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Field Release of the Beetle *Lilioceris egena* (Coleoptera: Chrysomelidae) for Classical Biological Control of Air Potato, *Dioscorea bulbifera* (Dioscoreaceae), in the Continental United States

Environmental Assessment, February 2021

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Contents

I. Purpose and Need for the Proposed Action

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Pests, Pathogens, and Biocontrol Permits (PPBP) is proposing to issue permits for release of the beetle *Lilioceris egena* (Weise) (Coleoptera: Chrysomelidae). *Lilioceris egena* would be used for the classical biological control of air potato, *Dioscorea bulbifera* L. (Dioscoreaceae), in the continental United States.

Classical biological control of weeds is a weed control method where natural enemies from a foreign country are used to reduce exotic weeds that have become established in the United States. Several different kinds of organisms have been used as biological control agents of weeds: insects, mites, nematodes, and plant pathogens. Efforts to study and release an organism for classical biological control of weeds consist of the following steps (TAG, 2016):

1. Foreign exploration in the weed's area of origin.
2. Host specificity studies.
3. Approval of the exotic agent by PPBP.
4. Release and establishment in areas of the United States invaded by the target weed.
5. Post-release monitoring.

This environmental assessment[1] (EA) has been prepared, consistent with USDA, APHIS' National Environmental Policy Act of 1969 (NEPA) implementing procedures (Title 7 of the Code of Federal Regulations (CFR), part 372). It examines the potential effects on the quality of the human environment that may be associated with the release of *L. egena* to control infestations of air potato within the continental United States. This EA considers the potential effects of the proposed action and its alternatives, including no action. Notice of this EA was made available in the Federal Register on January 8, 2021 for a 30-day public comment period. Fourteen comments were received on the EA by the close of the comment period. All comments were in favor of the proposed release of *L. egena.*

APHIS has the authority to regulate biological control organisms under the Plant Protection Act of 2000 (Title IV of Pub. L. 106–224). Applicants who wish to study and release biological control organisms into the United States must receive PPQ Form 526 permits for such activities. The PPBP received a permit application requesting environmental release of the beetle, *L. egena,* from China, and the PPBP is proposing to issue permits for this action. Before

[1] Regulations implementing the National Environmental Policy Act of 1969 (42 United States Code 4321 et seq.) provide that an environmental assessment "shall include brief discussions of the need for the proposal, of alternatives as required by section 102(2)(E), of the environmental impacts of the proposed action and alternatives, and a listing of agencies and persons consulted." 40 CFR § 1508.9.

permits are issued, the PPBP must analyze the potential impacts of the release of this agent into the contiguous United States.

The applicant's purpose for releasing *L. egena* is to reduce the severity of infestations of air potato in the contiguous United States. Air potato is a twining vine (65 feet long or greater) that often forms single species stands in Florida (Schmitz et al., 1997; Langeland and Craddock Burks, 1998; Gordon et al., 1999) and constitutes one of the most aggressive weeds introduced into the state (Hammer, 1998). Noted horticulturalist Henry Nehrling described his concern about the plant's invasiveness early in the 20th century, stating that "with the exception of the kudzu vine, I have never seen a more aggressive and dangerous vine in Florida" (Nehrling, 1933). Similar warnings were expressed in the 1970s, with recommendations to limit the planting of this ornamental species (Long and Lakela, 1976; Morton, 1976; Ward, 1977). By the 1980s, air potato was commonly growing in thickets, waste areas, and along hedges or fencerows in south and central Florida (Bell and Taylor, 1982). By the end of the 20th century, air potato was listed as a noxious weed by the Florida Department of Agricultural and Consumer Services (FDACS) (FL-EPPC, 2003). Air potato is considered the most serious type of environmental threat, described as a Category I weed by the Florida Exotic Pest Plant Council (FL-EPPC, 2003) as "invasive exotics that are altering native plant communities by displacing native species, changing community structure or ecological functions, or hybridizing with natives." Presently, air potato is well established in Florida, and is spreading into surrounding states (Raz, 2002) where it has the potential to severely disrupt entire ecosystems (Hammer, 1998).

Existing options for management of air potato, such as chemical and mechanical methods, provide only temporary solutions that require retreatment and are harmful to non-target species associated with the weed. *Lilioceris egena* is also expected to complement the activity of a previously released beetle, *Lilioceris cheni*, for biological control of air potato. For these reasons, the applicant has a need to release *L. egena,* a host-specific, biological control organism for the control of air potato, into the environment.

II. Alternatives

This section will explain the two alternatives available to the PPBP—no action and issuance of permits for environmental release of *L. egena*. Although the PPBP's alternatives are limited to a decision on whether to issue permits for release of *L. egena*, other methods available for control of air potato are also described. These control methods are not decisions to be made by the PPBP, and their use is likely to continue whether or not permits are issued for environmental release of *L. egena*, depending on the efficacy of *L. egena* to control air potato. These are methods presently being used to control air potato by public and private concerns.

A third alternative was considered, but will not be analyzed further. Under this third alternative, the PPBP would have issued permits for the field release of *L. egena*; however, the permits would contain special provisions or requirements concerning release procedures or mitigating measures. No issues have been raised that would indicate special provisions or requirements are necessary.

A. No Action

Under the no action alternative, PPBP would not issue permits for the field release of *L. egena* for the biological control of air potato. The release of this biological control agent would not take place. The following methods are presently being used to control air potato; these methods will continue under the "No Action" alternative and will likely continue even if permits are issued for release of *L. egena*, depending on the efficacy of the organism to control air potato.

1. Chemical Control

Chemical control of air potato vines requires repeated basal applications of herbicides (e.g., glyphosate, triclopyr), and these treatments need to be repeated over a two or three year period (Mullahey and Brown, 1999).

2. Mechanical Control

Removal of aboveground plants and bulbils by hand is a method of reducing air potato infestations (e.g., Duxbury et al., 2003). Hand removal was found to be as effective at controlling air potato as a combination of herbicide and hand pulling of air potato plants.

3. Biological Control

The Asian beetle *Lilioceris cheni* Gressitt and Kimoto was developed (Pemberton and Witkus, 2010) and released (Center et al., 2013) as a biological control agent of air potato. This beetle has been distributed throughout Florida and is dispersing at a rate of 8.2 kilometers (km)/year, with a maximum dispersal of 67 km from nearest release point (Overholt et al., 2016).

B. Issue Permits for Environmental Release of *Lilioceris egena.*

Under this alternative, the PPBP would issue permits for the field release of the beetle *L. egena* for the biological control of air potato. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures.

Biological Control Agent Information

1. Taxonomy and Description

Lilioceris egena is assigned to the insect order Coleoptera, family Chysomelidae, and subfamily Criocerinae. There are currently only two *Lilioceris* species in North America: the invasive *Lilioceris lilii* (Scopoli), and the biological control agent *L. cheni* (Center et al., 2013). A.S. Konstantinov, USDA-Agricultural Research Service, Systematic Entomology Laboratory, Beltsville, MD, determined the specimens collected by F.A. Dray and G. Witkus from air potato in southern China during May 2011 to be *Lilioceris egena*, a member of the *L. impressa* group (see Tishechkin et al., 2011). Specimens collected in Yunnan Province, China in May 2011 were deposited by A.S. Konstantinov at the National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Adult *L. egena* beetles are shiny black in color except for their red-reddish brown wing covers (elytra). Like other species in the subfamily Criocerinae, *L. egena* are elongate (1 centimeter (cm) long by 0.5 cm wide at the abdomen), with a narrow thorax and an even more narrow head with bulging eyes.

2. Geographical Range of *L. egena*

The genus *Lilioceris* is a widespread group that Berti and Rapilly (1976) proposed as originating in southern China/northern southeast Asia. The known distribution of *L. egena* includes China (Anhui, Yunnan, Fujian, Sichuan, and Hong Kong Provinces), India (Assam, Karnataka, and Uttarhand), Nepal (Province 3), Laos (Vientianne Province), Vietnam (Tây Nihn and Ho Chi Minh Provinces), and Singapore (Tishechkin et al., 2011). In addition, Warchalowski (2011) reports *L. egena* as occurring in Hainan, China and Taiwan.

3. Life History of *L. egena*

The average male *L. egena* beetle is only slightly longer-lived than the average female (126.2 ± 9.69 days vs. 110.7 ± 7.70 days) (± standard error (s.e.) hereafter); both have a maximum lifespan of 207 days. Females have a pre-egg laying period of 9.5 ± 0.75 days, and produce eggs for 95.3 ± 9.14 days. They deposit their eggs singly or in clutches of 2 to 14. *Lilioceris egena* eggs are frequently deposited on the undersides of aerial tubers, known as bulbils, that have fallen to the ground and split open, and there, the eggs are protected by the soil. The eggs may also be laid immediately above the soil/bulbil interface. Eggs are also deposited inside adult feeding holes in bulbils (Figure 1b). The eggs are attached to the bulbil with a gluey greenish substance exuded by the beetle that darkens over time. Eggs are occasionally found on nearby soil (Figure 1a), although it is unclear whether these are placed there by the females or represent eggs displaced when the bulbils are disturbed. Females only rarely oviposit (lay eggs) on air potato leaves.

Eggs are initially creamy yellow (Figure 1a), but darken (greenish-gray) as the larva inside develops (Figure 1b). The head capsule becomes visible through the end of the opaque egg midway through development, causing that end to appear to be darker than the rest of the egg (Figure 1b). Soon after, the developing larval eyes become visible as two distinct maroon spots. Neonate larvae eclose (hatch) from the eggs 4.8 days after oviposition.

Figure 1. *Lilioceris egena* eggs deposited (a) on soil at base of an air potato bulbil, and (b) in an adult feeding hole on a bulbil. Eggs in (a) are less than 24 hours old, whereas eggs in (b) are about 48 hours old. [Photos: F.A. Dray Jr.]

Neonates (newly hatched larvae) are translucent white with black legs, head capsules, and thoracic plates. As they age, the larvae become dark grey before pupation. The larvae feed singly, whether scraping the undersides of the foliage (Figure 2a) or mining within the bulbils (Figure 2b). The larvae prefer tender newly emerged air potato leaves, but can also eat older toughened leaves. Early instar larvae can consume air potato bulbils if there is an initial tear through itsthe bulbil surface, but more mature larvae can eat intact bulbils without difficulty.

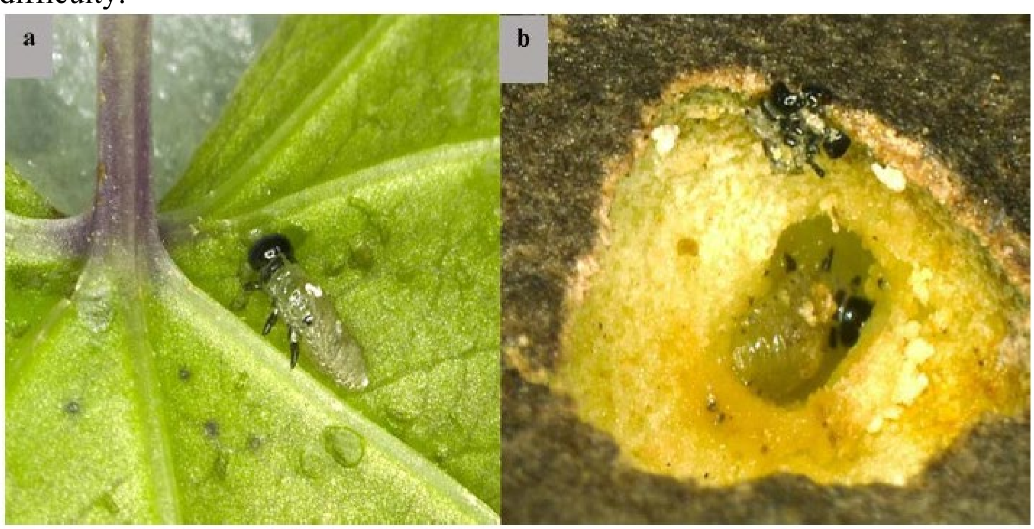

Figure 2. *Lilioceris egena* (a) newly hatched (neonate) larva feeding on underside of air potato leaf, and (b) 2nd instar larva beginning to tunnel into bulbil inside adult feeding scar. Note neonate exuvia on edge of adult feeding scar in (b). [Photos: F.A. Dray Jr.]

Although larvae can feed on foliage (Figure 2a), they prefer bulbils (Figure 2b), and develop through four instars (immature developmental stages) after which they exit the bulbils (Figure 3a). Neonates are unable to penetrate the periderm (outer ‘skin’) of the bulbil, so they use adult feeding holes (Figure 2b) to access the bulbil interior. Neonates have also been observed entering the bulbil via breaks in the periderm formed when the bulbil begins to sprout. Later (2nd and 3rd) instar larvae can penetrate the periderm and have also been observed cannibalizing younger instars. The peach-colored pre-pupae (4th instar; see Figure 3c) exit the bulbil and crawl to the soil. Although some form naked pupae (Figure 3b), the vast majority form a cocoon composed of a white substance secreted from their mouths. Soil adheres to this material as it hardens to a Styrofoam™-like texture thereby forming the puparium. A puparium is the hardened last larval skin that encloses the pupa. Puparia can be affixed to the undersides of the bulbil or can be free in the soil, and they often are found in clusters of 2–8 individuals (Figure 3d). Development from neonate to adult requires slightly less time on foliage compared to bulbils (27.4 ± 0.17 days for foliage vs. 28.9 ± 0.23 days for bulbils). However, a much greater proportion (62.9 vs. 44.3 percent) of larvae successfully complete development on bulbils.

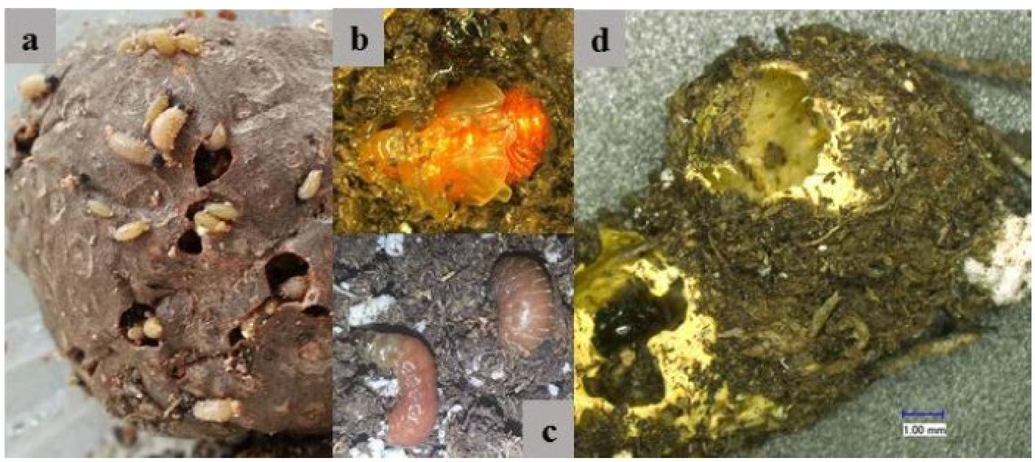

Figure 3. *Lilioceris egena* (a) 2nd to 4th instar larvae exiting air potato bulbil, (b) naked pupa, (c) pre-pupae, and (d) conjoined puparia. Note larval exuviae above pupa in (b) and at back of puparium on bottom left in (d). [Photos: F.A. Dray Jr.]

Egg production and adult emergence from pupae were greatly reduced during December and January indicating the existence of a reproductive diapause (a period of suspended development) that coincides both with cooler weather in the more temperate regions of *L. egena's* distribution and with the period when air potato is typically leafless with vines that have died back. The aerial tubers of air potato fall to the ground soon after the leaves fall, thereby becoming available on the ground where pupation occurs.

III. Affected Environment

A. Taxonomy and Description of Air Potato

Class:	Equisitopsida (Embryophytes)
Subclass:	Magnoliidae (Angiosperms)
Superorder:	Lilianae (monocots)
Order:	Dioscoreales
Family:	Dioscoreaceae (yams)
Genus:	*Dioscorea* L.
Species:	*bulbifera* L

Synonyms: *Helmia bulbifera* (L.) Kunth., *Dioscorea sativa* F.M. Bailey, *D. sativa* Thumb., *D. latifolia* Benth., *D. anthropophagorum* A. Chev. (Wilkin 2001, Gövaerts et al., 2007); *D. crispata* Roxb., *D. dicranandra* Donn. Sm., *D. heterophylla* Roxb., *D. hoffa* Cordem., *D. pulchella* Roxb., *D. tamnifolia* Salisb., *D. tenuiflora* Salisb., *Smilax decipens* Spreng. (Wunderlin et al., 2017); *D. oppositifolia* L., *D. papilaris* Blanco, *D. tunga* Hamilton (Coursey, 1967).

Common names: acom, air potato, air yam, ñame, ala-ala, hoi

Air potato is an herbaceous twining vine, growing 65 feet or more in length. Leaves are broadly cordate (heart shaped) and alternately arranged on stems. A distinguishing characteristic of air potato is that all leaf veins arise from the leaf base. Flowers are inconspicuous (Figure 4a), arising from leaf axils in panicles 4 inches long. Flowers are rarely seen in Florida. The fruit-type produced by female plants in the native range of air potato is a dry capsule which is pale brown at maturity (Figure 4a-c) (Coursey, 1967; Hamon et al., 1995; Raz, 2002). Seeds are winged, elongate, and are slightly curved at the point of attachment (Hamon et al., 1995; Raz, 2002) (Figure 4c). Seeds range in length from 12–22 millimeters (mm) (Raz, 2002). In Florida, vegetative reproduction is the primary mechanism of spread. This is through the formation of aerial tubers, or bulbils, which are formed in leaf axils.

Aerial tubers (bulbils; Figure 4d) may be produced throughout the active growing cycle of the plant, but tend to be more common later in the cycle when stem and leaf development is complete (Coursey, 1967; Miller, 2010). Bulbils are vegetative organs with a shape that is similar to a condensed stem (Coursey, 1967). One to four bulbils may be produced per leaf axil. Bulbils can reach 12 cm in length and have a potato-like appearance. New plants develop from bulbils, and these bulbils serve as a means of dispersal.

Bulbils produced by air potato in Florida are primarily of two types (Figure 4e) matching descriptions of Asian varieties of the plant (Martin, 1974). The majority of bulbils are dark coffee-colored with a bumpy texture (Figure 4e, right). Some plants, however, have been found to produce light tan or grey bulbils with smoother skin (Figure 4e, left; Hammer, 1998; Overholt et al., 2003). A third type of bulbil (Figure 4f) is very rare in Florida, but matches with descriptions of edible African varieties (Martin, 1974), especially *D. bulbifera* var. *anthropophagorum* (Matthews and Terauchi, 1994).

Figure 4. Reproductive structures of air potato: (a) herbarium specimen showing flowers and fruits, (b) close-up of ripening fruits on the vine, (c) close-up of dried fruits showing winged seed, (d) aerial tubers (bulbils) along stem at leaf axils, (e) smooth tan and bumpy brown bulbil types common in Florida, and (f) bulbil of edible African variety uncommon in Florida. [Photos: (a) Kew Herbarium, (b) N. Sasidharan, Kerala Forest Research Institute, (c) Kew Herbarium, (d) K. and F. Starr, Hawaii, (e) W. Overholt, University of Florida, and (f) D. Goodman, TheSurvivalGardener.com.]

B. Areas Affected by Air Potato

1. Native Range of Air Potato

Air potato is broadly pantropical, with two primary types - one African and one Asian - within the species (Terauchi et al., 1991; Figure 5). Chevalier (1936) believed that air potato was originally Asian (likely Chinese), transported to East Africa via Arabian merchants, and then to West Africa via Portuguese merchants. Although Chevalier (1936) may ultimately be correct about the Chinese origin, his assertion about the derivation of the two types is unsupported by the molecular data which suggests that they diverged about 10 million years ago during the Pliocene Epoch (Terauchi et al., 1991). Each type is quite diverse, with Miège (1982) listing 11 varieties in West Africa, and Yifeng et al. (2008) reporting four varieties in China. Both Terauchi et al.

(1991), and Zheng et al. (2006) proposed that Yunnan Province, China, was the center of diversification for Asian varieties of this species. The native range of air potato extends from Africa and Asia (including India) through Malaysia to Australia and the Pacific Islands (Prain and Burkill, 1936; Coursey, 1967; Wilson and Hamilton, 1988; Williams, 2012).

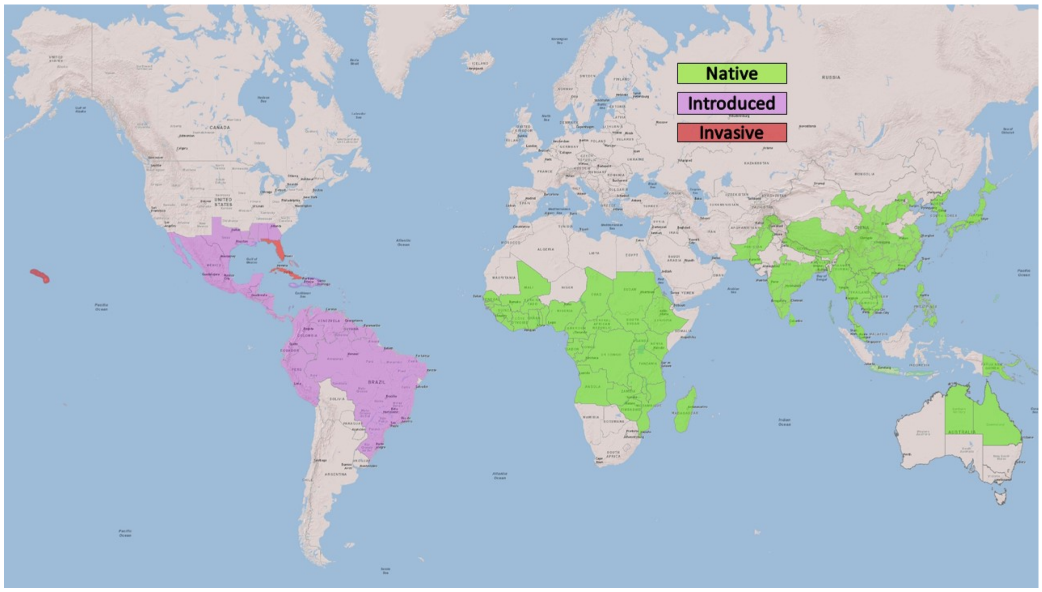

Figure 5. Worldwide distribution of *Dioscorea bulbifera* L. (air potato) (Dray, 2017).

2. Introduced Range of Air Potato

The earliest U.S. record for air potato is William Bartram's (1791) observation during 1777 of this vine in a garden in Mobile, Alabama. The plants he observed likely derived from Africa given that he described the bulbils as being kidney-shaped, a description that fits the African but not the Asian varieties (Martin, 1974; Matthews and Terauchi, 1994; see also Figure 4f). There is no evidence that these plants persisted into the present, however, and Croxton et al. (2011) found that the predominant varieties of air potato in Florida did not match plants tested from West Africa. The Florida types instead match plants from Asia and Oceania, including Hawaii (Croxton et al., 2011). These findings match well with USDA plant importation records showing that the earliest verifiable introduction of air potato into Florida was from Hawaii (as *D. sativa*) in March 1899 (USDA, 1900). Hillebrand (1888) described the bulbils of this species as "green globular bulbs" (a description that fits immature bulbils in Florida) and noted that this species is not native to Hawaii, but was introduced with many other species via Oceanian cultures. The common name "hoi" used in Hawaii (Hillebrand, 1888; Kinsey, 2016) is also used for this plant in Sumatra.

Since its introduction to Florida, air potato has aggressively spread throughout the state: from Baldwin County in the northwestern panhandle to Miami-Dade County at the southern tip of the state. Collections from herbaria and reports from state regional biologists listed 60 of 67 Florida counties infested with air potato in 2017 (Wunderlin et al., 2017; EDDMaps 2017; Figure 6), up from the 29 counties reported by Wheeler (2007). This species is reportedly naturalized in Georgia, Alabama, Mississippi, Louisiana, Texas, and Hawaii (Nesom and Brown, 1998; EDDMapS, 2017). Villaseñor and Espinosa-Garcia (2004) also report air potato from 10 states in Mexico.

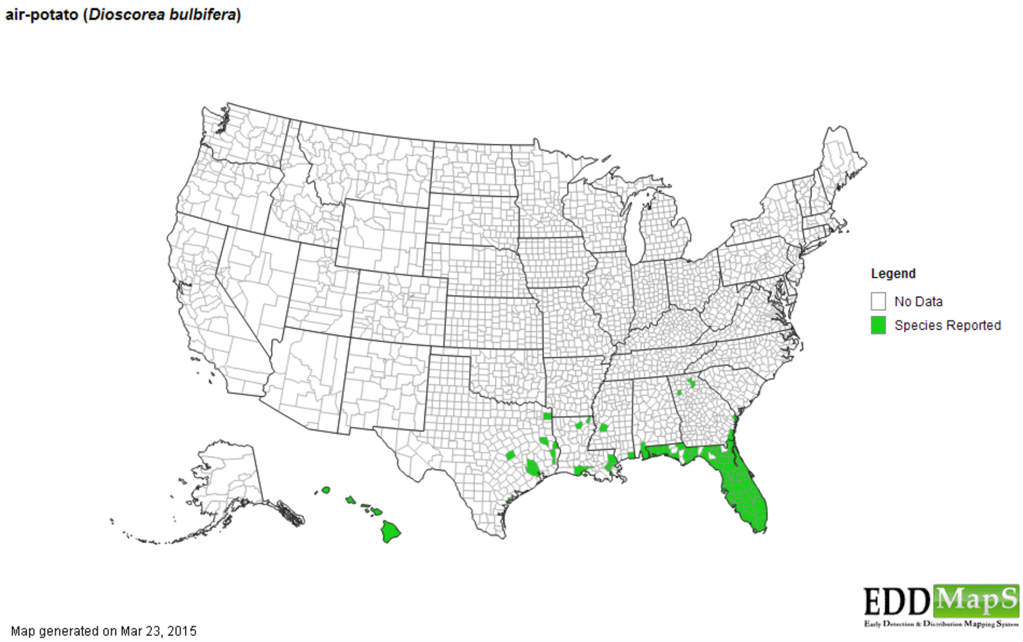

Figure 6. Distribution of *Dioscorea bulbifera* L. in the United States (EDDMapS, 2017).

Based on the known range of air potato in the United States (Figure 6), the plant can survive in areas with an average annual minimum temperature range of -12.2 to -9.5°C (10 to 15°F) — zone 8b on the USDA Hardiness Zone Map. Climatic data (minimum January temperature and annual rainfall) from locations where air potato is known to occur in Florida were extrapolated outside of Florida by Overholt et al. (2014) to estimate its potential distribution in the United States (Figure 7). These data suggest that air potato may be able to spread more extensively throughout the Gulf coast and along the Atlantic coast as far north as Charleston, South Carolina.

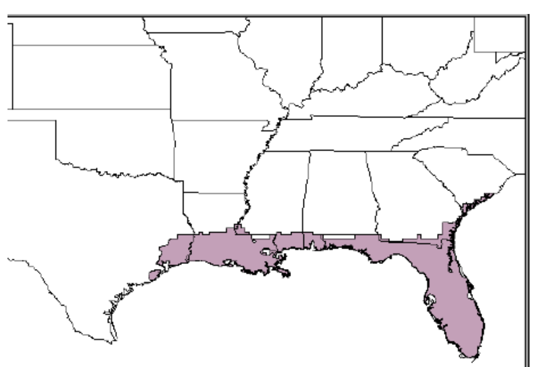

Figure 7. Potential range of air potato in North America (from Overholt et al., 2014).

3. Habitats Where Air Potato is Found in North America

In Florida, air potato is frequently found in tropical and subtropical hammocks but may also invade disturbed uplands, scrub, sinkholes, alluvial flood plain forests, urban lots (Schultz, 1993; Gann et al., 2001), pinelands (Langeland and Craddock-Burks, 1998), and hedges or fencerows (Bell and Taylor, 1982). Evidence also suggests that air potato aggressively exploits forest canopies damaged by hurricanes, thereby impeding the reestablishment of native species (Horvitz et al., 1998; Gordon et al., 1999).

C. Plants Related to Air Potato and Their Distribution

1. Native and Non-Native Relatives

The family Dioscoreaceae currently includes 644 species in four genera: *Dioscorea, Stenomeris, Tacca*, and *Trichopus* (Gövaerts et al., 2007; Viruel et al., 2016). The largest genus in the family, *Dioscorea*, contains approximately 600 species (Raz, 2002), most of which grow in the subtropics or tropics, with only a few species growing in temperate regions (Al-Shehbaz and Schubert, 1989; Raz, 2002). The *Dioscorea* genus is grouped into subgeneric sections (Uline, 1897; Kunth, 1924). Traditionally, air potato has been placed in section Opsophyton along with a few other tropical Old World (Africa, Asia and Europe) species (Kunth, 1924; Huber, 1998). The two native North American (north of Mexico) species, *D. floridana* (Florida yam) and *D. villosa* (wild yam), are assigned to the section Macropoda (Kunth, 1924; Raz, 2002). Raz (2016) suggests that these two U.S. *Dioscorea* species are separated in relatedness from air potato.

The native West Indian Dioscoreaceae are represented by 28 species of *Dioscorea*, 19 of which are found in *Rajania*, a subgenus recently merged into the genus *Dioscorea* (Raz, 2016). The center of origin for this group appears to be Cuba (Kunth, 1924; Raz, 2016).

Mexico harbors 73 species of *Dioscorea*, including the introduced *D. bulbifera* (air potato) and *D. alata*. None of the native species in Mexico are in the section Opsophyton to which air potato belongs, and so the Mexican species are also less closely related to air potato.

The remaining three genera in the family Dioscoreaceae represent the type genera for their former respective families. *Stenomeris* contains two species found only in Southeast Asia. Similarly, *Trichopus* contains one Asian species and one species native to Madagascar. However, the genus *Tacca* with 13 tropical species contains some New World (North, Central, and South America and nearby islands) representatives.

The family Dioscoreaceae is placed in the order Dioscoreales, a small order that has had its member families change considerably with DNA analysis (Judd et al., 2002). The order Dioscoreales was more recently redefined (Caddick et al., 2002a, 2002b; Judd et al., 2002; Gövaerts et al., 2007) to contain, in addition to the family Dioscoreaceae, the family Nartheciaceae and the tiny mycoparastic herbs in the family Burmanniaceae.

The family Burmanniaceae has 14 genera, three of which (*Burmannia, Apteria*, and *Thismia*) are represented in the plants of North American (Gövaerts et al., 2007). The genus *Burmannia* has three species in North America (*B. biflora, B. capitata*, and *B. flava*), all of which occur in Florida (Wunderlin et al., 2017). The genus *Apteria* has a single species which also occurs in Florida (*A. aphylla*) (Wunderlin et al., 2017), whereas *Thismia*'s single species is limited to a small area in northern Illinois (Gövaerts et al., 2007). The family Nartheciaceae contains five genera: three in North America (*Narthecium, Lophiola*, and *Aletris*), one limited to Japan and Korea (*Metanarthecium*), and one in northern South America (*Nietneria*) (Fuse et al., 2012). The genus *Aletris* has five species in North America (*A. aurea*, *A. bracteata*, *A. farinosa, A. lutea*, and *A. obovata*), all of which occur in Florida (Gövaerts et al., 2007; Wunderlin et al., 2017). The genus *Lophiola* has a single species (*L. aurea*) found from Nova Scotia south to Florida (Wunderlin et al., 2017). The genus *Narthecium* has seven species total, with two found in North America: one (*N. californicum*) from Oregon and California, and one (*N. americanum*) scattered from New Jersey to North Carolina (Gövaerts et al., 2007).

The subtropical order Pandanales is a sister group to the Dioscoreales in the most current analyses (Hertweck et al., 2015). It contains five families (APG IV, 2016): Cyclanthaceae which is found from Mesoamerica through South America, and in the West Indies; Pandanaceae which occurs in the Old World tropics and subtropics; Stemonaceae which is largely Asian and Australian, but contains a single North American species - the Florida (and southeastern U.S.) native *Croomia pauciflora* (Wunderlin et al., 2017); Triuridaceae which is scattered across the Old and New World tropics and has no North American representatives; and Velloziaceae which occurs in Africa, Asia, and South America.

2. Economically and Environmentally Important Relatives

The genus *Dioscorea* contains the true yams, several of which are important food crops in tropical and subtropical countries worldwide (Martin, 1974; Coursey, 1981; Prance and Nesbitt, 2005; Wheeler et al., 2007; FAO, 2017). None of the cultivated *Dioscorea* species are grown commercially in the continental United States (Wheeler et al., 2007; FAO, 2017), but a few are important commodities in the New World tropics. *Dioscorea alata* has historically been grown in the region and is still grown in the Bahamas (Correll and Correll, 1982), Cuba (Leon and Alain, 1974), Hispaniola (Liogier, 2000), Jamaica (Adams, 1972), Puerto Rico (Liogier and Martorell, 1982), and the Virgin Islands (USDA-NRCS, 2002). *Dioscorea cayenensis* (reported as *D. occidentalis* in Leon and Alain, 1974) is cultivated in Cuba, Jamaica, and Puerto Rico (Liogier and Martorell, 1982).

IV. Environmental Consequences

A. No Action

1. Impact of Air Potato

a. Animals

Air potato often dominates habitats that it invades. It negatively impacts wildlife dependent on native vegetation for forage, nesting, and cover.

b. Native Plants

Air potato poses a threat to natural areas because of its ability to trellis over and out-compete native vegetation for limited resources, especially sunlight (Figure 8; Schmitz, 1994; Langeland and Craddock Burks, 1998; Gordon et al., 1999). Wunderlin et al. (2017) lists over 75 species in Florida that have a vining habit. Given that air potato shares this growth form and invades a variety of plant communities, it seems likely that some of these native vines (e.g., *Dioscorea floridana*) are at risk of replacement by air potato.

Figure 8. *Dioscorea bulbifera* trellising over native palms, pines, hardwoods, and understory vegetation in Snyder Park, Broward County, Florida, during June 2012. [Photo: T. Center, U.S. Dept. Agriculture]

c. Human Health

No reports of detrimental health effects from air potato are known, although aerial bulbils of the main variety in Florida are reportedly poisonous (Martin, 1974).

d. Social and Recreational Uses

Children reportedly play with the aerial bulbils which, because of their size and weight, make them appealing to carry and throw – similar to snowballs in more temperate climatic regions (Pemberton, 2009). This and the unusual shapes of some bulbils promote their collection (Pemberton, 2009). However, this play and collection can result in the spread of bulbils and the vine.

e. Beneficial Uses

Air potato was introduced into Florida both as an ornamental and as a potential crop plant (Fairchild, 1938; Nehrling, 1944), but was never really a commercial success in either arena. *Dioscorea alata* is a popular ethnic West Indian food plant. Yet despite this crop's presence in over 400 gardens during the early 20th century (Young, 1923), commercial production failed to persist and home cultivation of this species in Florida today is uncommon. No purposeful cultivation of air potato or any other *Dioscorea* is known in Florida today, although one local survivalist does recommend wild *D. alata* (Good, 2016).

2. Impact from Use of Other Control Methods

The continued use of chemical, mechanical, and biological controls at current levels would be a result if the "no action" alternative is chosen. These environmental consequences may occur even with the implementation of the biological control alternative, depending on the efficacy of *L. egena* to reduce air potato populations in the contiguous United States.

a. Chemical Control

Herbicidal control with glyphosate applications in heavily infested areas (e.g., Fern Forest, Broward County, Florida) that included other invasive weeds such as Brazilian peppertree and bishop wood, cost $1,750/hectare (ha)/year. In this example, complete control was not achieved as re-sprouts continued despite three herbicide treatments over nearly two years. It has been estimated that five years or more of herbicidal treatments and monitoring would be required to achieve control. The herbicidal control method has additional costs as non-target species, especially native species, suffer damage from non-selective products.

b. Mechanical Control

Mechanical control is labor-intensive and provides only a temporary solution that requires constant retreatment as plants continue to sprout up from bulbils. Plants in isolated locations are difficult to access. Manual removal of air potato plants is harmful to the native plant species that is being climbed by the vine.

c. Biological Control

Foliar damage caused by *L. cheni* is credited with reducing abundance and overall biomass of air potato bulbils (Overholt et al., 2016). Despite the observed reductions attributable to *L. cheni*, bulbil production remains a serious concern because air potato is not known to reproduce sexually in North America, so the primary means of multiplying and spreading in Florida is via bulbils.

B. Issue Permits for Environmental Release of *Lilioceris egena*

1. Impact of *L. egena* on Nontarget Plants

Host specificity of *L. egena* to air potato has been demonstrated through scientific literature, field observations, and host range testing. If the candidate biological control agent only attacks one or a few plant species closely related to the target weed, it is considered to be very host-specific. Host specificity is an essential trait for a biological control organism proposed for environmental release.

a. Scientific Literature

Tishechkin et al. (2011) reports *L. egena* adults feeding on *Dioscorea subcalva*, a plant known only from Yunnan, Guangxi, Guizhou, and Chongqing Provinces in central China.

b. Field Observations

During collecting trips in China and Nepal, the permit applicant/researcher observed adult *L. egena* feeding only on *D. bulbifera* (air potato) (see also Center et al., 2013).

c. Host Range Testing

Quarantine host range testing was conducted to determine the specificity of *L. egena* for air potato and to determine if plants in the continental United States could be at risk of attack by *L. egena.*

(1) Site of Quarantine Studies in the United States

Quarantine host specificity studies were conducted at the Invasive Plant Research Laboratory, USDA Agricultural Research Service, 3225 College Avenue, Fort Lauderdale, FL 33314.

(2) Test Plant List

Test plant lists are developed by researchers for determining the host specificity of biological control agents of weeds in North America. Test plant lists are usually developed on the basis of phylogenetic relationships between the target weed and other plant species (Wapshere, 1974). It is generally assumed that plant species more closely related to the target weed species are at greater risk of attack than more distantly related species.

The host specificity test strategy as described by Wapshere (1974) is "a centrifugal phylogenetic testing method which involves exposing to the organism a sequence of plants from those most closely related to the weed species, progressing to successively more and more distantly related plants until the host range has been adequately circumscribed." Researchers do not pursue release of biological control agents that do not demonstrate high host specificity to the target weed.

For selecting the test plants for *L. egena*, the primary strategy followed the centrifugal phylogenetic method (Wapshere 1974) modified, in part, per Briese and Walker (2008). Host range of *L. egena* was determined through testing of 82 plant species in 46 families and 25 orders: 15 species within the Dioscoreaceae and 67 species outside of the Dioscoreaceae. Test plants within the Dioscoreaceae were chosen to represent the major taxonomic sections of the family with representatives in Florida and the West Indies, and species of economic importance. Additionally, the ornamental species *Tacca chantrieri* was tested, which is a member of the only other genus in the family Dioscoreaceae that occurs in Florida. Also, representative species were included whose tubers/corms/expanded rhizomes are economically important crops. This was important given that *L. egena* has proven to be an air potato storage organ (bulbil) specialist.

Representatives of the Burmanniaceae, sister family to Dioscoreaceae (Judd et. al., 2002; Merckx et al., 2006; 2010), were considered for testing – including representatives of the three *Burmannia* species and the single *Apteria* species that occurs in Florida (Wunderlin et al., 2017). However, these plants are tiny, short-lived, fungal parasites with thread-like flower stalks that rise only a few inches above the ground and thus were considered to be wholely inadequate to support any *L. egena* feeding and development.

Members of Nartheciaceae, the only other family in the order Dioscoreales, were also considered for testing – including the five species of *Aletris* and the single *Lophiola* species that occur in Florida but no sources of these species could be found. However, *Lophiola* and *Aletris* primarily inhabit locations with saturated soils, such as bogs, swamps, and moors (Fuse et al., 2012; Wunderlin et al., 2017) that would be unsuitable habitat for successful *L. egena* pupation.

The most closely related order to the Dioscoreales is the Pandanales (APG IV, 2016), represented in testing by *Pandanus tectorius*. Test plants outside the Dioscoreales/Pandanales group represented orders within superorder Lilianae with Florida natives, as well as families and orders within and outside the Lilianae that are economically important species and/or have tubers/corms/expanded rhizomes.

(3) Discussion of Host Specificity Testing

See appendix 1 for a complete description of host specificity test design and results.

The results of host range testing indicate that the beetle *Lilioceris egena* is highly specialized on its target host, *Dioscorea bulbifera* (air potato). Oviposition occurred only on this plant, and females tended to hold eggs while on air potato foliage only to lay eggs as soon as being placed upon bulbils. Thus, the ovipositional specificity occurs at the organ level within a single species, and not just the species as a whole. Neonates failed to develop on any plant species aside from air potato and developed better on air potato bulbils/tubers than on leaves. Further, in a preliminary choice test in which neonates were placed in arenas with leaves and bulbil slices, the larvae always moved onto the bulbils, even if placed directly on the leaves first. The reverse, larvae abandoning bulbils for leaves, never occurred. Finally, the data from 2nd/3rd instar larval trials suggest that late instar larvae from air potato leaves or bulbils may occasionally migrate to, and complete development on, a few *Dioscorea* congeners (*D. alata, D. cordata, D. trifida*) in areas of Florida and the Caribbean where these congeners are intermixed and the larvae cannot locate their preferred host. The exteme rarity of this occurance in the no choice trials (3 of 456 2nd/3rd instar larvae on Dioscoreaceae; less than 1 percent), which forced the larvae to stay on the non-target host, indicates that the likelihood of this occurring in nature is very low. However, even should this occur, the failure of adults to oviposit and neonates to develop on non-target plants assures that persistent populations could not develop on these non-targets.

2. Impact of *L. egena* on Air Potato

Release of *L. egena* is expected to directly impact air potato reproduction. Successful establishment of *L. egena* on air potato will complement effects already being realized by the release in 2011 of *L. cheni* (Center et al., 2013). Where *L. cheni* is already slowing growth and reducing air potato's dominance in invaded plant communities, production of vegetative propagules (i.e., bulbils) continues though at a reduced level (Overholt et al., 2016). Adult *L. egena* will contribute to foliar damage. More importantly, however, *L. egena*'s strong preference to lay eggs on air potato bulbils should lead to large numbers of bulbils being damaged by this beetle, thereby reducing their ability to sprout (Pemberton and Witkus, 2010). Vegetative propagation is the means by which this vine expands its geographic range, so damage to bulbils will likely restrict further spread of this invasive weed in Florida and the southern United States.

3. Animals

Reduction of air potato by *L. egena* could be beneficial to animals because it would reduce the potential of air potato to dominate animal habitats. Air potato negatively impacts wildlife dependent on native vegetation for forage, nesting, and cover.

4. Native Plants

Direct impact of the beetle will be restricted to the target weed, air potato. Adult feeding on native U.S., Caribbean, and Mesoamerican *Dioscorea* species found in close proximity to air potato is possible should heavy beetle infestations develop. Such damage would be short lived and only cosmetic; however, *L. egena*'s close connection to its host precludes oviposition and neonate development upon non-hosts, as demonstrated in host range testing.

Because air potato is uncommon in agricultural areas, except occasionally along fence lines, the primary benefits derived from release of *L. egena* will occur in natural areas. The beneficial indirect impact on native plants, will be substantial if *L. egena* results in reduction in air potato vine densities. Trellising of vines that smother trees and shrubs will be reduced, thereby fostering a more diverse canopy and mid-story plants. Population reductions of air potato will also, in conjunction with defoliation by *L. cheni*, promote light penetration to the forest floor thereby stimulating understory plant growth.

5. Human Health

Some varieties of air potato are cultivated for consumption in Asia and Africa, but this yam is not an important food crop anywhere in the New World (Asiedu and Sartie, 2010; FAO, 2017). Similarly, although dried bulbils/tubers are used in traditional medicines in Asia and Africa, air potato is not known to be used similarly in the United States or Caribbean. Many varieties are considerably bitter and can cause vomiting and diarrhea (Kawasaki et al., 1968; Martin, 1974; Telek et al., 1974; Webster et al., 1984; Bhandari and Kawabata, 2005), so control of the plant will reduce potential for human illness arising from consumption. Thus, control of this weed will not negatively affect human health, and may in fact have some small positive human health benefit.

No human health effects are known to be associated with *L. egena* or any other *Lilioceris* species.

6. Beneficial Uses

Lilioceris egena would reduce the quantity of bulbils for children to play with, collect, and throw.

7. Uncertainties Regarding the Environmental Release of *L. egena*

Once a biological control agent such as *L. egena* is released into the environment and becomes established, there is a slight possibility that it could move from the target plant (air potato) to attack nontarget plants. Host shifts by introduced weed biological control agents to unrelated plants are rare (Pemberton, 2000). Native species that are closely related to the target species are the most likely to be attacked (Louda et al., 2003). If other plant species were to be attacked by *L. egena*, the resulting effects could be environmental impacts that may not be easily reversed. Biological control agents such as *L. egena* generally spread without intervention by man. In principle, therefore, release of this biological control agent at even one site must be considered equivalent to release over the entire area in which potential hosts occur, and in which the climate is suitable for reproduction and survival. However, significant non-target impacts on plant populations from previous releases of weed biological control agents are unusual (Suckling and Sforza, 2014).

In addition, this agent may not be successful in reducing air potato populations in the contiguous United States. Worldwide, biological weed control programs have had an overall success rate of 33 percent; success rates have been considerably higher for programs in individual countries (Culliney, 2005). Actual impacts on air potato by *L. egena* will not be known until after release occurs and post-release monitoring has been conducted (see Appendix 2 for release protocol and post-release monitoring plan). It is expected that *L. egena* will damage bulbils, likely restricting further spread of air potato in Florida and the southern United States.

8. Cumulative Impacts

"Cumulative impacts are defined as the impact on the environment which results from the incremental impact of the action when added to other past, present and reasonably foreseeable future actions regardless of what agencies or person undertakes such other actions" (40 CFR 1508.7).

Other private and public concerns work to control air potato in invaded areas using available chemical, mechanical, and biological control methods. Release of *L. egena* is not expected to have any negative cumulative impacts in the continental United States because of its host specificity to air potato. Effective biological control of air potato will have beneficial effects for Federal, State, local, and private weed management programs, and may result in a long-term, non-damaging method to assist in the control of air potato.

9. Endangered Species Act

Section 7 of the Endangered Species Act (ESA) and ESA's implementing regulations require Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of federally listed threatened and endangered species or result in the destruction or adverse modification of critical habitat.

In the continental United States, there are no plants that are federally listed or proposed for listing in the family Dioscoreaceae, the same family as the target weed. However, in host range testing, minor feeding occurred on foliage or storage organs of some plants in the families Poaceae, Amaranthaceae, Apiaceae, Liliaceae, Convolvulaceae, Fabaceae, and Brassicaceae that contain federally listed and candidate plant species. Most of these plant species would not overlap with the projected distribution of air potato. Based on the host specificity of *L. egena* reported in testing, field observations, and in the scientific literature, APHIS has determined that environmental release of *L. egena* may affect, but is not likely to adversely affect listed plants in the families Poaceae, Amaranthaceae, Apiaceae, Liliaceae, Convolvulaceae, Fabaceae, and Brassicaceae. APHIS has also determined that the release of *L. egena* may affect beneficially the Stock Island tree snail, *Orthalicus reses*.

A biological assessment was prepared and submitted to the U.S. Fish and Wildlife Service (FWS) and is part of the administrative record for this EA (prepared by T.A. Willard, May 31, 2018, and revised November 6, 2018). APHIS requested concurrence from the FWS on these determinations, and received a concurrence letter dated February 21, 2019.

V. Other Issues

Consistent with Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-income Populations," APHIS considered the potential for disproportionately high and adverse human health or environmental effects on any minority populations and low-income populations. There are no adverse environmental or human health effects from the field release of *L. egena* and will not have disproportionate adverse effects to any minority or low-income populations.

Consistent with EO 13045, "Protection of Children from Environmental Health Risks and Safety Risks," APHIS considered the potential for disproportionately high and adverse environmental health and safety risks to children. No circumstances that would trigger the need for special environmental reviews are involved in implementing the preferred alternative. Therefore, it is expected that no disproportionate effects on children are anticipated as a consequence of the field release of *L. egena*.

EO 13175, "Consultation and Coordination with Indian Tribal Governments," was issued to ensure that there would be "meaningful consultation and collaboration with tribal officials in the development of Federal policies that have tribal implications…."

APHIS is consulting and collaborating with Indian tribal officials to ensure that they are well-informed and represented in policy and program decisions that may impact their agricultural interests in accordance with EO 13175.

VI. Agencies, Organizations, and Individuals Consulted

The Technical Advisory Group for the Biological Control Agents of Weeds (TAG) recommended the release of *L. egena* on March 30, 2018. The TAG members that reviewed the release petition (17-01) (Dray, 2017) included USDA representatives from the National Institute of Food and Agriculture, and Agricultural Research Service; U.S. Department of Interior's U.S. Geological Survey, Bureau of Land Management and U.S. Fish and Wildlife Service; U.S. Army Corps of Engineers; and representatives from California Department of Food and Agriculture (National Plant Board), Mexico Secretariat of Agriculture, Livestock, Rural Development, and Fisheries, and Agriculture and Agri-Food Canada.

This EA was prepared by personnel at APHIS and ARS. The addresses of participating APHIS units, cooperators, and consultants follow.

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Environmental and Risk Analysis Services
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Animal and Plant Health Inspection Service

Plant Protection and Quarantine
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Appendix 1. U.S. host-specificity testing methods and results (Dray, 2017).

Experimental Design

All 82 species of plants were included in foliage feeding and oviposition trials, and 33 of these were included in storage organ feeding and oviposition trials (see Table 1-1).

Source of plants

The *D. bulbifera* bulbils, or plants grown from bulbils, used in this study were collected either at Tree Tops Park, Davie FL (26.070434°N, -80.270151°W) or at Easterlin Park, Oakland Park, Florida (26.170197°N, -80.160279°W). Colonies of adult beetles were maintained on bulbils collected at these same sites. Some test plants remaining from the *L. cheni* host range studies (Pemberton and Witkus, 2010) and otherwise unobtainable (e.g., *Dioscorea altissima*) were incorporated into this present project but may not have been included in all trial types due to limited numbers (e.g., *Ipomoea pandurata*). Other plants were obtained from a variety of commercial sources; some as live plants, others as seed. A few species (e.g., *Smilax laurifolia)* were hand dug at field sites and repotted for cultivation. Foliage for trials of large trees was collected from species that are growing live on the USDA campus (e.g., *Ficus aurea*) or at nearby parks (e.g., *Salix caroliniana*). All field collected material was done so with permission of the appropriate land owners and with appropriate state and local permits.

Table 1-1. Plant species included in the host range trials with *Lilioceris egena*.

Order	Family	[1] Genus (Section[2]) species Authority[3]	Common names	Distribution/Status/Comments[4]
Category 1 - Genetic Type of the Target Weed Found in North America				
Dioscoreales	Dioscoreaceae	* *Dioscorea (Opsophyton) bulbifera* L.	air potato	OWT; *FL, GA, LA, Carib., M.Am.*
Category 2 (a) - Species in the Same Genus as the Target Weed: North American (excluding Mexico)				
Dioscoreales	Dioscoreaceae	*** *Dioscorea (Macropoda) floridana* Bartlett**	Florida yam	FL, GA, SC
	Dioscoreaceae	*** *Dioscorea (Macropoda) villosa* L.**	fourleaf yam, wild yam	FL north to Canada, west to Texas; /hort. (OH, Canada)
Category 2 (b) - Species in the Same Genus as the Target Weed: West Indian/Mesoamerican/South American				
Dioscoreales	Dioscoreaceae	*Dioscorea (Chondrocarpa) altissima* Lam.	dunguey	Brazil; *Puerto Rico*
	Dioscoreaceae	*Dioscorea (Rajania) cordata* (L.) Raz	himber	Puerto Rico, Cuba, Jamaica
	Dioscoreaceae	* *Dioscorea (Dematostemon) pilosiuscula* Bertero ex Spreng.	bulbous yam, air yam, dungeuy	Carib., M.Am., trop. S.Am.
	Dioscoreaceae	* *Dioscorea (Lynchnostemon) polygonoides* Humb. and Bonpl. ex Willd.	Jamaican bitter yam, mata gallina,	M.Am., trop. S.Am.
	Dioscoreaceae	* *Dioscorea (Macrogynodium) trifida* L.f.	yampi, cush-cush, mapuey, inhame, tabena, sacha papa	M.Am., trop. S.Am. /crop
Category 2 (c) - Species in the Same Genus as the Target Weed: Weedy and/or Exotic in U.S.				
Dioscoreales	Dioscoreaceae	* *Dioscorea (Enantiophyllum) alata* L.	purple yam, water yam, white yam, winged yam, name blanco	trop. Asia; *SE US, Puerto Rico, Virgin Islands* /crop (not US)
	Dioscoreaceae	* *Dioscorea (Enantiophyllum) cayenensis* Lam. *(combines subspp)*	yellow yam, Lagos yam, name amarillo	west and central Africa; *Carib., M.Am., S.Am.* /crop
	Dioscoreaceae	* *Dioscorea (Combilium) esculenta* (Lour.) Burkill	lesser yam, Asiatic yam, gan shu	trop. and subtrop. Asia; *Carib.* /crop
	Dioscoreaceae	* *Dioscorea (Enantiophyllum) oppositifolia* L.	nagaimo, Chinese yam	southern India; (where this species is listed as in U.S., the plants are actually *D. polystachya*)
	Dioscoreaceae	* *Dioscorea (Enantiophyllum) polystachya* Turcz.	Chinese yam, shan yao, cinnamon vine	temp. Asia; *FL north to MA, west to AR* (often listed under its synonym, *D. batatas*); /hort. (as *D. batatas*)
	Dioscoreaceae	* *Dioscorea (Opsophyton) sansibarensis* Pax	Zanzibar yam	trop. Africa; *FL* (but possibly eradicated)
Category 3 - Species in Other Genera in the Same Family (Dioscoreaceae) as the Target Weed				
Dioscoreales	Dioscoreaceae	* *Tacca chantrieri* André	batflower, devil flower	trop. Asia; /FL hort.
Category 4 - Threatened and Endangered Species in the Same Family (Dioscoreaceae) as the Target Weed				
Dioscoreales	Dioscoreaceae	There are no U.S. or Florida listed species in this family		
Category 5 - Species in Other Families in the Same Order (Dioscoreales) with Similarities to the Target Weed				
Dioscoreales	Burmanniaceae	Species in this family were unobtainable		

Order	Family	[1] Genus (Section[2]) species Authority[3]	Common names	Distribution/Status/Comments[4]
	Nartheciaceae	Species in this family were unobtainable		
		Category 6 (a) - Species in Closely Related Orders to the Target Weed		
Pandanales	Pandanaceae	*Pandanus tectorius* Park. ex Du Roi	variegated dwarf pandanus	Malesia, AU, Pacific Islands; /hort (often sold as *P. baptistii*)
		Category 6 (b) - Species in the Same SuperOrder (Lilianae) as the Target Weed		
Alismatales	Alismataceae	***Sagittaria latifolia* Willd.**	arrowhead, wapato	N.Am.
	Araceae	*Alocasia cucullata* (Lour.) Schott	dwarf elephant ear	trop. Asia; /crop
	Araceae	*Caladium bicolor* (Aiton) Vent.	angel wings, heart of Jesus, elephant ear	M.Am., S.Am.; *trop. India, trop. Africa* /hort.
	Araceae	** Colocasia esculenta* (L.) Schott	taro	Malaysia, AU, PNG; *India, Egypt, Africa, Carib., M.Am., S.Am., SE US;*
	Araceae	*Symplocarpus foetidus* Salisb.	skunk or swamp cabbage	eastern US to Canada
	Araceae	** Xanthosoma sagittifolium* (L.) Schott	arrowleaf elephant ear, nampi, malanga	NWT; /crop
	Araceae	*Zantedeschia aethiopica* (l.) Spreng.	calla lily, arum lily	southern Africa; *AU*
Arecales	Arecaceae	***Sabal palmetto* (Walt.) Lodd.**	cabbage palm	SE US, Bahamas, Cuba; /hort.
Asperagales	Amaryllidaceae	***Crinum americanum* L.**	bog lily, string lily	SE US, Mexico, Cuba, Jamaica; *Puerto Rico*
	Amaryllidaceae	*Zephyranthes minuta* (Kunth) D. Dietr.	pink rain lily	M.Am.; *Carib., SE US, HI*; /hort. [*Z. atamasco* (L.) Herb., *Z. simpsonii* Chapm., and *Z. treatiae* S.Watson are all FL state threatened]
Commelinales	Commelinaceae	*Tradescantia pallida* (Rose) D.R. Hunt	wandering jew, purple heart	Mexico; /hort.
	Pontedariaceae	***Pontederia cordata* L.**	pickerelweed	SE US
Poales	Cyperaceae	***Cladium mariscus* subsp. *jamaicense* (Crantz) Kük.**	sawgrass	M.Am., S.Am. SE US, Carib.; *trop. Africa, New Guinea, HI*
	Juncaceae	***Juncus effusus* L.**	soft rush, corkscrew rush	EU, Asia, Africa, N.Am., S.Am.; *AU, Madagascar, Pacific Is.*
	Musaceae	*Musa acuminata* Colla	wild banana	SE Asia; /crop
	Poaceae	*Saccharum officinarum* L.	sugar	SE Asia; /crop
	Poaceae	*Zea mays* L.	corn	Mexico; /crop
Zingiberales	Cannaceae	*Canna glauca* L.	canna lily	Carib., M.Am., S.Am., TX, LA; *OWT*
	Cannaceae	*Canna* cultivar *americanallis*var. *variegata*	variegated canna lily, Bengal tiger lily	uncertain, possibly India; *worldwide trop. and subtrop.* /hort.
	Costaceae	*Costus woodsonii* Maas	red button ginger, lipstick plant	M.Am; /hort.
	Heliconiaceae	*Heliconia bihai* (L.) L.	lobster claw, macawflower	northern S.Am., Carib.; /hort.
	Marantaceae	** Maranta arundinacea* L.	arrowroot, maranta, araru	M.Am., S.Am., Carib.; *FL, OWT* /crop
	Marantaceae	***Thalia geniculata* L.**	arrowroot, fireflag, alligatorflag	M.Am, S.Am., SE US, Carib., Africa
	Zingiberaceae	** Curcuma longa* L.	turmeric	trop. and subtrop. Asia; /crop

Order	Family	[1] Genus (Section[2]) species Authority[3]	Common names	Distribution/Status/Comments[4]
	Zingiberaceae	* *Hedychium coronarium* J. Koenig	white ginger lily	trop. Asia; *Carib., S.Am., SE US, HI* /hort.
	Zingiberaceae	* *Zingiber officinale* Roscoe	ginger	uncertain, possibly India; *worldwide* /crop
		Category 6 (c) - Species outside the Superorder (Lilianae) containing the Target Weed		
Apiales	Apiaceae	*Apium graveolens* L.	celery	uncertain; *worldwide* /crop
	Apiaceae	* *Daucus carota* L.	carrot	EU, SW Asia; *worldwide* /crop
	Araliaceae	* *Panax ginseng* C.A. Mey.	Asian ginseng	temp. Asia; /crop
Asterales	Asteraceae	* *Arctium lappa* L.	burdock, gobo, lappa	temp. EU, temp. Asia; *worldwide* /crop
Brassicales	Brassicaceae	* *Brassica rapa* L.	turnip	temp. Asia; *worldwide* /crop
	Brassicaceae	* *Raphanus sativus* L.	radish	possibly SE Asia; *worldwide* /crop
Caryophyllales	Amaranthaceae	* *Beta vulgaris* L.	beet	southern coastal EU, N Africa, W Asia; *worldwide* /crop
Fabales	Fabaceae	*Glycine max* (L.) Merr.	soybean	E Asia, AU; *worldwide* /crop
	Fabaceae	*Mimosa pudica* L.	sensitive plant, touch-me-not	M.Am., S.Am., Carib.; *pantropical*
	Fabaceae	* *Pachyrhizus erosus* (L.) Urb.	jicama, Mexican yam, Mexican turnip	Mexico; *trop. Asia* /crop
Gentianales	Rubiaceae	***Guettarda scabra* (L.) Vent.**	rough velvetseed, wild guave	Carib., FL, N S.Am.;
Laurales	Calycanthaceae	***Calycanthus floridus* L.**	Carolina allspice, sweet shrub	E US [FL state endangered]; *China*
Magnoliales	Annonaceae	***Annona glabra* L.**	pond apple	FL, Carib., M.Am., S.Am.; *AU, Sri Lanka*
Malphigiales	Chrysobalanaceae	***Chrysobalanus icaco* L.**	cocoplum	NWT, FL, trop. Africa; /hort.
	Euphorbiaceae	* *Manihot esculenta* Crantz	cassava, yuca, manioc, tapioca-root	Brazil; *OWT* /crop
Piperales	Aristolochiaceae	***Aristolochia tomentosa* Sims**	Dutchman's pipe vine	FL [state endangered] north to MA, west to MO; /hort.
Solanales	Convolvulaceae	* *Ipomoea batatas* (L.) Lam.	sweet potato	NWT; *worldwide trop. and warm temp.* /crop /hort. [*I. microdactyla* Griseb. and *I. tenuissima* Choisy are both FL state endangered]
	Convolvulaceae	***Ipomoea pandurata* (L.) G.F.W.Mey**.	man-of-the-earth, wild sweet potato	SE US;
		Category 7 (a) - Species on Which the Proposed Agent Has Been Recorded		
Dioscoreales	Dioscoreaceae	*Dioscorea subclava* Prain and Burkill is the only species aside from the the target from which *L. egena* has been reported		China; was unobtainable for the study
		Category 7 (b) - Species (or surrogates) on Which Congeners of the Proposed Agent Have Been Recorded[7]		
within Lilianae				
Asperagales	Amaryllidaceae	* *Allium cepa* L.	onion	uncertain, probably Asia; /crop
	Amaryllidaceae	* *Allium sativum* L.	garlic	uncertain, possibly central Asia; /crop
	Asparagaceae	*Asparagus densiflorus* (Kunth) Jessop	Sprenger's asparagus fern	S. Africa; *AU, Carib., FL, CA, HI*
	Asparagaceae	*Asparagus officinalis* L.	asparagus	N. Africa, EU, Asia; *worldwide* /crop

Order	Family	[1] Genus (Section[2]) species Authority[3]	Common names	Distribution/Status/Comments[4]
	Asphodelaceae	*Aloe vera* (L.) Burm.f.	aloe	unknown, possibly N Africa; *worldwide* /hort
	Iridaceae	***Sisyrinchium angustifolium* Mill.**	narrow-leaf blue-eyed grass	FL to Canada west to TX and MN
	Orchidaceae	*Bletilla striata* (Thunb,) Rchb.f.	hyacinth orchid	Japan, Korea, China, Myanmar; *FL* /hort.
Dioscoreales	Dioscoreaceae	** Dioscorea alata L.*	purple yam, water yam, white yam, winged yam, name blanco	trop. Asia; *SE US, Puerto Rico, Virgin Islands* /crop (not US)
Liliales	Liliaceae	*** *Lilium michauxii* Poir**	wild lily	FL north to VA, west to TX [FL state endangered, as are *L. iridollae* M.K.Henry and *L. superbum* L.; *L. catesbaei* Walter is FL state threatened]
	Liliaceae	* *Tricyrtis lasiocarpa* Matsum.	toad lily	Taiwan; /hort.
	Smilacaceae	***Smilax laurifolia* L.**	greenbrier	FL north to NJ and west to Arkansas, Cuba, Bahamas
Pandanales	Pandanaceae	*Pandanus tectorius* Park. ex Du Roi	variegated dwarf pandanus	Malesia, AU, Pacific Islands; /hort (often sold as *P. baptistii*)
Poales	Poaceae	*Triticum aestivum* L.	wheat	*worldwide*
non-Lilianae				
Apiales	Araliaceae	*Schefflera actinophylla* (Endl.) Harms	umbrella tree, octopus tree	AU, New Guinea, Java; *FL, HI, Carib.* /hort.
Asterales	Campanulaceae	***Lobelia cardinalis* L.**	cardinal flower	N.Am. [FL state threatened], M.Am., Columbia; /hort. [*L. boykinii* Torr. and A.Gray ex A.DC.is FL state endangered]
Caryophyllales	Polygonaceae	***Persicaria glabra* (Willd.) M. Gomez**	swamp smartweed	N.Am., elsewhere uncertain
Fabales	Fabaceae	*Senna (Cassia) ligustrina* (L.) H.S. Irwin and Barneby	privet cassia	OWT; *NWT* [*S. mexicana* (Jacq.) H.S.Irwin and Barneby var. *chapmanii* is FL state threatened]
Fagales	Betulaceae	*Corylus americana* Marshall	American hazelnut	eastern and central N.Am.
	Fagaceae	***Quercus virginiana* Miller**	southern live oak	SE US; [*Q. arkansana* Sarg. is FL state threatened]
Gentianales	Apocynaceae	***Asclepias tuberosa* L.**	butterfly milkweed	N.Am.
Lamiales	Verbenaceae	***Callicarpa americana* L.**	American beautyberry	Carib., SE US
Malpighiales	Salicaceae	***Salix caroliniana* Michx.**	coastal plain willow	Carib., SE US, M.Am. [*S. eriocephala* Michx. and *S. floridana* Chapm. are both FL state endangered]
Rosales	Moraceae	***Ficus aurea* Nutt.**	strangler fig	FL, Carib., M.Am.
Solanales	Solanaceae	* *Solanum tuberosum* L.	Irish potato	Peru; *worldwide* /crop
out of Magnoliidae (Angiosperms)				
Cycadales	Cycadaceae	*Cycas revoluta* Thunb.	Sago palm	Japan; *worldwide* /hort.

[1] Species preceded by an * were incorporated in storage organ trials as well as foliage trials.
[2] Raz (2016) was followed in using the *Dioscorea* sections erected by Kunth (1924) despite some deviations in the latter from currently accepted phylogenies.
[3] Florida native species in bold.
[4] Distribution of species are indicated as follows: OWT=Old World tropics/subtropics, NWT=New World tropics/subtropics, N.Am.=North America, M.Am.=Mesoamerica, S.Am.=South America, Carib.=Caribbean, EU=Europe, AU=Australia, Africa, Asia. U.S. state designations follow the standard two digit postal code (e.g., Florida=FL). Regions where a species is native are in regular Calibri font, whereas areas of naturalization/invasion are in *Cambria italics*. Horticultural species denoted as /hort. Agricultural species denoted as /crop.

Number of replicates

Each trial was usually replicated a minimum of five times, generally using five individual plants. In some cases, fewer than five individuals were available. In such cases multiple leaves were selected from one individual at random. Similarly, storage organs were divided into sections when necessary to achieve the desired five replicates. *Dioscorea altissma* represents an example of a few cases wherein the only specimens were left over from earlier *L. cheni* host specificity research, but developed a disease that prevented completion of all *L. egena* trials and replacement of the specimens was not possible. Data for such species are presented herein, but will appear incomplete.

Trial methodology

a. Multi-choice (choice minus control) adult storage organ feeding trials

Preliminary adult feeding trials were conducted on the storage organs of 25 species in 13 families (including Dioscoreaceae) and 10 orders (including Dioscoreales), using a multiple choice minus control (*D. bulbifera*) scenario (Table 1-1). These species were selected primarily to represent commercially available crop species that might be encountered by *L. egena* adults in Florida or the Caribbean. Each trial was composed of 9–10 plastic petri dishes (25 cm diameter x 10 cm deep), with each dish containing storage organs from three or four test plant species. A positive control of a separate container containing a *D. bulbifera* bulbil was run together with each trial. The storage organs were placed on moist filter paper and the dishes were sealed with Parafilm® to inhibit desiccation of the test materials. Storage organs that were too large for the containers were sectioned, with the cut ends sealed with Parafilm®. This assured that all beetles had to penetrate the storage organs' epidermal layers to feed, as they would in nature. Five beetles of uniform age were placed in each test arena and monitored for seven days, after which the storage organs were examined for the presence of eggs and scored for feeding damage on a qualitative scale of 0 (no feeding), 1 (a few scrapes), 2 (many scrapes and/or a few notches), 3 (many notches and/or holes), and 4 (burrowing inside bulb/tuber/etc.). These tests were replicated a minimum of five times using fresh storage organs each time. Data were analyzed using non-parametric multiple comparison tests available in SigmaPlot©12.3 (Systat 2011).

b. No choice adult storage organ feeding/ovipositional trials

Adult no choice storage organ trials were conducted on 33 plant species in 15 families and 11 orders (see Table 1-2). Storage organs were placed on moist filter paper in test arenas composed of either a plastic soup (11 cm diameter, 7 cm deep) or food storage (16 x 11 x 7 cm) container ventilated by cutting a rectangular hole in the lid which was then covered with 290 μ mesh screening. Three beetles (two females, one male) were placed in each test arena and monitored 30 days or until there were no live beetles in any test arena containing a non-target host, whichever was longer. Storage organs were replaced as needed (if they began to desiccate or decay). These storage organs were then scored for presence/absence of eggs and assessed for the amount of tissue consumed by all of the beetles in that trial. The latter was accomplished by developing a novel volumetric technique (Dray, in prep.) that required injecting water from a 0.3 mL syringe into the feeding scars/tunnels and then tallying the total across scars, tunnels, and storage organs.

Table 1-2. Species included in preliminary multi-choice adult feeding trials. Most (though not all, e.g. *I. pandurata*, *T. chantrieri*) are crop plants cultivated for their storage organs.

Order	Family	Genus (Section) species Authority[1]
Alismatales	Araceae	*Colocasia esculenta* (L.) Schott
Apiales	Apiaceae	*Apium graveolens* L.
	Apiaceae	*Daucus carota* L.
Asperagales	Amaryllidaceae	*Allium cepa* L.
	Amaryllidaceae	*Allium sativum* L.
Asterales	Asteraceae	*Arctium lappa* L.
Brassicales	Brassicaceae	*Brassica rapa* L.
	Brassicaceae	*Raphanus sativus* L.
Caryophyllales	Amaranthaceae	*Beta vulgaris* L.
	Fabaceae	*Pachyrhizus erosus* (L.) Urb.
Dioscoreales	Dioscoreaceae	*Dioscorea (Opsophyton) bulbifera* L.
	Dioscoreaceae	*Dioscorea (Macropoda) floridana* Bartlett
	Dioscoreaceae	*Dioscorea (Macropoda) villosa* L.
	Dioscoreaceae	*Dioscorea (Enantiophyllum) alata* L.
	Dioscoreaceae	*Dioscorea (Enantiophyllum) cayenensis* Lam. *(combines subspp cayenensis and rotundata)*
	Dioscoreaceae	*Dioscorea (Enantiophyllum) oppositifolia* L.
	Dioscoreaceae	*Dioscorea (Opsophyton) sansibarensis* Pax
	Dioscoreaceae	*Tacca chantrieri* André
Malphigiales	Euphorbiaceae	*Manihot esculenta* Crantz
Solanales	Convolvulaceae	*Ipomoea batatas* (L.) Lam.
	Convolvulaceae	*Ipomoea pandurata* (L.) G.F.W.Mey.
	Solanaceae	*Solanum tuberosum* L.
Zingiberales	Marantaceae	*Maranta arundinacea* L.
	Zingiberaceae	*Curcuma longa* L.
	Zingiberaceae	*Zingiber officinale* Roscoe

No choice adult foliage feeding/oviposition trials

Adult no choice foliage trials were conducted on 82 plant species in 46 families and 25 orders (Table 1-1). Trials were conducted in test arenas (Figure 1-1a,b) composed of plexiglass sleeves (8 cm diameter x 15 cm long) ventilated with four 5 cm holes drilled in the sides and covered with 290 μ mesh insect screening. The sleeves were fitted over leaves of live plants, with a soft foam bung inserted into the bottom of the sleeve and fitted around the plant stem. Another bung sealed the top after the insects were inserted into the sleeve (Figure 1-1b). Test arenas were held in place on bamboo stakes using rubber bands. Three beetles (two females, one male) were placed in each test arena and monitored 30 days or until there were no live beetles in any test arena containing a non-target host, whichever was longer.

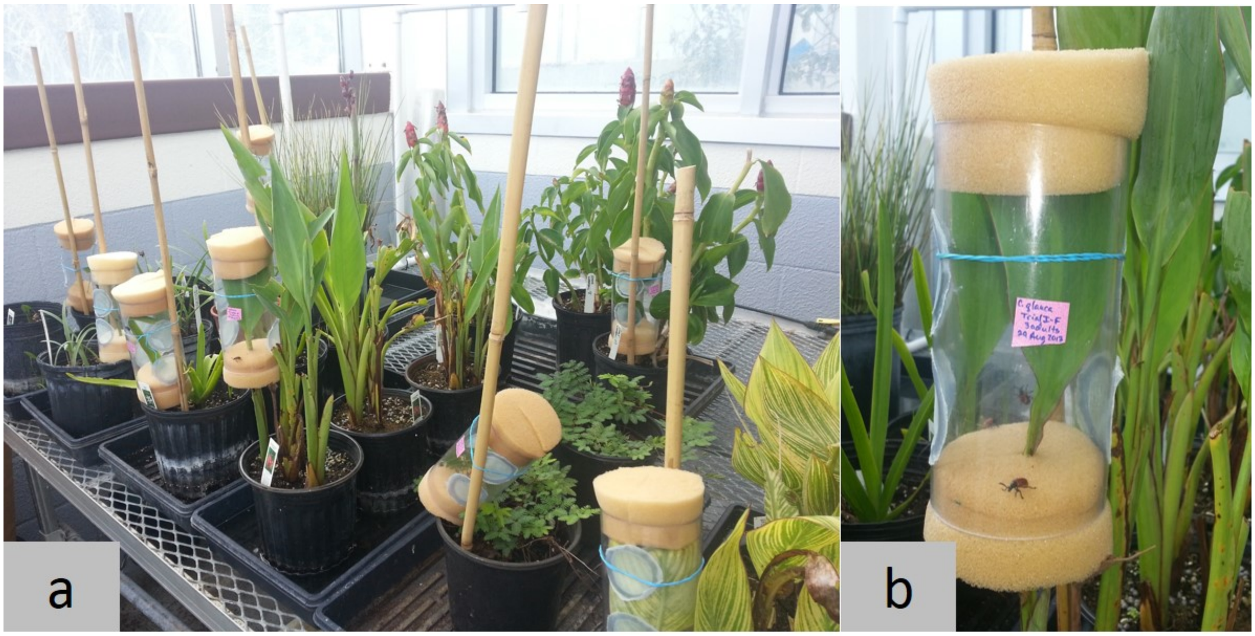

Figure 1-1. Plexiglass sleeve cages used for adult *L. egena* foliage feeding/oviposition trials; (a) test array, (b) individual test arena. [Photos: F.A. Dray Jr.]

Test arenas were moved onto fresh leaf material as needed. All leaves presented to the beetles were scored for presence/absence of eggs. Damaged leaves were subsequently pressed and dried, then scanned on a flatbed scanner. The resultant image was imported into the ImageJ software package (version 1.46r; Schneider et al., 2012) where the image was converted to 8-bit format. The paintbrush tool was used to draw lines filling in any gaps in the leaf perimeter. The image was then converted into binary (black and white) and the Analyze Particles function was applied twice. In the first analysis, the “include holes” option was selected, thereby providing a measurement of the entire area of the leaf prior to insect feeding. In the second analysis, the “include holes” option was deselected so that feeding damage was excluded from the overall area. By subtracting the second quantity from the first, a measure of the leaf material (mm^2) consumed by all of the beetles in a given trial could be obtained.

<u>No choice neonate storage organ feeding/developmental trials</u>

Neonate storage organ feeding/development trials were conducted on the same 33 plant species (see Table 1-2) as the adult storage organ trials. Naïve neonates were obtained by collecting *L. egena* eggs from the colonies and placing them on moist filter paper in small (9 cm diameter) petri dishes. The dishes were sealed with Parafilm® (to prevent desiccation and larval escape) and monitored for eclosion. Neonate mandibles are unable to penetrate the skin (epidermal +

peridermal layers) of *D. bulbifera* bulbils, so these larvae accessed the flesh (medulla) via adult feeding scars or breaks in the skin caused by eruption of the root radicle or cotyledon. Thus, to maximize the possibility that the neonates might feed on a proffered test plant, a portion of the skin from each storage organ was excised prior to their being placed on moist filter paper in the test arenas. These latter were composed of either a small (118 mL) Gladware® mini-round storage bowl ventilated with holes punched in the lid (Figure 1-2), a small plastic soup container (11 cm diameter x 7 cm high) or a plastic storage container (11 x 7 x 16 cm). To conduct the trials, freshly eclosed (<24 hour) neonates were transferred (using a fine 00 gauge paint brush) onto the bare patches of the storage organs and each trial was examined daily for larval mortality. Trials continued for 35 days, or until all larvae were dead and the positive controls (*D. bulbifera*) had produced adult beetles (whichever was longer). At the end of each trial, all puparia were dissected to check for the possibility that an adult beetle had formed but failed to emerge (pharate adult). As in the other storage organ trials, *L. egena* feeding was evaluated using the volumetric method previously described in the adult no choice storage organ trial section.

Figure 1-2. Example of an *L. egena* no choice neonate larval storage organ feeding/developmental trial.

<u>No choice neonate foliage feeding/developmental trials</u>

Neonate foliage feeding/development trials were conducted on the same 82 plant species (see Table 1-1) as the adult foliage trials. Naïve neonates were obtained for these trials in a similar manner as above. Freshly eclosed (<24 h) neonates were then transferred as before onto leaf material from the test plants. Generally, whole leaves were placed in the test arenas, but in rare cases it was only possible to use a section of larger leaves. The leaves were placed on moist filter paper in medium (15 cm diameter) petri dishes sealed with Parafilm® to prevent desiccation and larval escape. Larval mortality was monitored daily, and the filter paper was moistened and leaves replaced as needed. Trials continued for 35 days, or until all larvae were dead and the positive controls (*D. bulbifera*) had produced adult beetles (whichever was longer). At the end of each trial, all puparia were dissected to check for pharate adults. As in other foliage trials, feeding damage was assessed quantitatively using the leaf scan method previously described in the adult foliage trial section.

No choice 2nd/3rd instar larval storage organ feeding/development trials

These larval storage organ feeding/development trials were conducted on the same 33 plant species (see Table 1-2) as the adult storage organ trials. Larvae for these trials were obtained in two different ways. Initially, ages of test larvae were estimated by size, but for most studies, neonates were instead collected within 24 hours of eclosion and placed on thin (~1 cm) slices of *D. bulbifera* bulbil (5–10 neonates per slice) on moist filter paper in clear plastic salad containers (19 x 19 x 7 cm). The neonates were allowed to feed and develop for 4–5 days until they were 2nd or early 3rd instars.

Unlike the insects used in the previously described trials, these larvae were necessarily not naïve. Also, unlike the neonates, these larger larvae had mandibles capable of penetrating the bulbil's periderm (skin) and thus were transferred onto whole storage organs or sections of storage organs the ends of which were sealed by thermoplastic adhesive from a hot glue gun (preliminary tests showed no larval mortality resulted from the presence of the adhesive once it had cooled). This forced the larvae to access the flesh of the storage organs only after penetrating the periderm, to mimic conditions that a wandering larva might encounter in nature. Trials continued for 35 days or until all larvae were dead and the positive controls (*D. bulbifera*) had produced adult beetles (whichever was longer). At the end of each trial, all puparia were dissected to check for pharate adults. As in the other storage organ trials, *L. egena* feeding was evaluated quantitatively using the volumetric method developed.

No choice 2nd/3rd instar larval foliage feeding/development trials

These 2nd/3rd instar foliage feeding/development trials were conducted on the same 82 plant species (see Table 1-1) as the adult foliage trials. Larvae for these trials were obtained by collecting neonates as described above, placing them on thin (~ 1 cm) slices of *D. bulbifera* bulbil in clear plastic salad containers (19 x 19 x 7 cm), and allowing them to feed and develop for 4–5 days to the 2nd or early 3rd instar. As in the 2nd/3rd instar storage organ trials, these larvae were necessarily not naïve. The larvae were then transferred onto leaf material from the test plants. Generally, whole leaves were placed in the test arenas, but in rare cases it was only possible to use a section of a leaf. The leaves were placed on moist filter paper in medium-sized (15 cm diameter) petri dishes sealed with Parafilm® to prevent desiccation and larval escape (Figure 1-3). Larval mortality was monitored daily, and the filter paper was moistened and leaves replaced as needed. Trials continued for 35 days, or until all larvae were dead and the positive controls (*D. bulbifera*) had produced adult beetles (whichever was longer). At the end of each trial, all puparia were dissected to check for pharate adults. As in other foliage trials, feeding damage was assessed quantitatively using the leaf scan method described previously.

Figure 1-3. Example of an *L. egena* no choice 2nd/3rd instar larval foliage feeding/developmental trial. Note the extensive feeding on *D. bulbifera*.

<u>Two choice adult oviposition and development tests – adults on whole plants</u>

During the adult foliage trials, feeding on the Caribbean endemic *D. (Rajania) cordata* exceeded 10 percent of the leaf material presented to the beetles, the only plant besides *D. bulbifera* for which this was true. Despite failure of adults to live beyond 25 days on *D. cordata*, and the absence of oviposition on this plant, a side-by-side whole plant choice trial was conducted using these two species. To do so potted 1 m tall vines of each species were placed into three large (1 x 1 x 2 m) pop-up cages. Additionally, a pot with bulbils lying on the soil was placed into each cage. Each cage was inoculated with 5 pairs (one female, one male) of *L. egena*, and the trial ran for 7 days. The trial was scored for proportion of leaves of each species suffering feeding damage, the location of oviposition, and the locations of the adults at the conclusion of the trial.

<u>Ovipositional preference trials</u>

During preliminary foliage feeding trials, females only rarely produced or deposited eggs on *D. bulbifera* foliage. This prevented having a positive control for the ovipositional portion of the adult foliage trials. Thus, female discrimination of ovipositional substrates was assessed.

In the first test, females were removed from *D. bulbifera* leaves at the ends of a subset of the foliage feeding trials and moved them onto *D. bulbifera* bulbils. Any eggs produced were subsequently evaluated for viability by monitoring for eclosion of neonate larvae.

In a second experiment, female performance (feeding and oviposition) was compared on tubers versus bulbils, and whether the females would burrow through a substrate (soil or vermiculite) to locate, feed upon, and oviposit on tubers was also assessed. This experiment was conducted in three separate trials with slightly different conditions. The first trial used naïve adults (two females + one male) with a vermiculite substrate, the second trial used naïve adults with a heavier gardening soil as a substrate, and the third used fecund females (already ovipositing) with gardening soil as a substrate. Gardening soil was used in the latter two trials to better mimic conditions that the beetles would encounter in the field. Each trial included the following treatments: (a) a bulbil placed on the substrate surface as a positive control, (b) a tuber placed on the substrate surface, (c) a tuber buried 5 cm below the substrate surface, (d) a tuber buried at 10 cm below the surface, (e) a sprouting tuber buried at 10 cm below the soil surface, but with its stem emerging above the surface, and (f) a tuber buried at 25 cm below the surface. These depths were selected because tubers in the field are typically recovered 10–25 cm below the surface. Further, observations in China (FAD) suggest that these beetles and their congener, *L. cheni*, seek out very young sprouting vines during early spring and so *L. egena* may use this to locate underground food resources.

<u>Positive Controls</u>

D. bulbifera was included as a positive control in all host range trials.

<u>Rationale for Study Design and Execution</u>

The Asian beetle *L. egena* was first collected in China during May 2011 on an expedition that was focused on collecting the air potato leaf beetle *L. cheni* (Center et al., 2013). Efforts to maintain these insects during the expedition, and subsequently in the quarantine laboratory, revealed that these two beetles differed in their feeding predilections – whereas *L. cheni* was primarily a leaf feeder, adult *L. egena* fed both on foliage and on bulbils (Prain and Burkill, 1936; Martin, 1974). The latter are the primary means by which this invasive vine propagates in the United States. Thus, the decision was made to test *L. egena* both on foliage and on representative plant storage organs – especially those cultivated as food crops.

Unlike its congener *L. cheni*, which oviposits on leaves but also indiscriminately (Pemberton and Witkus, 2010), female *L. egena* prefer to deposit eggs in or on *D. bulbifera* bulbils and only occasionally oviposit on the undersides of the containers holding these bulbils. Thus, ovipositional trials were incorporated in adult feeding trials for this beetle. Preliminary reproductive studies showed a pre–ovipositional period of 7–10 days, so adult feeding trials with beetles younger than 7 days old were initiated – thereby assuring that potential egg production would be influenced by the proffered host plant. Further, to avoid possible effects of host plant imprinting, these adults were collected from pupation cages from which all food materials were removed prior to adult emergence, thereby assuring naïve adults were used. In a preliminary trial, freshly emerged naïve adults died after 22 days with no food or water. Therefore, adult trials were conducted for a minimum of 30 days. Males and females were differentiated based on gross morphological features (e.g., size and shape of abdomen), but this was an inexact science (~70 percent accuracy). The error was usually misidentifying a large male as female, so two putative females and one putative male were placed into each test arena to increase the likelihood of getting at least one complete mating pair.

Neonate and later instar larvae often abandon unsuitable host materials to search for more acceptable food. Thus, the potential exists for these stages to encounter and potentially feed upon novel food items. Therefore, in addition to the adult feeding trials, host trials both with neonates and with late 2^{nd}/early 3^{rd} instar larvae were conducted. Each was tested on foliage and on storage organs for the same reasons cited above for adult feeding trials.

Given that two distinct biogeographical populations were under colonization in quarantine, both biotypes were incorporated into the host range trials. The Chinese insects were tested against the entire suite of 82 plant species (Table 1-1). However, to avoid unnecessary duplication of effort (and associated time delays), the Nepalese insects were only tested against those species considered potentially most susceptible (the Dioscoreaceae). Substantive differences between the two biotypes in terms of responses to the Dioscoreaceae would have triggered an expanded list of host plants offered to the Nepalese biotype. This followed the protocols employed when releasing Chinese *L. cheni* after host trials had been conducted exclusively on Nepalese insects (see Center et al., 2013). Fortunately, the two *L. egena* biotypes performed similarly on the Dioscoreaceae, so further tests outside of this family with the Nepalese biotype were unnecessary. The Chinese and Nepalese data are reported separately within each of the tables, otherwise discussions and graphs are generally inclusive of the combined datasets.

Host Range Testing Results

<u>Multi-choice (choice minus control) adult storage organ feeding trials</u>

These multi-choice trials provided preliminary evidence that *L. egena* is host specific, given that the beetles ignored these initial test plant (predominantly crop) species (Table 1-3). The beetles produced minor surface "scrapes" on three species of *Dioscorea* (Table 1-3) compared with extensive tunneling inside *D. bulbifera* (both bulbils and tubers). Also, females oviposited only on *D. bulbifera* (Table 1-3).

An abundance of zero feeding scores caused us to collapse the data into the following groups for statistical analysis: (1) *D. bulbifera*, (2) native eastern U.S. Dioscoreaceae (*D. floridana* + *D. villosa*), (3) Dioscoreaceae crops (*D. cayenensis/rotundata* + *D. alata*), (4) other Dioscoreaceae (*D. oppositifolia* + *D. sansibarensis*, + *T. chantrieri*), and (5) non-Dioscoreaceae (all other species in Table 1-4). These data still demonstrated a non-normal distribution and had unequal variances, so they were analyzed using a Kruskal-Wallis One-way ANOVA on Ranks, with Dunn's Multiple Comparison test applied post-hoc. Results showed that the groups had highly divergent feeding scores (H=70.25, $P < 0.001$) - with *D. bulbifera* differing from each of the other 4 groups, but none of the rest differing among each other (Table 1-4).

Table 1-3. Summary results from multi-choice (choice minus control) adult storage organ feeding trials. The target weed (*D. bulbifera*) is highlighted in tan. See Table 1-2 for additional information (e.g., common names) on these species.

Order	Family	Genus (Section) species Authority	Replicates	Avg. feeding score[1]	Oviposition
Alismatales	Araceae	*Colocasia esculenta* (L.) Schott	5	0	no
Apiales	Apiaceae	*Apium graveolens* L.	4	0	no
	Apiaceae	*Daucus carota* L.	6	0	no
Asperagales	Amaryllidaceae	*Allium cepa* L.	4	0	no
	Amaryllidaceae	*Allium sativum* L.	4	0	no
Asterales	Asteraceae	*Arctium lappa* L.	4	0	no
Brassicales	Brassicaceae	*Brassica rapa* L.	4	0	no
	Brassicaceae	*Raphanus sativus* L. ("cherry belle" and "daikon")	8	0.3	no
Caryophyllales	Amaranthaceae	*Beta vulgaris* L.	4	0	no
	Fabaceae	*Pachyrhizus erosus* (L.) Urb.	5	0	no
Dioscoreales	Dioscoreaceae	*Dioscorea (Opsophyton) bulbifera* L.	10	3.5	yes
	Dioscoreaceae	*Dioscorea (Opsophyton) sansibarensis* Pax	5	0	no
	Dioscoreaceae	*Dioscorea (Macropoda) floridana* Bartlett	5	0.2	no
	Dioscoreaceae	*Dioscorea (Macropoda) villosa* L.	5	0	no
	Dioscoreaceae	*Dioscorea (Enantiophyllum) alata* L.	4	0	no
	Dioscoreaceae	*Dioscorea (Enantiophyllum) cayenensis* Lam. *(combines subspp cayenensis and rotundata)*	15	0.3	no
	Dioscoreaceae	*Dioscorea (Enantiophyllum) oppositifolia* L.	4	0.3	no
	Dioscoreaceae	*Tacca chantrieri* André	4	0	no
Malphigiales	Euphorbiaceae	*Manihot esculenta* Crantz	5	0	no
Solanales	Convolvulaceae	*Ipomoea batatas* (L.) Lam.	13	0	no
	Convolvulaceae	*Ipomoea pandurata* (L.) G.F.W.Mey.	4	0	no
	Solanaceae	*Solanum tuberosum* L.	5	0	no
Zingiberales	Marantaceae	*Maranta arundinacea* L.	4	0	no
	Zingiberaceae	*Curcuma longa* L.	4	0	no
	Zingiberaceae	*Zingiber officinale* Roscoe	4	0	no

[1] Qualitative feeding scored as: 0 - no feeding, 1 - a few scars, 2 - many scars and/or a few notches, 3 - many notches and/or holes, and 4 - burrowing inside bulb/tuber/etc.

Table 1-4. Comparison of the five groups of plant species (see text) subjected to multi-choice (choice minus control) adult *L. egena* feeding trials. Numbers in the upper quadrat (peach boxes) represent the Dunn's Multiple Comparison Q values for the various comparisons, those in the lower quadrat (green boxes) represent the associated significance values.

Groups	(1)	(2)	(3)	(4)	(5)
(1) *D. bulbifera*		2.970	3.621	3.956	4.769
(2) *D. floridana* + *D. villosa*	P<0.05		0.192	0.588	0.794
(3) *D. cayenensis/rotundata* + *D. alata*	P<0.05	NS		0.485	0.769
(4) other Dioscoreaceae	P<0.05	NS	NS		0.128
(5) non-Dioscoreaceae	P<0.05	NS	NS	NS	

<u>No choice adult storage organ feeding/ovipositional trials</u>

Naïve female *L. egena* produced eggs and oviposited only on storage organs of the target plant, *D. bulbifera* (Table 1-5) during the storage organ feeding/oviposition trials. In reproductive trials conducted separately from the feeding trials, Chinese females produced an average 8.4 ± 0.28 eggs/day, and a total of 931.0 ± 103.49 eggs/lifetime, on *D. bulbifera* bulbils.

Adult *L. egena* fed readily on *D. bulbifera* bulbils, with about 82 percent of Chinese and 92 percent of Nepalese biotype beetles surviving the 30-day trials. Overall, beetles fed non-host storage organs lived only about a third as long as those fed *D. bulbifera* bulbils (Table 1-5; Figure 1-4). However, a total of seven individuals (1 percent of the beetles placed on non-host plants) from a total of four different trials survived longer on three *Dioscorea* congeners (Table 1-5. None of these plants are native to Florida, but one (*D. trifida*) is native to Mesoamerica (Table 1-1). In each of these seven cases, the beetles on the *D. bulbifera* control out-lived the beetles on the congeners (Table 1-5). Trials were always terminated a few days after death of the last adult on non-host plants because adults on *D. bulbifera* can live 6 months or longer (one adult from an unrelated experiment has lived 373 days), so it was not necessary to maintain the adults from each trial until they died. The longest-lived non-control beetle (one on *D. polystachya*) consumed substantially less storage organ tissue than did the controls on *D. bulbifera* (Table 1-5), and none of these adults produced any eggs (Table 1-5).

Chinese and Nepalese beetles consumed the similar amounts of *D. bulbifera* bulbil tissue (2.845 ± 0.3698 vs 1.737 ± 0.4081 mL; Mann-Whitney U = 113.5, $P = 0.104$). Adult beetles fed native U.S. Dioscoreaceae (category 2a plants) and Dioscoreaceae native to the Caribbean and/or Mesoamerica (category 2b plants) consumed less than 1 percent of the storage organ tissue as those fed *D. bulbifera* bulbils (Table 1-5, Figure 1-5). Adults fed the remaining *Dioscorea* species (category 2c) consumed more tissue than those on other non-target species, but this still equaled only 6.2 percent of *D. bulbifera* bulbil tissue consumption in the controls (Table 1-5, Figure 1-5). There was virtually no feeding on storage organs from plants outside the Dioscoreaceae (Table 1-5, Figure 1-5).

Table 1-5. Results of the adult *L. egena* no choice bulbil feeding/oviposition trials. Uncolored rows represent host trials utilizing Chinese beetles, whereas gray colored rows represent host trials utilizing Nepalese beetles. The latter were only conducted on members of the order Dioscoreales. Tan colored rows represent summaries by TAG Category. See Table 1-1 for additional information regarding the test plant species.

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (avg ± s.e.)	Females produced eggs (Y/N)	Storage organ tissue consumed (mL) (avg ± s.e.)[2]
1	Dioscoreales	Dioscoreaceae	*Dioscorea bulbifera*	39	>30 ± --	Y	2.845 ± 0.3698
				9	>30 ± --	Y	1.737 ± 0.4081
			Category 1 summary	**48**	**>30 ± --**	**Y**	**2.291 ± 0.3148**
2a	Dioscoreales	Dioscoreaceae	*Dioscorea floridana*	6	10.3 ± 0.92	N	0.003 ± 0.0011
				5	10.8 ± 0.67	N	0.020 ± 0.0095
		Dioscoreaceae	*Dioscorea villosa*	6	10.8 ± 1.66	N	0.001 ± 0.0008
				5	11.3 ± 1.24	N	0.000 ± 0.0000
			Category 2a summary	**22**	**10.8 ± 0.57**	**N**	**0.006 ± 0.0027**
2b	Dioscoreales	Dioscoreaceae	*Dioscorea pilosiuscula*	5	9.3 ± 1.11	N	0.005 ± 0.0027
				5	9.3 ± 1.13	N	0.001 ± 0.0010
		Dioscoreaceae	*Dioscorea polygonoides*[3]	5	6.1 ± 0.44	N	0.000 ± 0.0000
				0	-- ± --	N	-- ± --
		Dioscoreaceae	*Dioscorea trifida*[3]	5	24.2 ± 9.95	N	0.108 ± 0.0474
				0	-- ± --	N	-- ± --
			Category 2b summary	**20**	**11.2 ± 2.82**	**N**	**0.019 ± 0.0152**
2c	Dioscoreales	Dioscoreaceae	*Dioscorea alata*	8	12.1 ± 3.60	N	0.108 ± 0.1081
				5	9.3 ± 1.63	N	0.043 ± 0.0320
		Dioscoreaceae	*Dioscorea cayenensis*[3]	6	10.1 ± 1.89	N	0.053 ± 0.0375
				0	-- --	N	-- --
		Dioscoreaceae	*Dioscorea esculenta*	5	11.7 ± 1.44	N	0.034 ± 0.0189
				5	8.8 ± 1.36	N	0.000 ± 0.0000
		Dioscoreaceae	*Dioscorea oppositifolia*	5	31.6 ± 14.30	N	0.864 ± 0.4155
				5	10.9 ± 0.76	N	0.186 ± 0.1050
		Dioscoreaceae	*Dioscorea polystachya*	7	28.7 ± 11.94	N	0.288 ± 0.2273
				5	17.3 ± 3.19	N	0.065 ± 0.0215
		Dioscoreaceae	*Dioscorea sansibarensis*	5	9.4 ± 0.59	N	0.000 ± 0.0000
				5	7.5 ± 0.44	N	0.000 ± 0.0000
			Category 2c summary	**61**	**14.0 ± 2.03**	**N**	**0.143 ± 0.0518**
3		Dioscoreaceae	*Tacca chantrieri*[3]	1	9.7 ± --	N	0.000 ± 0.0000
				5	9.6 ± 1.02	N	0.000 ± 0.0000
			Category 3 summary	**6**	**9.6 ± 0.83**	**N**	**0.000 ± 0.0000**
4	Dioscoreales	**No native N. American Dioscoreaceae are threatened or endangered (Neither U.S. nor Florida)**					
5	Dioscoreales	**No species available for testing, see text.**					
6a	Pandanales	**The only U.S. species [*Croomia pauciflora* (Nutt.) Torr. (Stemonaceae)] within this order has no storage organs**					
6b	Alismatales	Araceae	*Colocasia esculenta*	6	7.2 ± 0.65	N	0.000 ± 0.0000
		Araceae	*Xanthosoma sagittifolium*	5	7.5 ± 1.63	N	0.000 ± 0.0000
	Zingiberales	Marantaceae	*Maranta arundinacea*	7	10.6 ± 1.29	N	0.000 ± 0.0000
		Zingiberaceae	*Curcuma longa*	5	10.8 ± 1.85	N	0.000 ± 0.0000
		Zingiberaceae	*Hedychium coronarium*	5	7.5 ± 0.67	N	0.000 ± 0.0000

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (avg ± s.e.)	Females produced eggs (Y/N)	Storage organ tissue consumed (mL) (avg ± s.e.)[2]
		Zingiberaceae	*Zingiber officinale*	6	10.3 ± 1.41	N	0.000 ± 0.0000
		Category 6b (+7b) Lilianae summary		**57**	**9.6 ± 0.41**	**N**	**0.000 ± 0.0000**
6c	Apiales	Apiaceae	*Daucus carota*	6	9.4 ± 0.96	N	0.001 ± 0.0008
		Araliaceae	*Panax ginseng*	5	6.4 ± 0.69	N	0.000 ± 0.0000
	Asterales	Asteraceae	*Arctium lappa*	6	6.5 ± 0.75	N	0.000 ± 0.0000
	Brassicales	Brassicaceae	*Brassica rapa*	5	6.7 ± 0.62	N	0.000 ± 0.0000
		Brassicaceae	*Raphanus sativus*	6	8.0 ± 0.98	N	0.000 ± 0.0000
	Caryophyllales	Amaranthaceae	*Beta vulgaris*	6	7.9 ± 0.62	N	0.000 ± 0.0000
		Fabaceae	*Pachyrhizus erosus*	5	6.9 ± 1.05	N	0.000 ± 0.0000
	Malpighiales	Euphorbiaceae	*Manihot esculenta*	5	9.3 ± 1.27	N	0.000 ± 0.0000
	Solanales	Convolvulaceae	*Ipomoea batatas*	5	7.5 ± 0.47	N	0.000 ± 0.0000
		Category 6c (+ 7c) non-Lilianae summary		**55**	**7.8 ± 0.30**	**N**	**0.000 ± 0.0001**
7a	Monocot	Dioscoreaceae	*Dioscorea subclava*	**Known only from China, not available for testing.**			
7b	Asparagales	Amaryllidaceae	*Allium cepa*	7	9.5 ± 0.78	N	0.000 ± 0.0000
			Allium sativum	6	7.3 ± 0.69	N	0.000 ± 0.0000
	Dioscoreales	Dioscoreaceae	*Dioscorea alata*	13	10.7 ± 2.27	N	0.105 ± 0.0681
	Liliales	Liliaceae	*Lilium michauxii*	5	13.6 ± 1.93	N	0.000 ± 0.0000
		Liliaceae	*Tricyrtus lasiocarpa*	5	11.9 ± 1.49	N	0.000 ± 0.0000
		Category 7b Lilianae summary		**36**	**10.9 ± 0.94**	**N**	**0.044 ± 0.0262**
7c	Solanales	Solanaceae	*Solanum tuberosum*	6	9.3 ± 1.12	N	0.000 ± 0.0000
		Category 7 combined summary		**42**	**10.9 ± 0.67**	**N**	**0.044 ± 0.0190**

[1] Species from Category 7 also qualify as belonging in Category 6 (with the exception of *D. alata*, which is omitted) and so are included in the appropriate Category 6 summaries.

[2] Obtaining potential oviposition required having multiple individuals (2 females, 1 male) in each trial replicate. Thus, the leaf tissue consumed represents the combined feeding activity of the three adult beetles in each replicate.

[3] These plants died out part way through the trials, and additional plants could not be obtained to complete the studies.

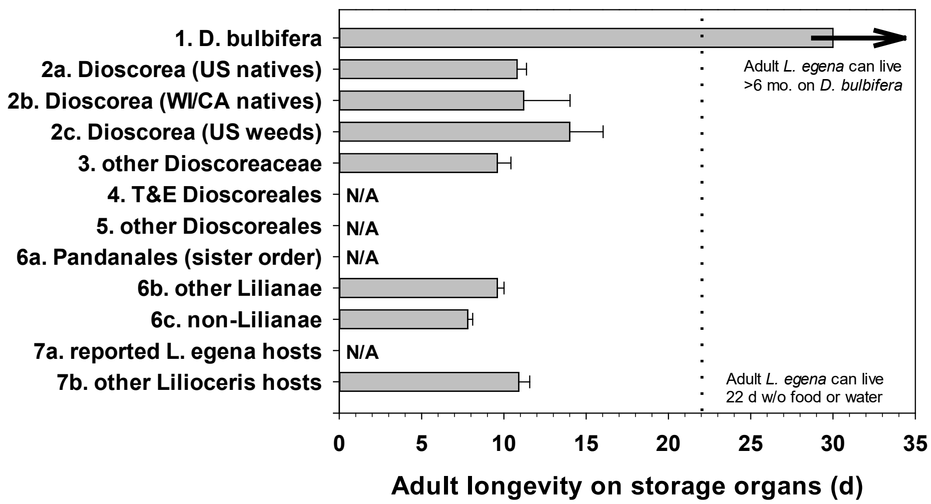

Figure 1-4. Adult *L. egena* longevity (mean ± s.e.) during storage organ feeding trials, presented as averages across TAG categories.

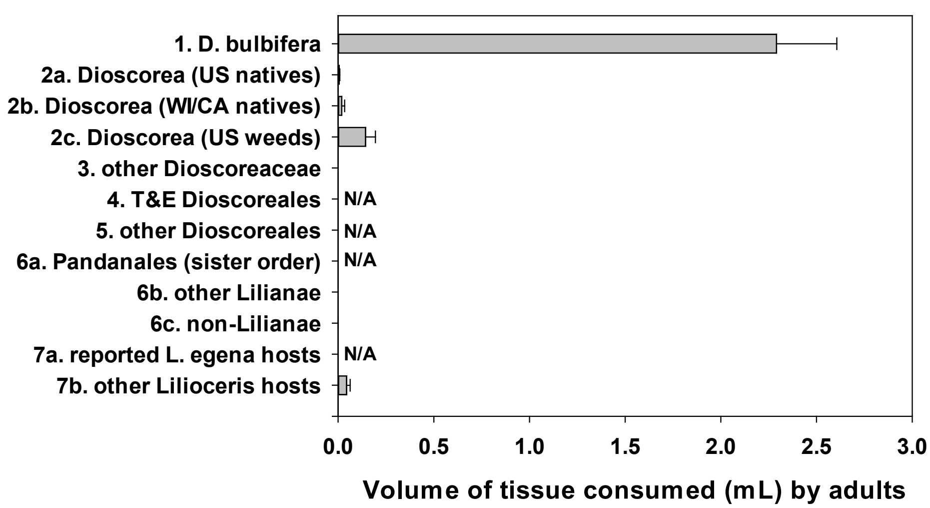

Figure 1-5. Adult *L. egena* tissue consumption (mean ± s.e.) during storage organ feeding trials, presented as averages across TAG categories.

No choice adult foliage feeding/oviposition trials

Female *L. egena* produced eggs on live *D. bulbifera* leaves in only five (three Chinese biotype and two Nepalese biotype) of the 81 (6.2 percent) successful adult feeding trials, and never produced eggs on other test plant species (Table 1-6). A total of 77 viable eggs were produced on *D. bulbifera* foliage in four of these five trials for an average of 0.03 eggs/female/day during the 30 days. In contrast, females in reproductive studies produced 251.5 ± 34.68 eggs/day over their first 30 days on bulbils. This suggests that the eggs deposited on foliage are an aberration.

Adult *L. egena* fed readily on *D. bulbifera* foliage, with about 75 percent of each biotype surviving the 30-day trials and consuming an average 14,839.9 ± 993.23 mm^2 of leaf tissue per trial (Table 1-6, Figures 1-6 and 1-7). In contrast, Chinese beetles survived an average of 23.2 ± 1.43 days on wheat (*Triticum aestivum*) and 22.7 ± 3.33 days on yucca (*Manihot esculenta*), but did not feed (Table 1-6). The closest congener in terms of beetle longevity was the U.S. native *D. villosa*, on which adults survived an average 18.7 ± 1.48 or 14.1 ± 3.29 days (Chinese vs. Nepalese beetles, respectively) - roughly 15 percent of the average lifespan of a beetle on *D. bulbifera* (Table 1-6). Beetles lived an average 15.7 ± 1.16 and 7.9 ± 1.33 days (Chinese vs. Nepalese beetles, respectively) on the other U.S. native, *D. floridana* (Table 1-6). In both of these cases, the beetles fed very little (Table 1-6). The closest host in terms of consumption was the West Indian endemic *D. cordata* on which *L. egena* consumed 1,196.2 ± 630.83 and 2,715.8 ± 1504.51 mm^2 (Chinese vs. Nepalese beetles, respectively) of available leaf tissue - roughly 8 and 18 percent, respectively, of consumption on *D. bulbifera* (Table 1-6).

On average, adult beetles consumed 95.5 ± 52.66 mm^2 of leaf tissue while surviving 14.1 ± 2.58 days on native U.S. Dioscoreaceae (category 2a plants) (Table 1-6, Figures 1-6 and 1-7). Adult beetles offered Dioscoreaceae native to the Caribbean and/or Mesoamerica (category 2b plants) consumed 546.0 ± 192.33 mm^2, but survived a shorter duration (9.2 ± 0.91 days) than on the U.S. natives (Table 1-6, Figures 1-6 and 1-7). Adults survived less than two weeks on other test plant species, including other Dioscoreaceae (categories 2c and 3), and consumed less than 1 percent of the leaf tissue that was consumed by *L.egena* on *D. bulbifera* (Table 1-6, Figures 1-6 and 1-7).

Failure of adult *L. egena* to oviposit upon, survive upon, or consume significant quantities of leaf tissue from plant species other than *D. bulbifera* supports the contention that this beetle has close host fidelity to air potato.

Table 1-6. Results of the adult *L. egena* no choice foliage feeding/oviposition trials. Uncolored rows represent host trials utilizing Chinese beetles, whereas gray colored rows represent host trials utilizing Nepalese beetles. The latter were only conducted on members of the order Dioscoreales. Tan colored rows represent summaries by TAG Category. See Table 1-1 for additional information regarding the test plant species.

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (mean ± s.e.)	Females produced eggs (Y/N)	Leaf tissue consumed (mm²) (mean ± s.e.)[2]
1	Dioscoreales	Dioscoreaceae	*Dioscorea bulbifera*	68	>30 ± --	Y	14981.9 ± 1082.3
				13	>30 ± --	N	14800.7 ± 2601.7
			Category 1 summary	**81**	**>30 ± --**	**Y**	**14839.9 ± 993.2**
2a	Dioscoreales	Dioscoreaceae	*Dioscorea floridana*	5	15.7 ± 1.16	N	55.3 ± 21.1
				5	7.9 ± 1.33	N	191.0 ± 54.3
		Dioscoreaceae	*Dioscorea villosa*	5	18.7 ± 1.48	N	6.9 ± 4.5
				5	14.1 ± 3.29	N	128.7 ± 69.6
			Category 2a summary	**20**	**14.1 ± 2.58**	**N**	**95.5 ± 52.7**
2b	Dioscoreales	Dioscoreaceae	*Dioscorea altissima*[3]	4	8.9 ± 2.03	N	318.7 ± 141.9
				0	-- --	N	-- --
		Dioscoreaceae	*Dioscorea cordata*	5	9.7 ± 3.80	N	1196.2 ± 630.8
				4	13.5 ± 4.72	N	2715.8 ± 1504.5
		Dioscoreaceae	*Dioscorea pilosiuscula*	5	15.7 ± 3.11	N	4.8 ± 3.5
				5	6.3 ± 0.65	N	0.0 ± 0.0
		Dioscoreaceae	*Dioscorea polygonoides*	5	4.6 ± 0.99	N	3.6 ± 2.2
				5	10.5 ± 2.49	N	26.6 ± 15.1
		Dioscoreaceae	*Dioscorea trifida*	5	7.1 ± 1.16	N	355.4 ± 346.5
				5	7.5 ± 1.18	N	513.9 ± 311.4
			Category 2b summary	**43**	**9.2 ± 0.91**	**N**	**546.0 ± 192.3**
2c	Dioscoreales	Dioscoreaceae	*Dioscorea alata*	5	10.9 ± 1.01	N	4.0 ± 3.4
				5	6.9 ± 1.94	N	0.0 ± 0.0
		Dioscoreaceae	*Dioscorea cayenensis*	10	10.1 ± 1.34	N	54.7 ± 39.2
				6	17.4 ± 9.74	N	175.9 ± 91.3
		Dioscoreaceae	*Dioscorea esculenta*	5	6.7 ± 1.28	N	0.5 ± 0.4
				5	6.9 ± 0.95	N	0.0 ± 0.0
		Dioscoreaceae	*Dioscorea oppositifolia*[3]	5	12.5 ± 1.84	N	4.7 ± 2.9
				0	-- --	N	-- --
		Dioscoreaceae	*Dioscorea polystachya*	5	6.9 ± 0.70	N	24.5 ± 7.8
				5	5.3 ± 0.58	N	3.7 ± 3.7
		Dioscoreaceae	*Dioscorea sansibarensis*	5	6.5 ± 1.34	N	6.7 ± 6.7
				5	6.7 ± 0.45	N	6.4 ± 4.2
			Category 2c summary	**56**	**9.0 ± 1.05**	**N**	**32.0 ± 12.3**
3	Dioscoreales	Dioscoreaceae	Tacca chantrieri	5	9.4 ± 1.35	N	13.8 ± 13.8
				4	10.8 ± 2.85	N	8.4 ± 8.4
			Category 3 summary	**9**	**9.8 ± 1.38**	**N**	**9.9 ± 8.1**
4	Dioscoreales	**No native N. American Dioscoreaceae are threatened or endangered (Neither U.S. nor Florida)**					
5	Dioscoreales	**No species available for testing, see text.**					
6a	Pandanales	Pandanaceae	*Pandanus tectorius*	5	7.0 ± 0.61	N	0.0 ± 0.0
6b	Alismatales	Alismataceae	*Sagittaria latifolia*	5	12.3 ± 1.93	N	0.0 ± 0.0
		Araceae	*Alocasia cuculata*	5	5.7 ± 1.85	N	0.0 ± 0.0

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (mean ± s.e.)	Females produced eggs (Y/N)	Leaf tissue consumed (mm^2) (mean ± s.e.)[2]
		Araceae	*Caladium bicolor*	5	8.1 ± 1.49	N	0.0 ± 0.0
		Araceae	*Colocasia esculenta*	7	9.5 ± 1.31	N	0.0 ± 0.0
		Araceae	*Symplocarpus foetidus*	5	6.5 ± 0.43	N	0.0 ± 0.0
		Araceae	*Xanthosoma sagittifolium*	5	11.2 ± 0.78	N	0.0 ± 0.0
		Araceae	*Zantedeschia aethiopica*	5	8.1 ± 0.70	N	0.0 ± 0.0
	Arecales	Arecaceae	*Sabal palmetto*	5	4.1 ± 0.07	N	0.0 ± 0.0
	Asperagales	Amaryllidaceae	*Crinum americanum*	5	17.7 ± 8.30	N	0.0 ± 0.0
		Amaryllidaceae	*Zephyranthes grandiflora*	5	7.7 ± 0.78	N	0.0 ± 0.0
	Commelinales	Commelinaceae	*Tradescantia pallida*	5	8.4 ± 1.00	N	0.2 ± 0.1
		Pontedariaceae	*Pontederia cordata*	5	8.7 ± 1.02	N	0.0 ± 0.0
	Poales	Cyperaceae	*Cladium jamaicense*	5	7.7 ± 0.67	N	0.0 ± 0.0
		Juncaceae	*Juncus effusus*	5	14.3 ± 7.28	N	0.0 ± 0.0
		Musaceae	*Musa acuminata*	5	7.7 ± 1.19	N	0.0 ± 0.0
		Poaceae	*Saccharum officinarum*	5	7.3 ± 1.27	N	1.3 ± 1.3
		Poaceae	*Zea mays*	5	4.7 ± 0.52	N	0.0 ± 0.0
	Zingiberales	Cannaceae	*Canna glauca*	5	13.1 ± 4.12	N	0.0 ± 0.0
		Cannaceae	*Canna americanallis*	5	15.4 ± 6.61	N	1.8 ± 1.8
		Costaceae	*Costus woodsonii*	5	15.6 ± 9.63	N	2.2 ± 2.2
		Heliconiaceae	*Heliconia caribaea*	5	13.1 ± 1.34	N	0.0 ± 0.4
		Marantaceae	*Maranta arundinacea*	5	5.7 ± 1.81	N	0.0 ± 0.0
		Marantaceae	*Thalia geniculata*	5	6.0 ± 1.37	N	0.1 ± 0.1
		Zingiberaceae	*Curcuma longa*	5	8.7 ± 1.21	N	0.0 ± 0.0
		Zingiberaceae	*Hedychium coronarium*	5	7.5 ± 0.79	N	0.0 ± 0.0
		Zingiberaceae	*Zingiber officinale*	5	6.5 ± 0.56	N	0.0 ± 0.0
		Category 6b (+7b) Lilianae summary		**192**	**9.6 ± 0.56**	**N**	**0.2 ± 0.1**
6c	Apiales	Apiaceae	*Apium graveolens*	5	6.7 ± 1.06	N	0.0 ± 0.0
		Apiaceae	*Daucus carota*	5	6.9 ± 0.76	N	0.0 ± 0.0
		Araliaceae	*Panax ginseng*	5	14.9 ± 1.96	N	1.9 ± 1.7
	Asterales	Asteraceae	*Arctium lappa*	5	12.1 ± 1.74	N	0.0 ± 0.0
	Brassicales	Brassicaceae	*Brassica rapa*	5	6.1 ± 0.97	N	0.0 ± 0.0
		Brassicaceae	*Raphanus sativus*	6	7.6 ± 1.39	N	3.9 ± 3.8
	Caryophyllales	Amaranthaceae	*Beta vulgaris*	6	7.6 ± 1.10	N	0.9 ± 0.9
	Fabales	Fabaceae	*Glycine max*	5	7.2 ± 0.20	N	0.0 ± 0.0
		Fabaceae	*Mimosa pudica*	5	13.0 ± 5.36	N	0.0 ± 0.0
		Fabaceae	*Pachyrhizus erosus*	5	7.4 ± 0.85	N	0.0 ± 0.0
	Gentianales	Rubiaceae	*Guettarda scabra*	5	5.8 ± 0.50	N	0.0 ± 0.0
	Laurales	Calycanthaceae	*Calycanthus floridus*	5	4.8 ± 0.74	N	0.0 ± 0.0
	Magnoliales	Annonaceae	*Annona glabra*	5	6.5 ± 0.83	N	0.0 ± 0.0
	Malphigiales	Chrysobalanaceae	*Chrysobalanus icaco*	6	8.9 ± 1.06	N	0.0 ± 0.0
		Euphorbiaceae	*Manihot esculenta*	5	22.7 ± 3.33	N	0.0 ± 0.0
	Piperales	Aristolochiaceae	*Aristolochia tomentosa*	5	5.7 ± 1.81	N	0.0 ± 0.0
	Solanales	Convolvulaceae	*Ipomoea batatas*	5	5.8 ± 0.57	N	0.0 ± 0.0
		Category 6c (+ 7c) non-Lilianae summary		**149**	**9.0 ± 0.54**	**N**	**0.3 ± 0.2**
7a	Monocot	Dioscoreaceae	*Dioscorea subclava*	**Known only from China, not available for testing.**			
7b	Asperagales	Amaryllidaceae	*Allium cepa*	5	7.5 ± 0.92	N	0.0 ± 0.0
		Amaryllidaceae	*Allium sativum*	5	6.9 ± 0.53	N	0.0 ± 0.0
		Asparagaceae	*Asparagus densiflorus*	5	4.9 ± 1.14	N	0.0 ± 0.0

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (mean ± s.e.)	Females produced eggs (Y/N)	Leaf tissue consumed (mm^2) (mean ± s.e.)[2]
		Asparagaceae	*Asparagus officinalis*	5	3.7 ± 0.24	N	0.0 ± 0.0
		Asphodelaceae	*Aloe vera*	5	10.5 ± 0.89	N	0.0 ± 0.0
		Iridaceae	*Sisyrinchium angustifolium*	5	9.3 ± 1.16	N	0.0 ± 0.0
		Orchidaceae	*Bletilla striata*	5	10.5 ± 1.33	N	0.0 ± 0.0
	Dioscoreales	Dioscoreaceae	*Dioscorea alata*	10	8.9 ± 1.22	N	4.0 ± 1.7
	Liliales	Liliaceae	*Lilium michauxii*	5	5.3 ± 0.21	N	0.0 ± 0.0
		Liliaceae	*Tricyrtus lasiocarpa*	5	15.1 ± 2.93	N	1.4 ± 1.4
		Smilacaceae	*Smilax laurifolia*	5	5.4 ± 0.77	N	0.0 ± 0.0
	Pandanales	Pandanaceae	*Pandanus tectorius*	5	7.0 ± 0.61	N	0.0 ± 0.0
	Poales	Poaceae	*Triticum aestivum*	5	23.2 ± 1.43	N	0.0 ± 0.0
			Category 7b Lilianae summary	**70**	**8.1 ± 0.67**	**N**	**0.2 ± 0.3**
7c	Apiales	Araliaceae	*Schefflera actinophylla*	5	9.0 ± 0.82	N	0.0 ± 0.0
	Asterales	Campanulaceae	*Lobelia cardinalis*	5	11.1 ± 0.69	N	0.0 ± 0.0
	Caryophyllales	Polygonaceae	*Persicaria glabra*	5	6.0 ± 0.61	N	0.0 ± 0.0
	Fabales	Fabaceae	*Senna ligustrina*	5	6.4 ± 0.40	N	0.0 ± 0.0
	Fagales	Betulaceae	*Corylus americana*	5	8.0 ± 0.55	N	0.0 ± 0.0
		Fagaceae	*Quercus virginiana*	5	15.1 ± 1.58	N	0.0 ± 0.0
	Gentianales	Apocynaceae	*Asclepias tuberosa*	5	10.1 ± 0.75	N	0.0 ± 0.0
	Lamiales	Verbenaceae	*Callicarpa americana*	5	7.9 ± 0.81	N	0.0 ± 0.1
	Malpighiales	Salicaceae	*Salix caroliniana*	5	11.1 ± 1.07	N	0.0 ± 0.0
	Rosales	Moraceae	*Ficus aurea*	5	8.7 ± 0.54	N	0.0 ± 0.0
	Solanales	Solanaceae	*Solanum tuberosum*	6	10.5 ± 3.27	N	0.1 ± 0.0
	Cycadales	Cycadaceae	*Cycas revoluta*	5	8.1 ± 1.26	N	0.0 ± 0.0
			Category 7c non-Lilianae summary	**61**	**9.3 ± 0.48**	**N**	**0.0 ± 0.0**
			Category 7 combined summary	**131**	**8.5 ± 0.42**	**N**	**0.1 ± 0.1**

[1] Species from Category 7 also qualify as belonging in Category 6 (with the exception of *D. alata*, which is omitted) and so are included in the appropriate Category 6 summaries.

[2] Obtaining potential oviposition required having multiple individuals (2 females, 1 male) in each trial replicate. Thus, the leaf tissue consumed represents the combined feeding activity of the three adult beetles in each replicate.

[3] These plants died out part way through the trials, and additional plants could not be obtained to complete studies.

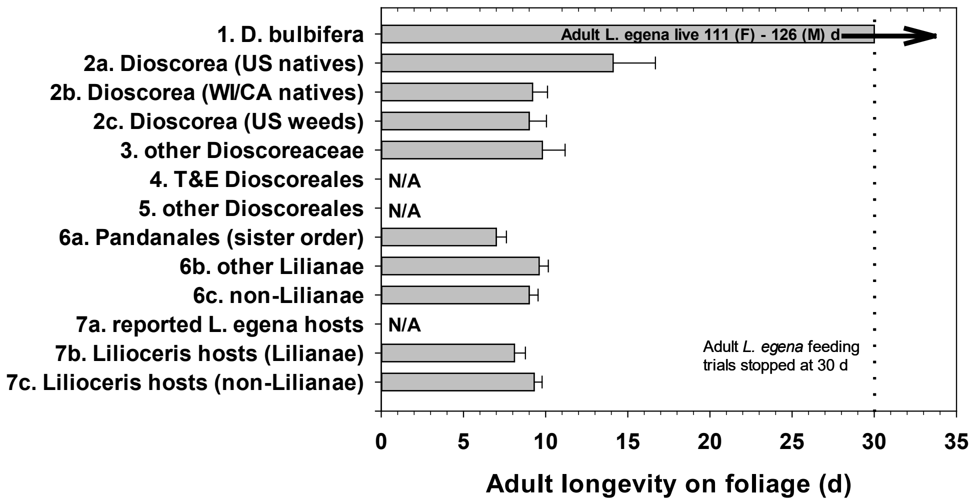

Figure 1-6. Adult *L. egena* longevity (mean ± s.e.) during foliage feeding trials, presented as averages across TAG categories.

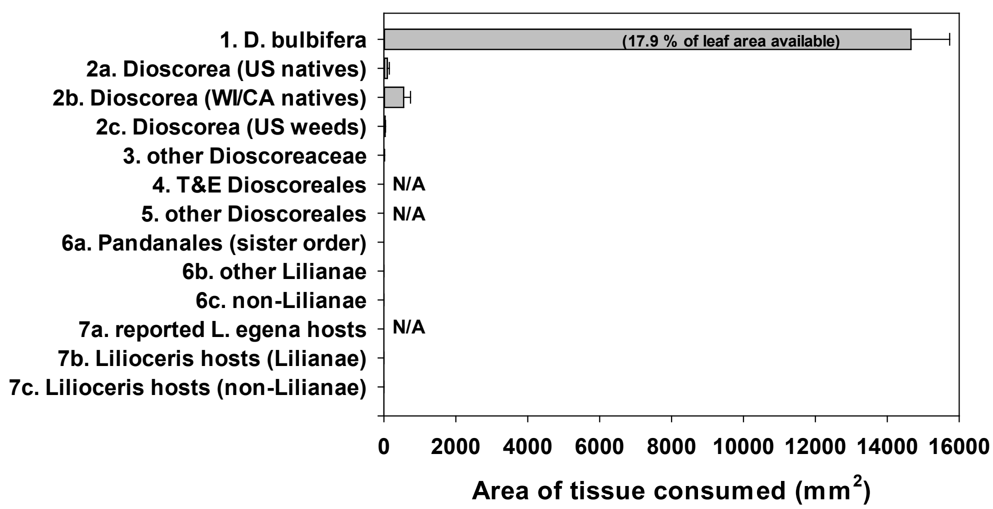

Figure 1-7. Adult *L. egena* leaf tissue consumption (mean ± s.e.) during foliage feeding trials, presented as averages across TAG categories.

<u>No choice neonate storage organ feeding/developmental trials</u>

Neonates placed on non-target storage organs usually quickly abandoned the proffered host after minor taste-testing as evidenced by the positions of dead neonates which were usually scattered throughout the testing arenas but seldom found on the storage organs. In contrast, neonates placed on *D. bulbifera* generally moved very little prior to initiating feeding and instead began burrowing into the *D. bulbifera* bulbil (or tuber), forming extensive tunnels where they fed until exiting for pupation. Neonates developing on *D. bulbifera* consumed an average of 1.81 ± 0.22 mL of plant tissue, whereas the maximum consumed on any other species was 0.02 ± 0.02 mL on *P. erosus* (jicama) which constituted 1 percent of the average damage on *D bulbifera*. Most test plants remained undamaged (Table 1-7, Figure 1-8).

Whereas 62.9 percent of the *L. egena* neonates on *D. bulbifera* storage organs developed into adults, none of the neonates completed development on any non-target storage organs (Table 1-7, Figure 1-9). Average developmental time for *L. egena* neonates on *D. bulbifera* was 29.0 ± 0.39 days, whereas most neonates on non-targets survived less than 3 days (Table 1-7, Figure 1-9). The longest lived neonates other than those on *D. bulbifera* were two that survived 8 days on *P. erosus* (jicama) and one that survived 7 days on *D. polystachya* (cinnamon vine), but all three failed to molt and become 2nd instar larvae.

These results suggest that storage organs of the non-target species lack the cues necessary to stimulate neonate feeding, and are nutritionally inadequate to permit neonates to develop.

Table 1-7. Outcomes of *Lilioceris egena* no choice neonate storage organ feeding/developmental trials. Uncolored rows represent host trials utilizing Chinese beetles, whereas gray colored rows represent host trials utilizing Nepalese beetles. The latter were only conducted on members of the order Dioscoreales. Tan colored rows represent summaries by TAG Category.

TAG Category	Order	Family	Species	Total reps	Longevity (d) (mean ± s.e.)	F_1 adults produced (mean ± s.e.)	Storage organ tissue consumed (mL) (mean ± s.e.)[2]
1	Dioscoreales	Dioscoreaceae	*Dioscorea bulbifera*	35	29.0 ± 0.40	3.2 ± 0.1	1.810 ± 0.2167
				12	29.4 ± 1.01	3.5 ± 0.3	1.866 ± 0.2644
			Category 1 summary	**47**	**29.2 ± 0.39**	**3.3 ± 0.1**	**1.838 ± 0.1710**
2a	Dioscoreales	Dioscoreaceae	*Dioscorea floridana*	4	1.3 ± 0.13	0.0 ± 0.0	0.004 ± 0.0013
				5	1.2 ± 0.12	0.0 ± 0.0	0.000 ± 0.0000
		Dioscoreaceae	*Dioscorea villosa*	5	1.4 ± 0.10	0.0 ± 0.0	0.000 ± 0.0000
				5	1.0 ± 0.00	0.0 ± 0.0	0.000 ± 0.0000
			Category 2a summary	**19**	**1.2 ± 0.06**	**0.0 ± 0.0**	**0.001 ± 0.0004**
2b	Dioscoreales	Dioscoreaceae	*Dioscorea pilosiuscula*	5	1.4 ± 0.27	0.0 ± 0.0	0.000 ± 0.0000
				5	1.0 ± 0.04	0.0 ± 0.0	0.000 ± 0.0000
		Dioscoreaceae	*Dioscorea polygonoides*	5	2.7 ± 0.27	0.0 ± 0.0	0.004 ± 0.0023
				5	1.0 ± 0.00	0.0 ± 0.0	0.000 ± 0.0000
		Dioscoreaceae	*Dioscorea trifida*	5	2.3 ± 0.74	0.0 ± 0.0	0.004 ± 0.0010
				5	2.9 ± 0.26	0.0 ± 0.0	0.024 ± 0.0128
			Category 2b summary	**30**	**1.9 ± 0.20**	**0.0 ± 0.0**	**0.005 ± 0.0025**
2c	Dioscoreales	Dioscoreaceae	*Dioscorea alata*	5	1.4 ± 0.18	0.0 ± 0.0	0.001 ± 0.0010
				5	2.3 ± 0.68	0.0 ± 0.2	0.003 ± 0.0020
		Dioscoreaceae	*Dioscorea cayenensis*[3]	4	1.5 ± 0.22	0.0 ± 0.0	0.002 ± 0.0012
				0	-- --	-- --	-- --
		Dioscoreaceae	*Dioscorea esculenta*	5	1.0 ± 0.00	0.0 ± 0.0	0.000 ± 0.0000
				5	3.3 ± 0.46	0.0 ± 0.0	0.000 ± 0.0000
		Dioscoreaceae	*Dioscorea oppositifolia*	4	1.2 ± 0.12	0.0 ± 0.0	0.002 ± 0.0012
				5	1.1 ± 0.12	0.0 ± 0.0	0.000 ± 0.0000
		Dioscoreaceae	*Dioscorea polystachya*	5	3.1 ± 0.23	0.0 ± 0.0	0.001 ± 0.0000
				5	1.3 ± 0.20	0.0 ± 0.0	0.001 ± 0.0010
		Dioscoreaceae	*Dioscorea sansibarensis*	6	1.0 ± 0.03	0.0 ± 0.0	0.000 ± 0.0002
				5	1.0 ± 0.00	0.0 ± 0.0	0.000 ± 0.0000
			Category 2c summary	**54**	**1.7 ± 0.14**	**0.0 ± 0.0**	**0.001 ± 0.0003**
3		Dioscoreaceae	*Tacca chantrieri*	5	1.2 ± 0.20	0.0 ± 0.0	0.000 ± 0.0000
				5	3.4 ± 0.24	0.0 ± 0.0	0.000 ± 0.0000
			Category 3 summary	**10**	**2.3 ± 0.40**	**0.0 ± 0.0**	**0.000 ± 0.0000**
4	Dioscoreales	**No native N. American Dioscoreaceae are threatened or endangered (Neither U.S. nor Florida)**					
5	Dioscoreales	**No species available for testing, see text.**					
6a	Pandanales	**The only U.S. species [*Croomia pauciflora* (Nutt.) Torr. (Stemonaceae)] within this order has no storage organs**					
6b	Alismatales	Araceae	*Colocasia esculenta*	5	1.0 ± 0.04	0.0 ± 0.0	0.000 ± 0.0000
		Araceae	*Xanthosoma sagittifolium*	6	1.7 ± 0.19	0.0 ± 0.0	0.001 ± 0.0008
	Zingiberales	Marantaceae	*Maranta arundinacea*	5	1.0 ± 0.00	0.0 ± 0.0	0.000 ± 0.0000
		Zingiberaceae	*Curcuma longa*	5	1.0 ± 0.00	0.0 ± 0.0	0.000 ± 0.0000
		Zingiberaceae	*Hedychium coronarium*	5	1.0 ± 0.00	0.0 ± 0.0	0.000 ± 0.0000

		Zingiberaceae	*Zingiber officinale*	5	1.4 ± 0.21	0.0 ± 0.0	0.000 ± 0.0000
		Category 6b (+7b) Lilianae summary		**51**	**1.3 ± 0.07**	**0.0 ± 0.0**	**0.000 ± 0.0001**
6c	Apiales	Apiaceae	*Daucus carota*	5	2.1 ± 0.05	0.0 ± 0.0	0.000 ± 0.0002
		Araliaceae	*Panax ginseng*	5	1.0 ± 0.00	0.0 ± 0.0	0.000 ± 0.0000
	Asterales	Asteraceae	*Arctium lappa*	5	2.0 ± 0.31	0.0 ± 0.0	0.000 ± 0.0000
	Brassicales	Brassicaceae	*Brassica rapa*	5	1.6 ± 0.17	0.0 ± 0.0	0.000 ± 0.0000
		Brassicaceae	*Raphanus sativus*	4	1.4 ± 0.18	0.0 ± 0.0	0.000 ± 0.0000
	Caryophyllales	Amaranthaceae	*Beta vulgaris*	5	1.5 ± 0.14	0.0 ± 0.0	0.000 ± 0.0002
		Fabaceae	*Pachyrhizus erosus*	5	1.6 ± 0.56	0.0 ± 0.0	0.022 ± 0.0220
	Malpighiales	Euphorbiaceae	*Manihot esculenta*	5	1.0 ± 0.00	0.0 ± 0.0	0.000 ± 0.0000
	Solanales	Convolvulaceae	*Ipomoea batatas*	5	1.8 ± 0.35	0.0 ± 0.0	0.000 ± 0.0000
		Category 6c (+ 7c) non-Lilianae summary		**49**	**1.5 ± 0.09**	**0.0 ± 0.0**	**0.002 ± 0.0021**
7a	Monocot	Dioscoreaceae	*Dioscorea subclava*		**Known only from China, not available for testing.**		
7b	Asparagales	Amaryllidaceae	*Allium cepa*	5	2.0 ± 0.25	0.0 ± 0.0	0.000 ± 0.0000
			Allium sativum	5	1.1 ± 0.08	0.0 ± 0.0	0.000 ± 0.0000
	Dioscoreales	Dioscoreaceae	*Dioscorea alata*	5	1.4 ± 0.18	0.0 ± 0.0	0.001 ± 0.0010
	Liliales	Liliaceae	*Lilium michauxii*	5	1.0 ± 0.00	0.0 ± 0.0	0.000 ± 0.0000
		Liliaceae	*Tricyrtus lasiocarpa*	5	1.9 ± 0.21	0.0 ± 0.0	0.000 ± 0.0002
		Category 7b Lilianae summary		**25**	**1.5 ± 0.11**	**0.0 ± 0.0**	**0.000 ± 0.0002**
7c	Solanales	Solanaceae	*Solanum tuberosum*	5	1.5 ± 0.23	0.0 ± 0.0	0.000 ± 0.0000
		Category 7 combined summary		**30**	**1.5 ± 0.10**	**0.0 ± 0.0**	**0.000 ± 0.0002**

[1] Species from Category 7 also qualify as belonging in Category 6 (with the exception of *D. alata*, which is omitted) and so are included in the appropriate Category 6 summaries.

[2] Each trial replicate contained three 2nd/3rd instar larvae. Although survivorship could be tracked individually, tissue consumption could not. Thus, the leaf tissue consumed represents the combined feeding activity of the three larvae in each replicate.

[3] These plants died out part way through the trials, and additional plants could not be obtained to complete the studies.

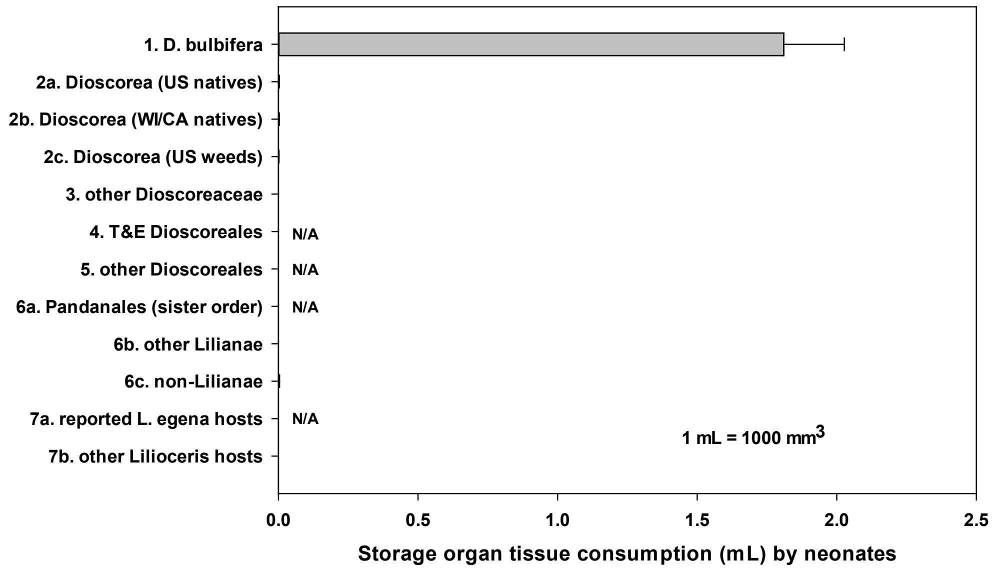

Figure 1-8. Consumption (mean ± s.e.) of storage organ tissues by *Lilioceris egena* neonates, averaged across TAG categories.

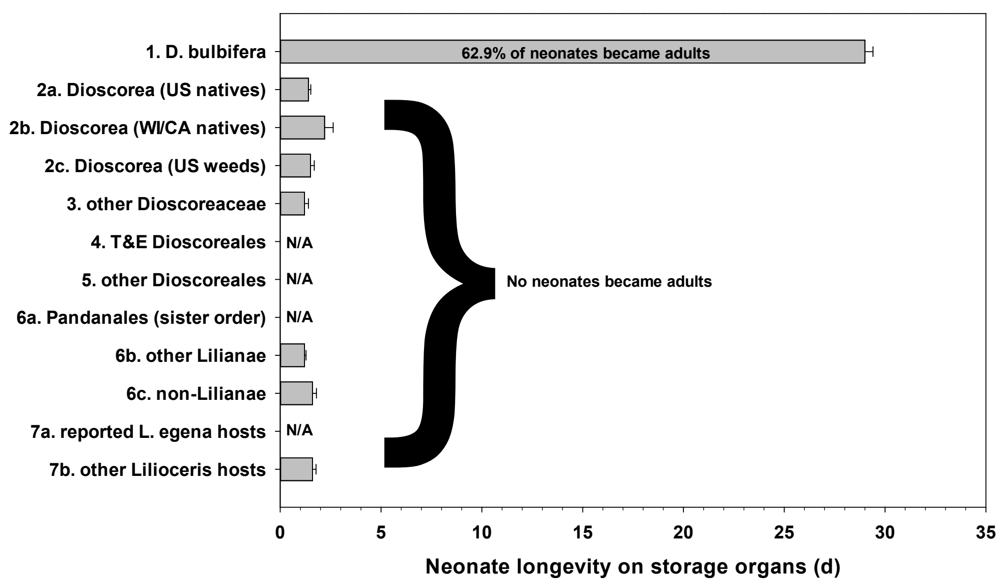

Figure 1-9. Survivorship (mean ± s.e.) of neonate *Lilioceris egena* on storage organs of test plants, averaged across TAG categories.

No choice neonate foliage feeding/developmental trials

Neonates placed on foliage other than *D. bulbifera* leaves exhibited the same wandering behavior as those placed on storage organs. Some neonates scraped a small bit of tissue from the food resource prior to abandoning it, usually within minutes. As before, dead neonates were seldom found on the test leaves, but instead were elsewhere in the testing arenas.

In contrast, neonates placed on *D. bulbifera* generally wandered a bit initially, presumably seeking a storage organ, but then settled into feeding on the leaf (Table 1-8). Neonates developing on *D. bulbifera* consumed an average of 9,082.6 ± 554.9 mm^2 of leaf tissue. In contrast, the maximum consumed on any other species was 56.7 ± 41.0 mm^2 (on *D. alata*), and most test plants remained undamaged (Table 1-8, Figure 1-10).

An average of 47.8 percent of the *L. egena* neonates developed into adults on *D. bulbifera* leaves (as compared to the 62.9 percent on bulbils) while no neonates completed development on any non-target foliage (Table 1-8, Figure 1-11). Average developmental time for *L. egena* neonates on *D. bulbifera* was 28.0 ± 0.4 days, whereas neonates on any other plant species generally survived less than 3 days (Table 1-8, Figure 1-11). The longest lived neonates, other than those on *D. bulbifera*, were one on *D. cordata* that survived 10 days and two that survived 6 days – one on *D. cordata* and one on *D. alata*, but none of these molted to become 2^{nd} instar larvae.

Thus, the neonate foliage feeding/development trials further supports the contention that *L.egena* is an air potato specialist, and the lower survivorship on foliage versus bulbils indicates this beetle is primarily adapted to the storage organs of its host.

Table 1-8. Outcomes of *Lilioceris egena* no choice neonate foliage feeding/developmental trials. Uncolored rows represent host trials utilizing Chinese beetles, whereas gray colored rows represent host trials utilizing Nepalese beetles. The latter were only conducted on members of the order Dioscoreales. Tan colored rows represent summaries by TAG Category.

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (mean ± s.e.)	F_1 adults produced (mean ± s.e.)	Leaf tissue consumed (mm^2) (mean ± s.e.)[2]
1	Dioscoreales	Dioscoreaceae	*Dioscorea bulbifera*	78	28.5 ± 0.4	2.3 ± 0.2	8828.0 ± 594.2
				9	27.4 ± 0.5	2.7 ± 0.4	9337.2 ± 1577.3
			Category 1 summary	**87**	**28.0 ± 0.4**	**2.5 ± 0.2**	**9082.6 ± 554.9**
2a	Dioscoreales	Dioscoreaceae	*Dioscorea floridana*	6	1.8 ± 0.3	0.0 ± 0.0	0.4 ± 0.1
				6	1.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
		Dioscoreaceae	*Dioscorea villosa*	5	1.6 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
				6	1.6 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
			Category 2a summary	**23**	**1.6 ± 0.1**	**0.0 ± 0.0**	**0.1 ± 0.0**
2b	Dioscoreales	Dioscoreaceae	*Dioscorea altissima*[3]	2	1.4 ± 0.4	0.0 ± 0.0	0.4 ± 0.4

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (mean ± s.e.)	F_1 adults produced (mean ± s.e.)	Leaf tissue consumed (mm^2) (mean ± s.e.)[2]
				0	-- --	-- --	-- --
		Dioscoreaceae	*Dioscorea cordata*	6	3.1 ± 0.6	0.0 ± 0.1	4.0 ± 3.5
				6	1.7 ± 0.2	0.0 ± 0.0	0.4 ± 0.3
		Dioscoreaceae	*Dioscorea pilosiuscula*	7	1.8 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
				6	1.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
		Dioscoreaceae	*Dioscorea polygonoides*	7	2.3 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
				6	1.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
		Dioscoreaceae	*Dioscorea trifida*	5	1.8 ± 0.3	0.0 ± 0.2	0.1 ± 0.1
				5	1.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
			Category 2b summary	**50**	**1.8 ± 0.1**	**0.0 ± 0.0**	**0.5 ± 0.4**
2c	Dioscoreales	Dioscoreaceae	*Dioscorea alata*	5	2.5 ± 0.7	0.0 ± 0.0	56.7 ± 41.0
				7	1.7 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
		Dioscoreaceae	*Dioscorea cayenensis*	8	1.8 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
				8	1.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Dioscoreaceae	*Dioscorea esculenta*	7	1.9 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
				6	1.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
		Dioscoreaceae	*Dioscorea oppositifolia*[3]	5	1.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
				0	-- -- --	-- -- --	-- -- --
		Dioscoreaceae	*Dioscorea polystachya*	6	1.9 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
				5	1.5 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
		Dioscoreaceae	*Dioscorea sansibarensis*	5	0.9 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
				5	1.4 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
			Category 2c summary	**67**	**1.6 ± 0.1**	**0.0 ± 0.0**	**4.1 ± 3.3**
3	Dioscoreales	Dioscoreaceae	Tacca chantrieri	5	1.7 ± 0.4	0.0 ± 0.0	0.2 ± 0.2
				7	1.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
			Category 3 summary	**12**	**1.5 ± 0.2**	**0.0 ± 0.0**	**0.1 ± 0.1**
4	Dioscoreales	**No native N. American Dioscoreaceae are threatened or endangered (Neither U.S. nor Florida)**					
5	Dioscoreales	**No species available for testing, see text.**					
6a	Pandanales	Pandanaceae	*Pandanus tectorius*	5	1.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
6b	Alismatales	Alismataceae	*Sagittaria latifolia*	5	1.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Araceae	*Alocasia cuculata*	5	1.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		Araceae	*Caladium bicolor*	5	1.7 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
		Araceae	*Colocasia esculenta*	8	1.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
		Araceae	*Symplocarpus foetidus*	5	1.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
		Araceae	*Xanthosoma sagittifolium*	5	1.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Araceae	*Zantedeschia aethiopica*	5	1.8 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Arecales	Arecaceae	*Sabal palmetto*	5	1.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Asperagales	Amaryllidaceae	*Crinum americanum*	8	1.9 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
		Amaryllidaceae	*Zephyranthes grandiflora*	5	1.5 ± 0.4	0.0 ± 0.0	0.0 ± 0.0

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (mean ± s.e.)	F_1 adults produced (mean ± s.e.)	Leaf tissue consumed (mm^2) (mean ± s.e.)[2]
	Commelinales	Commelinaceae	*Tradescantia pallida*	5	1.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Pontedariaceae	*Pontederia cordata*	5	1.6 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Poales	Cyperaceae	*Cladium jamaicense*	5	1.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Juncaceae	*Juncus effusus*	5	1.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Musaceae	*Musa acuminata*	5	4.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Poaceae	*Saccharum officinarum*	9	1.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Poaceae	*Zea mays*	7	1.8 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Zingiberales	Cannaceae	*Canna glauca*	5	0.8 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Cannaceae	*Canna americanallis*	5	1.0 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
		Costaceae	*Costus woodsonii*	5	0.9 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
		Heliconiaceae	*Heliconia caribaea*	5	1.8 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
		Marantaceae	*Maranta arundinacea*	5	1.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Marantaceae	*Thalia geniculata*	6	1.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
		Zingiberaceae	*Curcuma longa*	5	1.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		Zingiberaceae	*Hedychium coronarium*	10	1.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Zingiberaceae	*Zingiber officinale*	5	1.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Category 6b (+7b) Lilianae summary		**215**	**1.4 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
6c	Apiales	Apiaceae	*Apium graveolens*	5	1.9 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
		Apiaceae	*Daucus carota*	5	1.6 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
		Araliaceae	*Panax ginseng*	3	1.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
	Asterales	Asteraceae	*Arctium lappa*	5	1.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	Brassicales	Brassicaceae	*Brassica rapa*	5	1.7 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
		Brassicaceae	*Raphanus sativus*	5	1.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Caryophyllales	Amaranthaceae	*Beta vulgaris*	6	1.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Fabales	Fabaceae	*Glycine max*	5	1.4 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
		Fabaceae	*Mimosa pudica*	5	1.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Fabaceae	*Pachyrhizus erosus*	5	1.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Gentianales	Rubiaceae	*Guettarda scabra*	5	1.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	Laurales	Calycanthaceae	*Calycanthus floridus*	5	1.8 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Magnoliales	Annonaceae	*Annona glabra*	5	2.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	Malphigiales	Chrysobalanaceae	*Chrysobalanus icaco*	5	1.4 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
		Euphorbiaceae	*Manihot esculenta*	5	1.8 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	Piperales	Aristolochiaceae	*Aristolochia tomentosa*	5	1.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Solanales	Convolvulaceae	*Ipomoea batatas*	5	1.6 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Category 6c (+ 7c) non-Lilianae summary		**147**	**1.5 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**
7a	Monocot	Dioscoreaceae	*Dioscorea subclava*		**Known only from China, not available for testing.**		
7b	Asperagales	Amaryllidaceae	*Allium cepa*	6	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		Amaryllidaceae	*Allium sativum*	6	1.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
		Asparagaceae	*Asparagus densiflorus*	5	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (mean ± s.e.)	F_1 adults produced (mean ± s.e.)	Leaf tissue consumed (mm^2) (mean ± s.e.)[2]
		Asparagaceae	*Asparagus officinalis*	5	1.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Asphodelaceae	*Aloe vera*	5	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		Iridaceae	*Sisyrinchium angustifolium*	5	1.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Orchidaceae	*Bletilla striata*	5	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Dioscoreales	Dioscoreaceae	*Dioscorea alata*	12	2.1 ± 0.4	0.0 ± 0.0	28.4 ± 18.0
	Liliales	Liliaceae	*Lilium michauxii*	5	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		Liliaceae	*Tricyrtus lasiocarpa*	10	1.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Smilacaceae	*Smilax laurifolia*	5	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Pandanales	Pandanaceae	*Pandanus tectorius*	5	1.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	Poales	Poaceae	*Triticum aestivum*	5	1.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
			Category 7b Lilianae summary	**79**	**1.5 ± 0.1**	**0.0 ± 0.0**	**0.0 ± 0.0**
7c	Apiales	Araliaceae	*Schefflera actinophylla*	5	1.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Asterales	Campanulaceae	*Lobelia cardinalis*	5	1.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	Caryophyllales	Polygonaceae	*Persicaria glabra*	5	1.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	Fabales	Fabaceae	*Senna ligustrina*	5	1.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	Fagales	Betulaceae	*Corylus americana*	5	1.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Fagaceae	*Quercus virginiana*	5	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Gentianales	Apocynaceae	*Asclepias tuberosa*	5	1.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	Lamiales	Verbenaceae	*Callicarpa americana*	8	1.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Malpighiales	Salicaceae	*Salix caroliniana*	5	1.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Rosales	Moraceae	*Ficus aurea*	5	1.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	Solanales	Solanaceae	*Solanum tuberosum*	5	1.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	Cycadales	Cycadaceae	*Cycas revoluta*	5	2.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
			Category 7c non-Lilianae summary	**63**	**1.4 ± 0.1**	**0.0 ± 0.0**	**0.0 ± 0.0**
			Category 7 combined summary	**142**	**1.5 ± 0.0**	**0.0 ± 0.0**	**0.0 ± 0.0**

[1] Species from Category 7 also qualify as belonging in Category 6 (with the exception of *D. alata*, which is omitted) and so are included in the appropriate Category 6 summaries.

[2] Each trial replicate contained three 2nd/3rd instar larvae. Although survivorship could be tracked individually, tissue consumption could not. Thus, the leaf tissue consumed represents the combined feeding activity of the three larvae in each replicate.

[3] These plants died out part way through the trials, and additional plants could not be obtained to complete the studies.

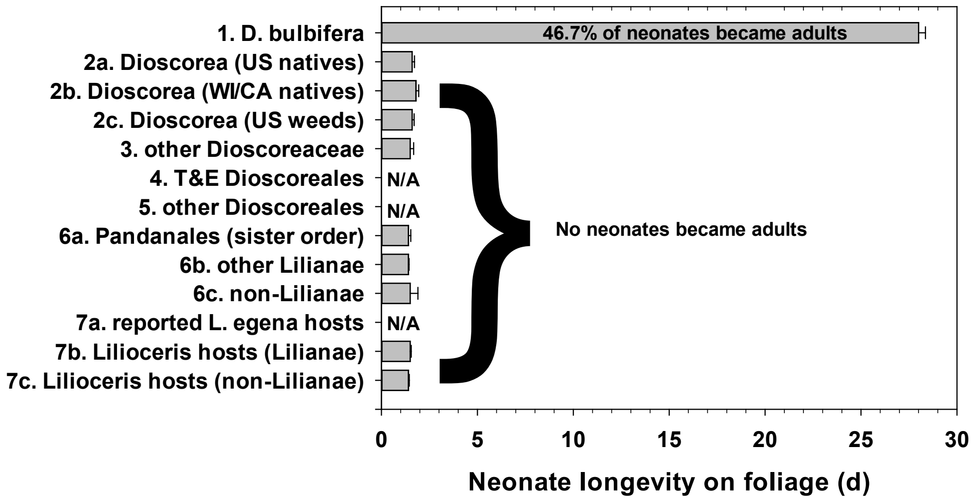

Figure 1-10. Neonate *Lilioceris egena* developmental period/longevity (mean ± s.e.) on foliage, averaged across TAG categories.

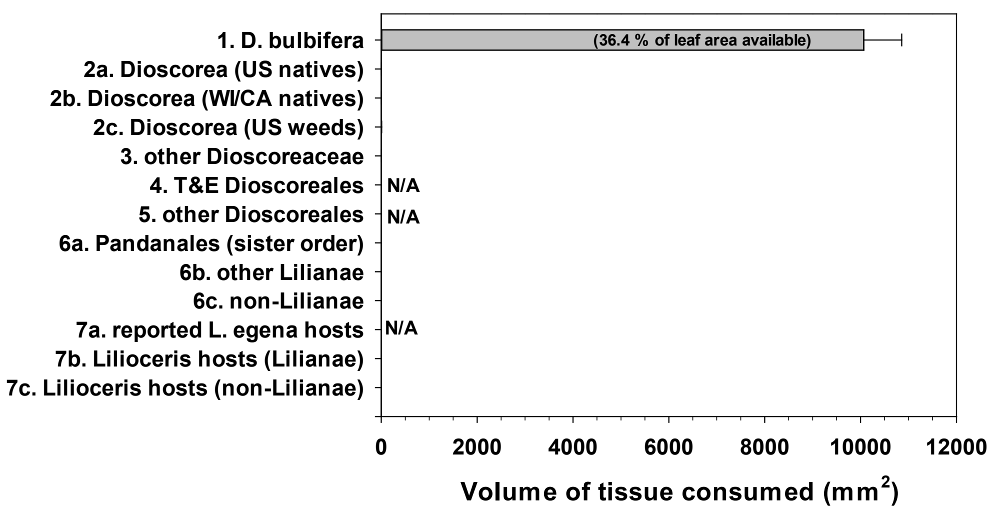

Figure 1-11. Neonate *Lilioceris egena* leaf tissue consumption (mean ± s.e.), averaged across TAG categories.

No choice $2^{nd}/3^{rd}$ instar storage organ feeding/development trials

Roughly two-thirds (67.9 percent) of $2^{nd}/3^{rd}$ instars developed into adults when fed *D. bulbifera* storage organs, requiring an average of 25.2 ± 0.57 days to complete development. An additional 5.4 percent of the larvae pupated, but failed to produce adults. In contrast, larvae fed non-target hosts generally survived a week or less, and only one (0.01 percent of all $2^{nd}/3^{rd}$ instars included in these trials) developed into an adult (Table 1-9). This single individual was a Nepalese 3^{rd} instar exposed to bulbils of *D. alata* (an Asian native, cultivated in the Caribbean and

Mesoamerica, and invasive in Florida). The larvae in these trials were not naïve, and had been feeding and developing in *D. bulbifera* bulbils prior to being moved onto the storage organs of test plants. Such a scenario makes it likely this lone 3rd instar had acquired sufficient fat reserves to survive and complete development without feeding upon the test plant. All-in-all, though, no other 2nd/3rd instars pupated or completed development on plants other than *D. bulbifera* (Table 1-9, Figure 1-12).

Aside from *D. bulbifera*, three trials produced substantial damage (Table 1-9, Figure 1-13). Larvae in two of the trials on tuberous jicama (*Pachyrhizus erosus*) roots consumed 4.65 and 4.80 mL (per trial, respectively) of tissue, roughly three times the amount of tissue consumed in successful *D. bulbifera* trials (Table 1-9). These jicama-fed larvae (three total) died after molting from 2nd to 3rd instar. The large consumption coupled with failure to pupate or complete development indicates that the jicama roots are nutritionally inadequate for *L. egena*, but may lack feeding deterrants. Similarly, two larvae in a single *D. oppositifolia* trial molted into third instars, but then failed to develop further despite consuming a toal of 2.750 mL of tissue.

The data from these no choice 2nd/3rd instars storage organ feeding/development trials suggests the possibility that late instars from eggs deposited upon *D. bulbifera* bulbils could occassionally migrate to, and complete development on *D. alata* bulbils in areas of Florida and the Caribbean where the two species are intermixed. However, the failure of adults to oviposit and failure of neonates to develop on *D. alata* suggests that persistent populations would not develop on this plant.

Table 1-9. Outcomes of *Lilioceris egena* no choice 2nd/3rd instar larval storage organ feeding/developmental trials. Uncolored rows represent host trials utilizing Chinese beetles, whereas gray colored rows represent host trials utilizing Nepalese beetles. The latter were only conducted on members of the order Dioscoreales. Tan colored rows represent summaries by TAG Category.

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (avg ± s.e.)	F_1 adults produced (avg ± s.e.)	Storage organ tissue consumed (mL) (avg ± s.e.)[2]
1	Dioscoreales	Dioscoreaceae	*Dioscorea bulbifera*	43	25.8 ± 0.72	2.0 ± 0.1	1.615 ± 0.1105
				11	24.7 ± 0.38	1.9 ± 0.3	1.775 ± 0.2478
			Category 1 summary	**54**	**25.2 ± 0.57**	**2.0 ± 0.1**	**1.695 ± 0.1015**
2a	Dioscoreales	Dioscoreaceae	*Dioscorea floridana*	5	2.9 ± 0.66	0.0 ± 0.0	0.018 ± 0.0143
				5	3.1 ± 0.23	0.0 ± 0.0	0.000 ± 0.0000
		Dioscoreaceae	*Dioscorea villosa*	5	3.3 ± 0.32	0.0 ± 0.0	0.010 ± 0.0100
				5	2.7 ± 0.36	0.0 ± 0.0	0.000 ± 0.0000
			Category 2a summary	**20.0**	**3.0 ± 0.18**	**0.0 ± 0.0**	**0.004 ± 0.0028**
2b	Dioscoreales	Dioscoreaceae	*Dioscorea pilosiuscula*	5	2.0 ± 0.38	0.0 ± 0.0	0.022 ± 0.0058
				5	4.5 ± 0.45	0.0 ± 0.0	0.028 ± 0.0166
		Dioscoreaceae	*Dioscorea polygonoides*	5	5.8 ± 1.11	0.0 ± 0.0	0.105 ± 0.0371
				5	3.5 ± 0.17	0.0 ± 0.0	0.049 ± 0.0175
		Dioscoreaceae	*Dioscorea trifida*	5	3.7 ± 0.43	0.0 ± 0.0	0.137 ± 0.1136
				5	3.4 ± 0.99	0.0 ± 0.0	0.002 ± 0.0012

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (avg ± s.e.)	F_1 adults produced (avg ± s.e.)	Storage organ tissue consumed (mL) (avg ± s.e.)[2]
			Category 2b summary	**30.0**	**3.8 ± 0.32**	**0.0 ± 0.0**	**0.050 ± 0.0195**
2c	Dioscoreales	Dioscoreaceae	*Dioscorea alata*	6	5.0 ± 0.37	0.0 ± 0.0	0.138 ± 0.0225
				5	3.1 ± 1.82	0.2 ± 0.2	0.138 ± 0.1256
		Dioscoreaceae	*Dioscorea cayenensis*[3]	5	1.9 ± 0.20	0.0 ± 0.0	0.005 ± 0.0050
				0	-- --	-- --	-- --
		Dioscoreaceae	*Dioscorea esculenta*	5	2.7 ± 0.33	0.0 ± 0.0	0.003 ± 0.0030
				5	3.6 ± 0.75	0.0 ± 0.0	0.014 ± 0.0068
		Dioscoreaceae	*Dioscorea oppositifolia*	5	4.8 ± 1.09	0.0 ± 0.0	0.834 ± 0.5479
				5	5.9 ± 0.27	0.0 ± 0.0	1.651 ± 0.2433
		Dioscoreaceae	*Dioscorea polystachya*	5	4.6 ± 0.54	0.0 ± 0.0	0.261 ± 0.1217
				5	5.1 ± 0.69	0.0 ± 0.0	0.000 ± 0.0000
		Dioscoreaceae	*Dioscorea sansibarensis*	11	1.5 ± 0.27	0.0 ± 0.0	0.066 ± 0.0267
				5	1.9 ± 0.08	0.0 ± 0.0	0.097 ± 0.0238
			Category 2c summary	**62.0**	**3.6 ± 0.27**	**0.0 ± 0.0**	**0.285 ± 0.0749**
3		Dioscoreaceae	*Tacca chantrieri*[3]	5	4.3 ± 0.42	0.0 ± 0.0	0.014 ± 0.0083
				0	-- --	-- --	-- --
			Category 3 summary	**5**	**4.3 ± 0.42**	**0.0 ± 0.0**	**0.014 ± 0.0083**
4	Dioscoreales	**No native N. American Dioscoreaceae are threatened or endangered (Neither U.S. nor Florida)**					
5	Dioscoreales	**No species available for testing, see text.**					
6a	Pandanales	**The only U.S. species [*Croomia pauciflora* (Nutt.) Torr. (Stemonaceae)] within this order has no storage organs**					
6b	Alismatales	Araceae	*Colocasia esculenta*	5	2.5 ± 0.20	0.0 ± 0.0	0.000 ± 0.0002
		Araceae	*Xanthosoma sagittifolium*	5	5.3 ± 1.02	0.0 ± 0.0	0.011 ± 0.0077
	Zingiberales	Marantaceae	*Maranta arundinacea*	5	3.9 ± 0.94	0.0 ± 0.0	0.000 ± 0.0000
		Zingiberaceae	*Curcuma longa*	5	2.7 ± 0.07	0.0 ± 0.0	0.000 ± 0.0000
		Zingiberaceae	*Hedychium coronarium*	5	1.3 ± 1.28	0.0 ± 0.0	0.000 ± 0.0002
		Zingiberaceae	*Zingiber officinale*	5	2.1 ± 0.25	0.0 ± 0.0	0.003 ± 0.0030
			Category 6b (+7b) Lilianae summary	**49.0**	**3.0 ± 0.21**	**0.0 ± 0.0**	**0.003 ± 0.0011**
6c	Apiales	Apiaceae	*Daucus carota*	5	4.1 ± 0.66	0.0 ± 0.0	0.014 ± 0.0070
		Araliaceae	*Panax ginseng*	5	2.5 ± 0.29	0.0 ± 0.0	0.000 ± 0.0000
	Asterales	Asteraceae	*Arctium lappa*	5	3.6 ± 0.80	0.0 ± 0.0	0.000 ± 0.0000
	Brassicales	Brassicaceae	*Brassica rapa*	10	3.1 ± 0.49	0.0 ± 0.0	0.004 ± 0.0026
		Brassicaceae	*Raphanus sativus*	5	3.5 ± 0.50	0.0 ± 0.0	0.204 ± 0.1231
	Caryophyllales	Amaranthaceae	*Beta vulgaris*	5	5.6 ± 0.39	0.0 ± 0.0	0.017 ± 0.0118
		Fabaceae	*Pachyrhizus erosus*	5	7.0 ± 1.66	0.0 ± 0.0	1.893 ± 1.1564
	Malpighiales	Euphorbiaceae	*Manihot esculenta*	5	4.0 ± 0.38	0.0 ± 0.0	0.000 ± 0.0000
	Solanales	Convolvulaceae	*Ipomoea batatas*	5	2.6 ± 0.32	0.0 ± 0.0	0.000 ± 0.0000
			Category 6c (+ 7c) non-Lilianae summary	**55.0**	**3.8 ± 0.27**	**0.0 ± 0.0**	**0.191 ± 0.1194**
7a	Monocot	Dioscoreaceae	*Dioscorea subclava*	**Known only from China, not available for testing.**			
7b	Asparagales	Amaryllidaceae	*Allium cepa*	5	3.1 ± 0.95	0.0 ± 0.0	0.012 ± 0.0097
			Allium sativum	4	3.3 ± 0.16	0.0 ± 0.0	0.000 ± 0.0000
	Dioscoreales	Dioscoreaceae	*Dioscorea alata*	6	5.0 ± 0.37	0.0 ± 0.0	0.138 ± 0.0225
	Liliales	Liliaceae	*Lilium michauxii*	5	3.9 ± 0.48	0.0 ± 0.0	0.000 ± 0.0000
		Liliaceae	*Tricyrtus lasiocarpa*	5	2.0 ± 0.00	0.0 ± 0.0	0.000 ± 0.0000
			Category 7b Lilianae summary	**25**	**3.5 ± 0.30**	**0.0 ± 0.0**	**0.035 ± 0.0129**
7c	Solanales	Solanaceae	*Solanum tuberosum*	5	3.3 ± 0.33	0.0 ± 0.0	0.000 ± 0.0000
			Category 7 combined summary	**30.0**	**3.5 ± 0.25**	**0.0 ± 0.0**	**0.030 ± 0.0110**

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (avg ± s.e.)	F_1 adults produced (avg ± s.e.)	Storage organ tissue consumed (mL) (avg ± s.e.)[2]

[1] Species from Category 7 also qualify as belonging in Category 6 (with the exception of *D. alata*, which is omitted) and so are included in the appropriate Category 6 summaries.
[2] Each trial replicate contained three 2nd/3rd instar larvae. Although survivorship could be tracked individually, tissue consumption could not. Thus, the leaf tissue consumed represents the combined feeding activity of the three larvae in each replicate.
[3] These plants died out part way through the trials, and additional plants could not be obtained to complete the studies.

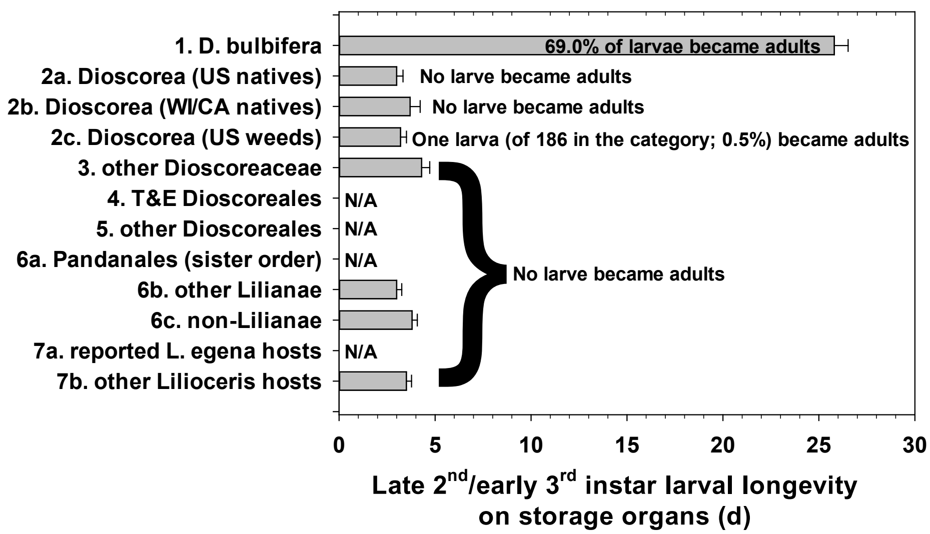

Figure 1-12. Developmental period/longevity (mean ± s.e.) of 2nd/3rd instar *Lilioceris egena* larvae on plant storage organs, averaged across TAG categories.

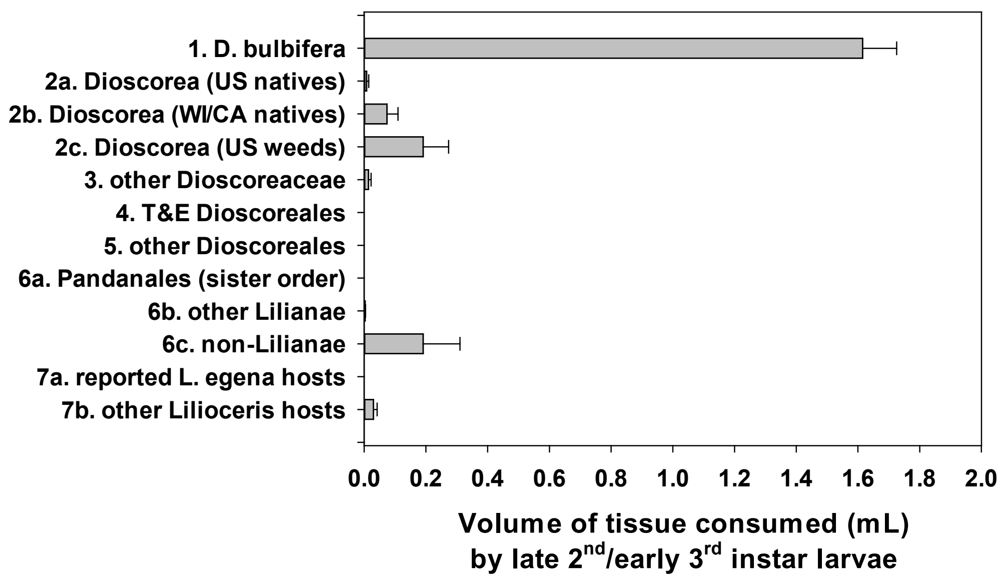

Figure 1-13. Consumption of plant storage organ tissues (mean ± s.e.) by 2nd/3rd instar *Lilioceris egena* larvae, averaged across TAG categories.

<u>No choice 2nd/3rd instar larval foliage feeding/development trials</u>

Roughly two-thirds of 2nd/3rd instar *L. egena* larvae produced adults when fed *D. bulbifera* foliage, requiring 21.6 ± 0.3 days to complete development (Table 1-10, Figure 1-14). Given that these larvae had developed 4–5 days from eclosion, this developmental period is consistent with the neonate developmental data (Table 1-8).

Two adults were produced on plants aside from *D. bulbifera* in these trials, though neither was on a species found in the United States. A single adult was produced on *Dioscorea cordata* (a West Indian endemic), and another on *Dioscorea trifida* (Mesoamerica to northern South America) (both category 2b plants). Each adult represents 2.8 percent of the larvae offered these plants. Once again, the ability of a 2nd/3rd instar *L. egena* larva to occasionally finish development on another species in the genus *Dioscorea* is unsurprising, given the larvae in these trials were not naïve but had been feeding and developing in *D. bulbifera* bulbils prior to being moved onto the foliage of test plants. Such a scenario makes it likely that an occasional 3rd instar larva would be produced with sufficient fat reserves to complete development even without feeding upon the test plant.

In general, 2nd/3rd instar *L. egena* larvae died within a week of being placed on test plant foliage (Table 1-10, Figure 1-14). Similar to the neonate larvae, the 2nd/3rd instar larvae frequently abandoned the proffered host plant to seek a more suitable food resource. The extended lifespan, as compared to neonates, may have been a result of fat reserves built up while developing on a *D. bulbifera* bulbil slice prior to being transferred onto the test plant. This conclusion is supported by the fact that average *L. egena* 2nd/3rd instar larval consumption of non-target

Dioscorea species (categories 2a, 2b, 2c) was very low (1–15 percent) when compared to the amount of *D. bulbifera* leaf material consumed, thus making it highly unlikely these older larvae would have survived for 7 days without the "jump start" that the production technique provided.

Results from this set of trials suggests that there is a small chance that late 3rd instar *L. egena* larvae which initiate development on *D. bulbifera* bulbils or leaves could possibly transfer and complete development on the leaves of a couple of *Dioscorea* congeners. However, given the extreme ovipositional preference of female *L. egena* for *D. bulbifera* storage organs, and the inability of neonates to develop on anything other than *D. bulbifera*, it is unlikely that a persistent population could develop on any other *Dioscorea* species (Dray, 2017).

Table 1-10. Outcomes of *Lilioceris egena* no choice 2nd/3rd instar larval foliage feeding/developmental trials. Uncolored rows represent host trials utilizing Chinese beetles, whereas gray colored rows represent host trials utilizing Nepalese beetles. The latter were only conducted on members of the order Dioscoreales. Tan colored rows represent summaries by TAG Category.

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (avg ± s.e.)	F1 adults produced (avg ± s.e.)	Leaf tissue consumed (mm^2) (avg ± s.e.)[2]
1	Dioscoreales	Dioscoreaceae	*Dioscorea bulbifera*	66	23.0 ± 0.3	2.1 ± 0.1	7255.6 ± 410.4
				11	20.2 ± 0.0	1.8 ± 0.3	7698.4 ± 1598.6
			Category 1 summary	**77**	**21.6 ± 0.3**	**1.9 ± 0.1**	**7479.3 ± 420.8**
2a	Dioscoreales	Dioscoreaceae	*Dioscorea floridana*	7	3.8 ± 0.5	0.0 ± 0.0	33.8 ± 9.7
				5	3.9 ± 0.6	0.0 ± 0.0	155.9 ± 118.1
		Dioscoreaceae	*Dioscorea villosa*	7	4.4 ± 0.6	0.0 ± 0.0	106.2 ± 70.9
				5	4.4 ± 0.7	0.0 ± 0.0	288.3 ± 115.1
			Category 2a summary	**24**	**4.0 ± 0.3**	**0.0 ± 0.0**	**150.1 ± 28.7**
2b	Dioscoreales	Dioscoreaceae	*Dioscorea altissima*[3]	1	3.0 ± 0.0	0.0 ± 0.0	89.3 ± 0.0
				0	-- ----	-- -- --	-- -- --
		Dioscoreaceae	*Dioscorea cordata*	7	5.3 ± 1.1	0.1 ± 0.1	209.3 ± 141.1
				5	5.1 ± 0.6	0.0 ± 0.0	1051.5 ± 289.9
		Dioscoreaceae	*Dioscorea pilosiuscula*	6	3.2 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
				5	4.3 ± 0.3	0.0 ± 0.0	1.1 ± 1.1
		Dioscoreaceae	*Dioscorea polygonoides*	6	5.3 ± 0.6	0.0 ± 0.0	225.0 ± 126.7
				5	5.0 ± 0.4	0.0 ± 0.0	1051.4 ± 611.1
		Dioscoreaceae	*Dioscorea trifida*	5	4.5 ± 1.3	0.2 ± 0.2	945.0 ± 663.9
				7	4.4 ± 0.8	0.0 ± 0.0	1029.7 ± 544.2
			Category 2b summary	**47**	**4.6 ± 0.3**	**0.0 ± 0.0**	**549.4 ± 102.7**
2c	Dioscoreales	Dioscoreaceae	*Dioscorea alata*	6	3.7 ± 0.4	0.0 ± 0.0	371.5 ± 325.5
				5	4.8 ± 0.5	0.0 ± 0.0	13.4 ± 13.4
		Dioscoreaceae	*Dioscorea cayenensis*	9	3.4 ± 0.3	0.0 ± 0.0	19.1 ± 15.5
				7	4.4 ± 0.3	0.0 ± 0.0	27.6 ± 21.1
		Dioscoreaceae	*Dioscorea esculenta*	5	3.9 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
				7	4.5 ± 0.4	0.0 ± 0.0	7.4 ± 7.2
		Dioscoreaceae	*Dioscorea oppositifolia*[3]	5	4.0 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
				0	-- ----	-- -- --	-- -- --
		Dioscoreaceae	*Dioscorea polystachya*	5	5.0 ± 0.8	0.0 ± 0.0	282.2 ± 215.4
				7	4.7 ± 0.3	0.0 ± 0.0	465.8 ± 247.8
		Dioscoreaceae	*Dioscorea sansibarensis*	5	1.9 ± 0.1	0.0 ± 0.0	14.7 ± 9.8
				7	2.0 ± 0.2	0.0 ± 0.0	46.6 ± 26.0
			Category 2c summary	**68**	**3.8 ± 0.2**	**0.0 ± 0.0**	**115.9 ± 45.8**
3	Dioscoreales	Dioscoreaceae	Tacca chantrieri	6	2.9 ± 0.5	0.0 ± 0.0	7.7 ± 7.7

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (avg ± s.e.)	F_1 adults produced (avg ± s.e.)	Leaf tissue consumed (mm^2) (avg ± s.e.)[2]
				7	2.3 ± 0.4	0.0 ± 0.0	7.0 ± 3.6
			Category 3 summary	**13**	**2.6 ± 0.3**	**0.0 ± 0.0**	**7.3 ± 5.0**
4	Dioscoreales	**No native N. American Dioscoreaceae are threatened or endangered (Neither U.S. nor Florida)**					
5	Dioscoreales	**No species available for testing, see text.**					
6a	Pandanales	Pandanaceae	*Pandanus tectorius*	5	1.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
6b	Alismatales	Alismataceae	*Sagittaria latifolia*	5	2.8 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
		Araceae	*Alocasia cuculata*	6	6.1 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
		Araceae	*Caladium bicolor*	6	5.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0
		Araceae	*Colocasia esculenta*	5	3.0 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
		Araceae	*Symplocarpus foetidus*	6	4.3 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
		Araceae	*Xanthosoma sagittifolium*	5	3.8 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
		Araceae	*Zantedeschia aethiopica*	5	4.9 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
	Arecales	Arecaceae	*Sabal palmetto*	5	4.7 ± 0.6	0.0 ± 0.0	0.0 ± 0.0
	Asperagales	Amaryllidaceae	*Crinum americanum*	5	3.4 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
		Amaryllidaceae	*Zephyranthes grandiflora*	5	2.9 ± 0.9	0.0 ± 0.0	0.0 ± 0.0
	Commelinales	Commelinaceae	*Tradescantia pallida*	5	3.4 ± 0.7	0.0 ± 0.0	0.1 ± 0.1
		Pontedariaceae	*Pontederia cordata*	5	4.0 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
	Poales	Cyperaceae	*Cladium jamaicense*	5	4.7 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Juncaceae	*Juncus effusus*	5	3.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
		Musaceae	*Musa acuminata*	5	2.9 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Poaceae	*Saccharum officinarum*	5	4.9 ± 0.6	0.0 ± 0.0	0.0 ± 0.0
		Poaceae	*Zea mays*	5	4.6 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
	Zingiberales	Cannaceae	*Canna glauca*	5	4.2 ± 0.6	0.0 ± 0.0	1.3 ± 0.6
		Cannaceae	*Canna americanallis*	5	3.8 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
		Costaceae	*Costus woodsonii*	5	2.6 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
		Heliconiaceae	*Heliconia caribaea*	5	5.8 ± 0.6	0.0 ± 0.0	0.3 ± 0.2
		Marantaceae	*Maranta arundinacea*	5	3.7 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
		Marantaceae	*Thalia geniculata*	5	6.6 ± 0.4	0.0 ± 0.0	0.9 ± 0.9
		Zingiberaceae	*Curcuma longa*	10	4.4 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
		Zingiberaceae	*Hedychium coronarium*	5	4.5 ± 0.6	0.0 ± 0.0	0.0 ± 0.0
		Zingiberaceae	*Zingiber officinale*	5	5.5 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
			Category 6b (+7b) Lilianae summary	**210**	**4.0 ± 0.1**	**0.0 ± 0.0**	**0.1 ± 0.0**
6c	Apiales	Apiaceae	*Apium graveolens*	7	4.1 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
		Apiaceae	*Daucus carota*	7	4.9 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
		Araliaceae	*Panax ginseng*	5	3.7 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
	Asterales	Asteraceae	*Arctium lappa*	5	1.9 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	Brassicales	Brassicaceae	*Brassica rapa*	7	5.1 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
		Brassicaceae	*Raphanus sativus*	5	3.5 ± 0.9	0.0 ± 0.0	0.0 ± 0.0
	Caryophyllales	Amaranthaceae	*Beta vulgaris*	6	4.9 ± 0.7	0.0 ± 0.0	0.0 ± 0.0

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (avg ± s.e.)	F_1 adults produced (avg ± s.e.)	Leaf tissue consumed (mm^2) (avg ± s.e.)[2]
	Fabales	Fabaceae	*Glycine max*	5	4.3 ± 0.6	0.0 ± 0.0	0.0 ± 0.0
		Fabaceae	*Mimosa pudica*	5	3.7 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
		Fabaceae	*Pachyrhizus erosus*	7	4.8 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
	Gentianales	Rubiaceae	*Guettarda scabra*	5	4.3 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
	Laurales	Calycanthaceae	*Calycanthus floridus*	5	2.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	Magnoliales	Annonaceae	*Annona glabra*	5	2.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Malphigiales	Chrysobalanaceae	*Chrysobalanus icaco*	5	3.2 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
		Euphorbiaceae	*Manihot esculenta*	5	3.9 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Piperales	Aristolochiaceae	*Aristolochia tomentosa*	5	2.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	Solanales	Convolvulaceae	*Ipomoea batatas*	6	4.1 ± 0.6	0.0 ± 0.0	5.4 ± 5.4
		Category 6c (+ 7c) non-Lilianae summary		**152**	**4.1 ± 0.1**	**0.0 ± 0.0**	**0.2 ± 0.2**
7a	Monocot	Dioscoreaceae	*Dioscorea subclava*		**Known only from China, not available for testing.**		
7b	Asperagales	Amaryllidaceae	*Allium cepa*	5	3.6 ± 0.8	0.0 ± 0.0	0.0 ± 0.0
		Amaryllidaceae	*Allium sativum*	6	3.8 ± 0.5	0.0 ± 0.0	0.8 ± 0.8
		Asparagaceae	*Asparagus densiflorus*	5	4.7 ± 0.8	0.0 ± 0.0	0.0 ± 0.0
		Asparagaceae	*Asparagus officinalis*	5	2.9 ± 0.3	0.0 ± 0.0	0.1 ± 0.0
		Asphodelaceae	*Aloe vera*	5	2.7 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
		Iridaceae	*Sisyrinchium angustifolium*	5	4.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
		Orchidaceae	*Bletilla striata*	5	3.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Dioscoreales	Dioscoreaceae	*Dioscorea alata*	11	4.2 ± 0.4	0.0 ± 0.0	371.5 ± 229.2
	Liliales	Liliaceae	*Lilium michauxii*	5	3.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
		Liliaceae	*Tricyrtus lasiocarpa*	5	4.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		Smilacaceae	*Smilax laurifolia*	5	2.7 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Pandanales	Pandanaceae	*Pandanus tectorius*	5	3.4 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
	Poales	Poaceae	*Triticum aestivum*	5	3.6 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
		Category 7b Lilianae summary		**72**	**4.2 ± 0.1**	**0.0 ± 0.0**	**6.73 ± 0.9**
7c	Apiales	Araliaceae	*Schefflera actinophylla*	5	5.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
	Asterales	Campanulaceae	*Lobelia cardinalis*	5	3.7 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
	Caryophyllales	Polygonaceae	*Persicaria glabra*	5	4.5 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
	Fabales	Fabaceae	*Senna ligustrina*	5	3.3 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Fagales	Betulaceae	*Corylus americana*	5	5.9 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
		Fagaceae	*Quercus virginiana*	5	5.7 ± 0.9	0.0 ± 0.0	0.0 ± 0.0
	Gentianales	Apocynaceae	*Asclepias tuberosa*	5	2.9 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
	Lamiales	Verbenaceae	*Callicarpa americana*	5	4.5 ± 1.1	0.0 ± 0.0	0.0 ± 0.0
	Malpighiales	Salicaceae	*Salix caroliniana*	5	3.7 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
	Rosales	Moraceae	*Ficus aurea*	5	4.6 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
	Solanales	Solanaceae	*Solanum tuberosum*	4	2.9 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
	Cycadales	Cycadaceae	*Cycas revoluta*	5	5.3 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
		Category 7c non-Lilianae summary		**57**	**4.5 ± 0.2**	**0.0 ± 0.0**	**0.0 ± 0.0**

TAG Category	Order	Family	Species[1]	Total reps	Longevity (d) (avg ± s.e.)	F_1 adults produced (avg ± s.e.)	Leaf tissue consumed (mm^2) (avg ± s.e.)[2]
			Category 7 combined summary	**129**	**4.4 ± 0.1**	**0.0 ± 0.0**	**6.7 ± 0.4**

[1] Species from Category 7 also qualify as belonging in Category 6 (with the exception of *D. alata*, which is omitted) and so are included in the appropriate Category 6 summaries.

[2] Each trial replicate contained three 2nd/3rd instar larvae. Although survivorship could be tracked individually, tissue consumption could not. Thus, the leaf tissue consumed represents the combined feeding activity of the three larvae in each replicate.

[3]These plants died out part way through the trials, and additional plants could not be obtained to complete the studies.

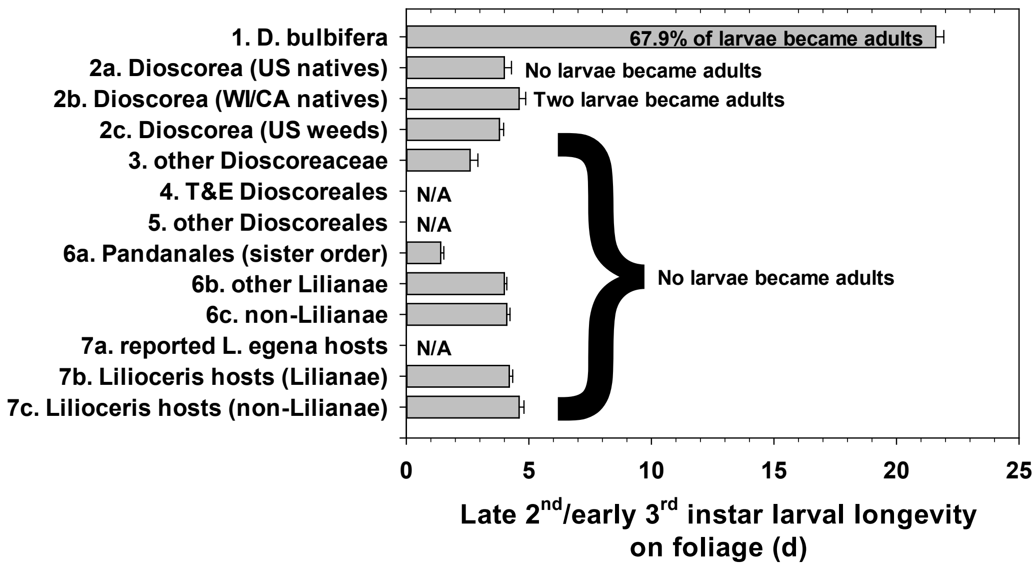

Figure 1-14. Developmental period/longevity (mean ± s.e.) of 2nd/3rd instar *Lilioceris egena* larvae on foliage, averaged across TAG categories.

<u>Two choice adult oviposition and development tests – adults on whole plants.</u>

In this choice test, *L. egena* preferred *D. bulbifera* versus *D. cordata* in each of the three parameters tested. The beetles damaged an average of 83.6 percent of the *D. bulbifera* leaves available to them, as compared to 11.4 percent of the *D. cordata* leaves available. Two-thirds of the *D. bulbifera* bulbils in the cages were damaged, and females only oviposited on these bulbils. Finally, 60 percent of the adult beetles that were recovered were found on *D. bulbifera* leaves, with another 20 percent on the bulbils. The remaining 20 percent of the adults were recovered from *D. coradata* leaves. These data suggest that should *L. egena* migrate to regions of the Caribbean where *D. bulbifera* and *D. cordata* co-occur in close proximity, the beetle would likely cause some cosmetic damage to *D. cordata*. However, the absence of oviposition on *D. cordata* (both in this trial and in the adult no choice foliage trials, Table 1-6), 100 percent neonate mortality on *D. cordata*, and this species' absence of above ground storage organs means that persistent populations would not establish on this plant.

Ovipositional preference trials

Females rarely oviposited on live *D. bulbifera* leaves during the feeding trials (6.2 percent of trials), whereas they oviposited on *D. bulbifera* bulbils in 90.3 percent of the storage organ feeding trials. Also, oviposition occurred in 45 of 49 cases (91.8 percent) in which the females were immediately moved from *D. bulbifera* leaves onto *D. bulbifera* bulbils at the conclusion of foliage feeding trials, usually within 24–48 hours. This confirms that the females used in these trials were capable of producing eggs, but did not oviposit until placed on their preferred ovipositional host. From these data, the researchers conclude that female *L. egena* are so closely adapted to their host plant, *D. bulbifera*, that acceptable ovipositional substrates are restricted to the air potato vine's storage organs (bulbils and tubers).

A follow-up question was whether *L. egena* females would demonstrate any difference in ovipositional success between subterranean tubers and aerial bulbils (Figure 1-15a). In this series of experiments, *L. egena* adults fed equally as well on tubers (Figure 1-15b) as bulbils when at the surface, but did not burrow to locate tubers underground (Figure 1-15c). Likewise, the females demonstrated no ovipositional preference between the two storage organs (Figure 1-15d), but failed to burrow to locate tubers underground. Finally, when placed on exposed patches (4.6 cm^2) of tubers and bulbils that were otherwise buried in the soil, neonates developed equally well on either storage organ (Figure 1-15e). Thus, although tubers are an acceptable food and developmental substrate for these beetles, it is likely that the primary impact will be on bulbils because *L. egena* requires tubers to be exposed before the beetles will attack them.

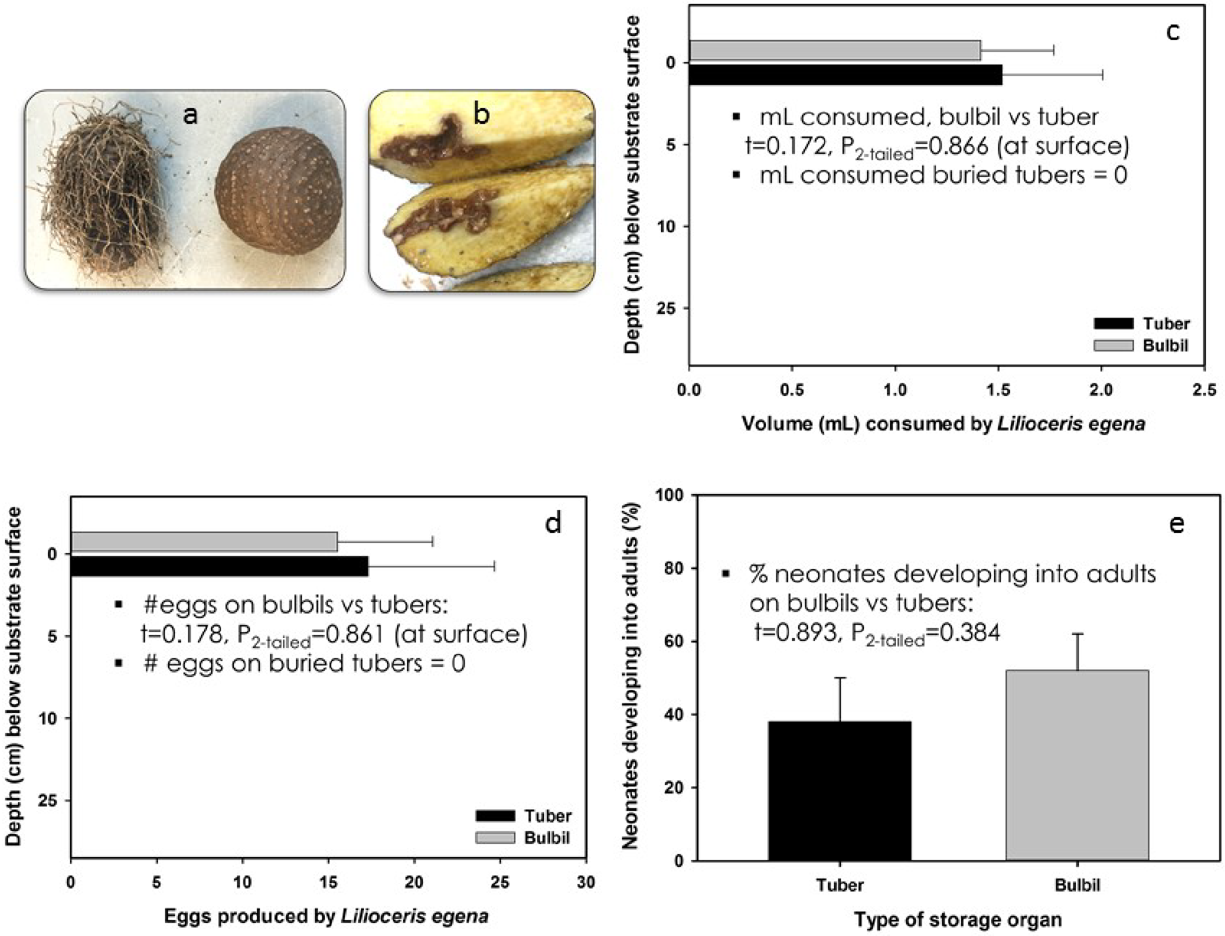

Figure 1-15. Comparison of adult *Lilioceris egena* performance on *Dioscorea bulbifera* tubers vs bulbils (a) showed that adult feeding damage in tubers (b) did not differ from that in bulbils (c). Similarly, neither was preferred as an ovipositional substrate (d), and neonates developed equally well on both (e).

<u>Researcher's conclusions (Dray, 2017)</u>

The results of these host range trials provide strong evidence that the beetle, *Lilioceris egena,* is specialized on its target host, *Dioscorea bulbifera* (air potato). Oviposition occurred only on this plant, and females tended to hold eggs while on air potato foliage only to initiate oviposition as soon as being placed upon bulbils (and presumedly, exposed subterranean tubers). Thus, the ovipositional specificity occurs at the organ level within a single species, and not just the species as a whole. Neonates failed to develop on any plant species aside from *D. bulbifera* and developed better on *D. bulbifera* bulbils/tubers than on leaves. Further, in a preliminary choice test (not otherwise reported herein) in which neonates were placed in arenas with leaves and bulbil slices, the larvae always moved onto the bulbils, even if placed directly on the leaves first. The reverse, larvae abandoning bulbils for leaves, never occurred. Finally, the data from $2^{nd}/3^{rd}$ instar larval trials suggest that late instar larvae from *D. bulbifera* leaves or bulbils might on occasionally migrate to, and complete development on, a few *Dioscorea* congeners (*D. alata, D. cordata, D. trifida*) in areas of Florida and the Caribbean where these congeners are intermixed and the larvae cannot locate their preferred host. The exteme rarity of this occurance in the no choice trials (3 of 456 $2^{nd}/3^{rd}$ instar larvae on Dioscoreaceae; <1 percent), which forced the larvae to stay on the non-target host, indicates that the likelihood of this occurring in nature is very low. However, even should this occur, the aforementioned failure of adults to oviposit and neonates to develop on non-target plants assures that persistent populations could not develop on these non-target plant species. Accordingly, *L. egena* appears to be a specific insect.

Appendix 2. Release Protocol and Post-Release Monitoring Plan for *Lilioceris egena* (Dray, 2017).

Release Protocol

Releases will be made of individuals descended from the Chinese and Nepalese colonies used in the host range trials reported herein. These colonies have already been cleared of diseases, natural enemies, and the possibility of cryptic species.

The initial insects for release will be reared in the USDA-ARS Fort Lauderdale quarantine facility (APHIS Facility #106)**.**

Initial releases will be composed of adults because they are long-lived, readily collected, easily transported, and less likely than immature stages to be targeted by generalist predators. Later releases may include larvae/egg-infested bulbils, however. Releases will be made in long-term research plots where *D. bulbifera* bulbils are abundant and where impacts by the beetle can be readily measured. These data will be compared to similar control plots where no releases will be made. Releases will be made in southern, central, and northern Florida. A recent study with the already released congener *L. cheni* (Lake et al., 2018), found 85 percent successful establishment with releases of 100 individuals. Thus, 100 *L. egena* will initially be released per site and adjusted as observations dictate.

The insects will be released as soon after PPBP provides a permit as practical. The insects enter a reproductive diapause phase during late November through mid-February, so releases would not occur during that season.

Post-Release Monitoring

The permittee and colleagues at the USDA Invasive Plant Research Lab will conduct monitoring with cooperation from local land managers. APHIS, the permitting agency, does not have any involvement in post-release monitoring.

None of the insects that feed on *D. bulbifera* in Florida damage the bulbils, which is *L. egena*'s primary food resource. Thus, presence or absence of bulbil damage (rather than leaf damage) will serve as the principle indicator for establishment, as will presence or absence of the insects. Of course, *L. egena*'s congener, *L. cheni*, was released against *D. bulbifera* in Florida during 2011 (Center et al., 2013) and has been spread throughout state (Overholt et al., 2016). Therefore, distinguishing between the two during field surveys will be important. Larvae of the two species are largely indistinguishable in appearance. However, immature *L. egena* will generally be found inside the bulbils, whereas immature *L. cheni* are found on the foliage and are not known to burrow into bulbils. The adults of the two species are similar in appearance: shiny black in color except for red to reddish brown elytra, having elongate bodies (1 cm long by 0.5 cm wide at the abdomen), with narrow thoraxes and even more narrow heads with bulging eyes**.** The wing coloration makes them relatively easy to spot in the field, but offers little aid in parsing between the two species. However, they can be distinguished by the presence or absence of setae on the metasternal plate. This characteristic may be too subtle for the casual observer, but with a modicum of training even citizen-scientists should be able to detect the differences.

Treatment plots where *Lilioceris* will be released and control plots where it will not be released have been established in southern, central, and northern Florida. Given that *L. cheni* is already producing impacts in terms of reduced vine densities and bulbil production, monitoring will focus on proportions of damaged bulbils as a primary measure of *L. egena* impact. A subset of damaged and undamaged bulbils from these research plots will be returned to the lab where they will be planted and allowed to germinate. Germination success, and vine and propagule biomass produced by germinating bulbils, will be compared between damaged (or possibly across multiple damage levels) and undamaged bulbils. These data can then be extrapolated back to the field sites using the field bulbil damage data. Adult beetles will be collected and the proportions of *L. egena* to *L. cheni* will be calculated. Also, subsets of damaged bulbils will be returned to the lab and dissected to provide estimates of larval abundance. Realized (i.e., ecological) host range will be evaluated by infesting *D. bulbifera* and other Dioscoreaceae at a controlled field site with *L. egena* in a manner simiar to Lake et al. (2015) and monitoring for dispersal from and damage to non-hosts including the Florida natives *Dioscorea floridana* and *D. villosa*.