way ANOVA by feeding treatment and plant species followed by Tukey's test (PROC GLM, SAS Institute, 2011). Survival analysis was used to evaluate the longevity of the beetles in the laboratory consumption study (PROC LIFETEST, SAS Institute, 2011). The data were initially stratified by naïve and fed treatments, after which each feeding treatment was analyzed separately and stratified by plant species.

Table 2
Total number of naïve *L. cheni* adults counted on test plant species in the six blocks and proportion of beetles on *D. bulbifera*. Stems of the *D. bulbifera* plants were cut 48 h post-release and a new *D. bulbifera* plant was added to each block 121 h post-release.

	Hours post-release										
	3	23	26	29	47	53	72	102	120	146	167
Dioscorea alata L.	3	1	1	0	0	0	1	0	2	0	0
Dioscorea polystachya Turcz.	1	1	2	2	1	0	0	0	0	0	0
Dioscorea floridana Bartlett	6	2	2	2	1	1	0	0	0	0	0
Dioscorea villosa L.	5	0	0	0	0	0	0	0	0	0	0
Dioscorea bulbifera L.	20	24	20	27	22	17	16	8	19	12	11
Dioscorea sansibarensis Pax	4	1	2	1	0	1	0	0	0	0	0
Tacca chantrieri André	7	0	2	1	0	0	0	0	0	0	0
Plastic plant	1	0	0	0	0	0	0	0	0	0	0
Total	47	29	29	33	24	19	17	8	21	12	11
Proportion of beetles on *D. bulbifera*	0.43	0.83	0.69	0.82	0.92	0.89	0.94	1.00	0.90	1.00	1.00

Beetle feeding damage in the spillover experiment was analyzed using a two-way ANOVA by field site and plant species (PROC GLM, SAS Institute, 2011). Violations of the assumption of normality in the ANOVA tests were disregarded if the residuals were normally distributed (Glass et al., 1972).

3. Results

3.1. Field host-specificity experiment

Marked beetles held at the laboratory did not exhibit higher mortality than unmarked beetles. The number of beetles on the seven non-target plants did not differ in the naïve ($F_{6,39} = 0.64$, $P = 0.7001$, Table 2) or fed feeding treatments ($F_{6,23.2} = 1.86$, $P = 0.1310$, Table 3). Beetle presence on all non-targets declined over time (fed: $F_{8,225} = 23.04$, $P < 0.0001$; naïve: $F_{10,311} = 14.76$, $P < 0.0001$) but did so with conflicting patterns producing a significant interaction between time and non-target plant species (fed: $F_{48,239} = 2.12$, $P = 0.0001$ and naïve: $F_{60,319} = 1.46$, $P = 0.0216$). The five non-target congeneric *Dioscorea* species did not differ from the plastic plant in their ability to attract or retain *L. cheni* adults. Fed and unfed beetles on non-target plants did not differ by block (fed: $F_{5,25.1} = 0.62$, $P = 0.6869$; naïve: $F_{5,42.3} = 1.19$, $P = 0.3318$).

The total number of beetles on the non-target plants as a group was calculated for each block and sample time, and was compared to the total number of beetles on *D. bulbifera*. More beetles from both feeding treatments were observed on *D. bulbifera* than the non-target group (fed: $F_{1,11} = 12.98$, $P = 0.0042$; naïve: $F_{1,8.27} = 10.62$, $P = 0.0111$, Fig. 1). Beetles decreased significantly over time (fed: $F_{8,15.3} = 5.89$, $P = 0.0015$; naïve: $F_{10,17.4} = 4.45$, $P = 0.0032$), and did so differently between *D. bulbifera* and the non-target group (fed: $F_{8,15.3} = 2.75$, $P = 0.0423$; naïve: $F_{10,17.4} = 4.42$, $P = 0.0034$). There was no significant block effect (fed: $F_{5,11.9} = 1.46$, $P = 0.2722$; naïve: $F_{5,13.2} = 1.78$, $P = 0.1862$, Fig. 1). In both fed and naïve treatments, beetle numbers were higher on non-target plants at the first sample point, but then decreased overall in subsequent samples; this decrease started within 3 h of the release of the fed beetles (Fig. 1). During the survey 3 h post-release 43% of beetles in the naïve treatment and 71% of beetles in the fed treatment were observed on *D. bulbifera* (Tables 2 and 3). By 47 h post-release more than 90% of the beetles that remained in the plots were found on *D. bulbifera* (Tables 2 and 3). Beetles did not immediately disperse from *D. bulbifera* after the stems were cut and the plants wilted slowly in the field. The number of beetles on the non-target plants did not increase after the *D. bulbifera* stems were cut. Beetle numbers had declined in the plots prior to addition of the new potted *D. bulbifera* plants, and did not increase thereafter (Tables 2 and 3). No *L. cheni* eggs or larvae were found on non-target plants, but very limited test feeding was observed on three non-target species: *D. floridana*, *D. villosa*, and *D. sansibarensis*.

3.2. Laboratory consumption study

Liliocerис cheni adults consumed significantly more *D. bulbifera* per beetle per day than the non-target species ($F_{3,39} = 75.63$, $P < 0.0001$, Fig. 2) and 8.6 times more *D. bulbifera* than *D. floridana*. Fed and naïve treatments did not differ in the leaf area that they consumed ($F_{1,39} = 1.07$, $P = 0.3064$, Fig. 2). Neither fed nor naïve beetles consumed any *D. villosa*. Fed beetles survived longer than naïve beetles overall in the laboratory consumption study (Wilcoxon test, $\chi^2 = 6.4073$, $df = 1$, $P = 0.0114$). The survival

Table 3
Total number of fed *L. cheni* adults counted on test plant species in the six blocks and proportion of beetles on *D. bulbifera*. Stems of the *D. bulbifera* plants were cut 26 h post-release and a new *D. bulbifera* plant was added to each block 96 h post-release.

	Hours post-release								
	1	3	22	28	47	77	95	121	142
Dioscorea alata L.	12	2	0	0	0	0	0	0	0
Dioscorea polystachya Turcz.	3	1	0	0	0	0	0	0	1
Dioscorea floridana Bartlett	5	2	1	0	0	0	0	0	0
Dioscorea villosa L.	5	2	0	1	0	0	0	0	0
Dioscorea bulbifera L.	30	29	24	17	19	8	10	8	9
Dioscorea sansibarensis Pax	12	5	2	2	2	0	0	1	0
Tacca chantrieri André	3	0	0	0	0	0	0	0	0
Plastic plant	1	0	0	0	0	0	0	0	0
Total	71	41	27	20	21	8	10	9	10
Proportion of beetles on *D. bulbifera*	0.42	0.71	0.89	0.85	0.90	1.00	1.00	0.89	0.90

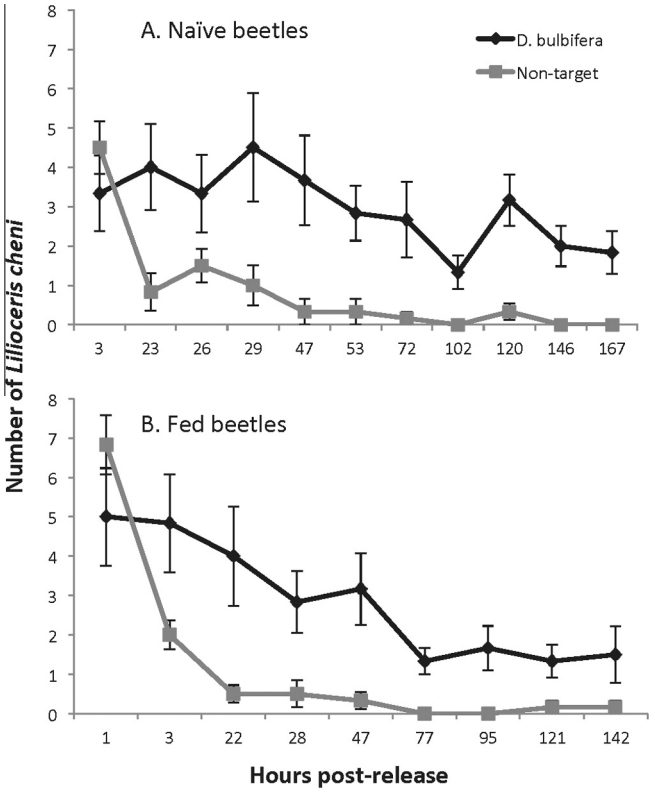

Fig. 1. Number of *L. cheni* (mean ± SEM) recovered on *D. bulbifera* and all non-target plants in the (A) naïve, and (B) fed beetle treatments. Eight naïve beetles were placed at the base of each plant on 14 August 2013 and five fed beetles were placed at the base of each plant on 15 August 2014.

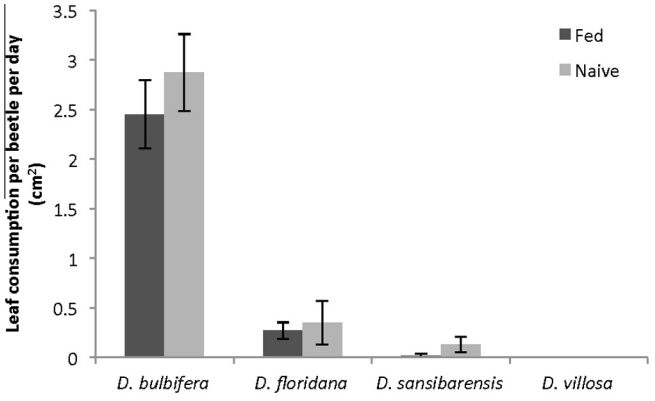

Fig. 2. Leaf consumption per beetle per day (mean ± SEM) by beetles in the two feeding treatments in the laboratory consumption study.

function for *L. cheni* differed by plant species within the naïve (Wilcoxon test, $\chi^2 = 28.1252$, $df = 3$, $P < 0.0001$, Fig. 3A) and fed treatments (Wilcoxon test, $\chi^2 = 19.1250$, $df = 3$, $P = 0.0003$, Fig. 3B). Beetles consuming *D. bulbifera* survived longer than beetles on the non-targets in both feeding treatments (Fig. 3).

3.3. Spillover experiment

After 8 d in the field, 83% of the *D. bulbifera* plants had *L. cheni* eggs and/or larvae present but none were found on *D. floridana*. The beetles fed more heavily on *D. bulbifera* than *D. floridana* ($F_{1,30} = 147.83$, $P < 0.0001$, Fig. 4). Beetles in Fort Pierce fed less than the other two sites ($F_{2,30} = 6.07$, $p = 0.0061$, Fig. 4) and there

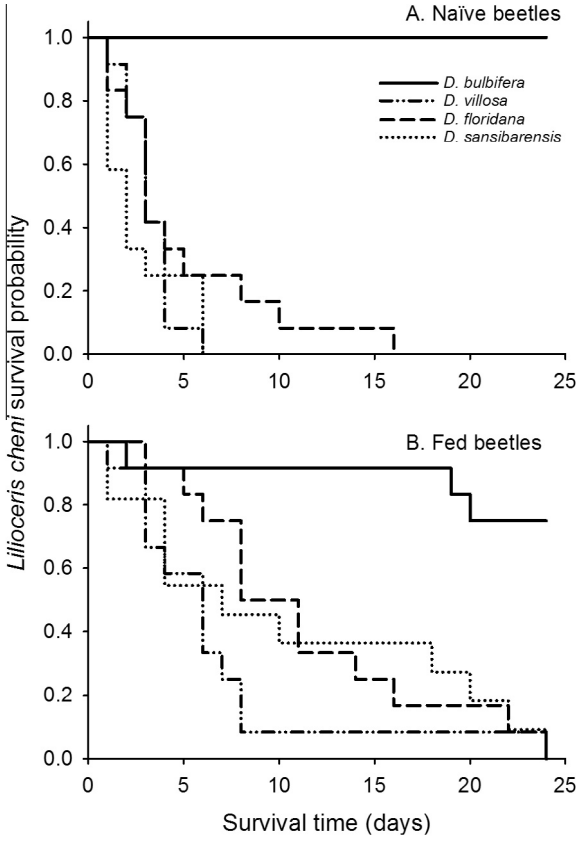

Fig. 3. Kaplan–Meier survival curves for *Lilioceris cheni* in the (A) naïve and (B) fed beetle treatments in the laboratory consumption study. The experiment ended on day 24 when live beetles were only present on *D. bulbifera*.

was an interaction between site and feeding score ($F_{2,30} = 6.07$, $P = 0.0061$). Beetles rapidly attacked the potted *D. bulbifera* plants and were present on at least one *D. bulbifera* plant in each field site within the 30 min needed to install the plants. All 18 *D. floridana* plants had minor test feeding. This test feeding was observed on the *D. floridana* plants in Fort Pierce after 24 h in the field, but when the plants were collected 7 d later there did not appear to be any additional feeding damage.

4. Discussion

During the field host-specificity experiment, *L. cheni* demonstrated a clear preference for the target host *D. bulbifera*, regardless of whether or not they were offered *D. bulbifera* prior to the experiment. Minor test feeding occurred on three non-targets. A follow-up laboratory consumption study with these non-targets and *D. bulbifera* indicated that *L. cheni* consumes significantly more and survives longer on *D. bulbifera* than the non-targets. Furthermore, an additional field study verified that the native *D. floridana* is not at risk for spillover damage when it occurs sympatrically with *D. bulbifera* supporting large populations of *L. cheni*.

The process of host-plant selection can be split into two general phases: searching and contact testing (Schoonhoven et al., 2005). Before making contact with the plant, insects detect plant volatiles and visual cues (Finch and Collier, 2000). In the field study, utilizing all host-selection behaviors in the presence of the host plant and non-targets, *L. cheni* rapidly abandoned non-target plants and either left the study area or alighted on another plant, often a *D. bulbifera* test plant. We observed beetles climbing to the top of test plants before taking flight. While contacting the plants, these beetles could explore physical and chemical traits that could

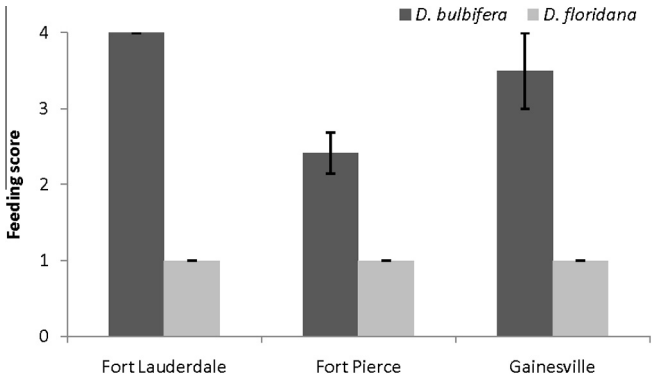

Fig. 4. *Lilioceris cheni* feeding score (mean ± SEM) on *D. bulbifera* and *D. floridana* after 8 d at the three field sites (0, no damaged leaves; 1, minor test feeding on a few leaves; 2, feeding on half the leaves; 3, feeding on most leaves; 4, extensive damage on most leaves).

not be evaluated from a distance (Chapman and Bernays, 1989; Schoonhoven et al., 2005). This can involve extensive examination of the plant via contact chemoreceptors located on the tarsi, antennae, ovipositor or mouthparts (Bernays and Chapman, 1994; Schoonhoven et al., 2005). Many insects decide to reject or continue examining a plant based on physical and chemical cues from the surface of the plant (Bernays and Chapman, 1994). Contact testing may be followed by a test bite to release chemicals from the plant interior (Schoonhoven et al., 2005).

During the field host-specificity test, some *L. cheni* were attracted to non-target plants and test feeding occurred on *D. floridana*, *D. sansibarensis*, and *D. villosa*. Test bites are typically small, and the insect may hold the plant material in its preoral cavity for a longer period of time than during normal feeding. If the insect accepts the food, it usually follows the test bite with extensive feeding (Schoonhoven et al., 2005). The low beetle numbers, lack of a continued presence of the beetles, and absence of extensive feeding damage suggest that *L. cheni* rejected the non-targets after test feeding.

An induction of food preference (food imprinting) has been shown in some insects, including Coleoptera (Bernays, 1995; Heard, 2000; Szentesi and Jermy, 1990). The response of *L. cheni* adults to *D. bulbifera* and non-targets did not differ based on whether or not they had fed on *D. bulbifera* prior to field or laboratory experiments. The use of fed and naïve insects addressed the potential bias of food imprinting as well as the chance for a false positive if an insect feeds on a non-target during no-choice testing due to food deprivation (Heard, 2000; Withers et al., 2000). The results of the adult no-choice feeding consumption study presented here are identical to the results of laboratory no-choice tests with Chinese biotype larvae (i.e. some feeding on *D. floridana* and *D. sansibarensis*, but no feeding on *D. villosa*; Center et al., 2013). The lack of any feeding by *L. cheni* on *D. villosa* in the laboratory consumption study indicates that the test feeding observed in the field was likely caused by another insect species.

Beetles in the fed treatment survived longer than beetles in the naïve treatment in the laboratory consumption study. Presumably, beetles that consumed *D. bulbifera* before the start of the experiment acquired more fat reserves than beetles that were restricted to the honey and Gatorade solution, and thus survived longer. Beetles in the fed treatment only survived for 11 d on *D. floridana*. In contrast, *L. cheni* held under similar conditions in environmental chambers on *D. bulbifera* can live for more than 5 mo (Manrique et al., unpublished data).

We observed minor test feeding on *D. floridana* plants during the field host-specificity experiment and on all *D. floridana* plants used in the spillover study. Some beetles are clearly attracted to the plant, alight on it, and test feed, but reject the plant after tasting it. At the Fort Pierce site, test feeding was present after 24 h in the field but did not appear to increase over the next 7 d. This implies that the response to a novel plant is brief. Blaney and Simmonds (1985) demonstrated that male *Locusta migratoria* nymphs that initially rejected a non-host plant after test feeding learned to recognize that plant by its surface properties, and could reject the plant solely based on surface cues after multiple exposures. Further study is required to determine if similar changes in rejection behavior are occurring in *L. cheni* after multiple exposures to a non-target.

During population outbreaks of *Galerucella calmariensis* L. and *G. pusilla* Duft. (Coleoptera: Chrysomelidae), biological control agents of *Lythrum salicaria* L., purple loosestrife, spillover damage occurred on several non-target species when the target weed was severely defoliated (Blossey et al., 1994, 2001; Schooler et al., 2003). In some sites, newly emerged adults caused this spillover damage, but when these same individuals emerged from overwintering the following spring they only fed on purple loosestrife (Blossey et al., 1994, 2001). *Lilioceris cheni* is long-lived and has multiple overlapping generations in the introduced range; thus, for most of the growing season of *D. bulbifera* a portion of the *L. cheni* population will consist of inexperienced adults (unpublished data). Given the minor extent of test feeding in areas with high densities of *L. cheni*, it is possible that inexperienced adults are responsible for this feeding damage.

Neither biotype of *L. cheni* was able to complete development on any non-target plant (Center et al., 2013; Pemberton and Witkus, 2010). Based on the experiments reported here, we conclude that the Chinese biotype of *L. cheni* is host-specific to *D. bulbifera* and does not pose a spillover risk to the native *D. floridana*. Ongoing experiments are evaluating differences in the biology of the Chinese and Nepalese biotypes of *L. cheni* and will contribute to the design of a comprehensive release strategy for control of *D. bulbifera*.

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