Chapter 32 Classical Biological Control of Air Potato Vine, *Dioscorea bulbifera*, Infestations in Florida

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NON-TECHNICAL SUMMARY

The air potato vine (or air yam), *Dioscorea bulbifera*, a member of the Old-World yam family (Dioscoreales: Dioscoreaceae), was introduced to the United States over two centuries ago and has become one of the most serious exotic invasive weeds in Florida. It vigorously invades disturbed and undisturbed habitats under public and private ownership across Florida, southern Georgia, Alabama, Mississippi, Louisiana, and Texas. Research shows the vine grows up to 25 cm (9 in) per day and branches profusely. These attributes enable air potato to climb up and over other vegetation, smothering trees, shrubs, and understory plants. It produces numerous aerial tubers (known as bulbils) as it grows, which fall to the ground when the vines die back in winter. The bulbils and underground tubers sprout in spring and repeat the seasonal growth cycle. Herbicidal, mechanical, and cultural methods used by land managers are costly, provide only temporary relief, and can cause damage to non-target plants in the area. In contrast to chemical controls, biological control agents offer a self-sustaining, environmentally friendly, and cost-efficient method for managing this weed. A biological control program against air potato was started in 2002 after a leaf feeding beetle, *Lilioceris cheni* (Coleoptera: Chrysomelidae), from air potato's native range in Nepal was accidentally discovered. Later, the same beetle was discovered in China. Extensive testing at the USDA-ARS Invasive Plant Research Laboratory in Fort Lauderdale, Florida showed that Nepalese and Chinese beetles are highly specific, so federal and state regulatory agencies approved their release in the United States. Adults and larvae feed on air potato leaves, ultimately causing vines to die early and significantly reducing bulbil production. The beetles are relatively less effective at controlling air potato vines in urban and suburban areas, where mosquito spraying programs interfere with the beetles' life cycle. Still, they are very effective in rural areas, federal, state, and local parks, and other natural areas with no or limited spraying.

Rayamajhi, M. B. and F. A. Dray Jr. 2022. Classical biological control of air potato vine, *Dioscorea bulbifera*, infestations in Florida, pp. 390–401. *In:* Van Driesche, R. G., R. L. Winston, T. M. Perring, and V. M. Lopez (eds.). *Contributions of Classical Biological Control to the U.S. Food Security, Forestry, and Biodiversity.* FHAAST-2019-05. USDA Forest Service, Morgantown, West Virginia, USA. https://bugwoodcloud.org/resource/files/23194.pdf

HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

Dioscorea bulbifera (air potato, air yam; Dioscoreales: Dioscoreaceae) is an herbaceous vine (**Fig. 1**) of Afro-Asian origin (Burkill, 1960; Martin, 1974; Tindall, 1993). The first recorded introduction was in Florida in 1905, although there is evidence of *D. bulbifera* vines in a garden in Mobile, Alabama in 1777 (Bartram, 1998). Introduced into the United States as potential resources for food and medicine, several species of *Dioscorea* have escaped cultivation and now grow in the wild. Ironically, the air potato is the most widespread and best known of these, but it is also the only species whose extremely bitter foliage and tubers (Bhandari and Kawabata, 2005) render it largely inedible. Nehrling (1944, and see also Morton, 1976), an early Florida horticulturist, worried about the vine's potential to become invasive, as proved to be the case. Over time, *D. bulbifera* has become one of the most aggressive noxious weeds in the southeastern United States, particularly in Florida (Hammer, 1998). It has also become established in Hawaii, the West Indies, and Meso-America (Schultz, 1993; Gordon, 1998; Nesom and Brown, 1998; Center et al., 2013; Overholt et al., 2016; EDDMapS, 2020).

Figure 1. Typical *Dioscorea bulbifera* vine mats affecting natural areas with (a) relatively old dark green vines trellising and blanketing tree canopies, and (b) showing relatively young growth bearing vigorously growing foliage with expanding leaves and succulent stem-tips, smothering shrubby vegetation. (a: M. Rayamajhi, USDA-ARS; b: Jorge Leidi)

Burkill (1960) recognized two types of *D. bulbifera* (African and Asian-Polynesian), whose ranges may have become disjunct due to the desiccation of southwestern Asia about 10 million years ago. Martin (1974) also recognized two types, noting that edible varieties common in Africa produced angular bulbils (vegetative propagules produced on leaf axils). In contrast, the globular or spherical bulbils of Asia/Oceania were very bitter (Bhandari and Kawabata, 2005). Terauchi et al. (1991), using RFLP analysis, identified nine discrete chloroplast genomes that clustered into the same African vs. Asian/Oceania dichotomy. RAPD analysis (Ramser et al., 1996) further supports this dichotomy. Originally, *D. bulbifera* vines from Florida were thought to be African in origin (Overholt et al., 2003), and there are reports of an angular variety in the state. However, Florida vines primarily produce two varieties with globular/spherical bulbils: one with brown-colored, rough skin and another with tan-colored, smooth skin (**Fig. 2**; see also Fig. 1 in Terauchi et al., 1991). More recent chloroplast studies by Croxton et al. (2011) confirmed that both common morphotypes in Florida are of Asian origin, as their bulbil shapes suggest. Common garden studies showed these two bulbil types produce indistinguishable vines in terms of leaf morphology and growth phenology, but the bulbils produced on these vines remain true to their parental morphotypes (Rayamajhi et al., 2016, 2021).

Figure 2. Vegetative propagules (bulbils) of *Dioscorea bulbifera* (left) brown and (right) tan morphotypes. (M. Rayamajhi, USDA-ARS)

Dioscorea bulbifera plants are dioecious but produce only pistillate (female) inflorescences in Florida and thus do not produce seeds (Gordon, 1998). Flowering is rare and occurs towards the end of the growing season. The lack of sexual reproduction in North America means that its spread occurs exclusively via the aerial tubers (i.e., bulbils) vegetatively produced in leaf axils. These bulbils weigh from <1 g to >1 kg (<0.035 oz to 2.2 lb) and are produced from summer through early fall (Langeland et al., 2008; Overholt et al., 2016; Rayamajhi et al., 2016).

In the continental United States, air potato vines die during autumn when the leaves and most bulbils drop to the ground (Coursey, 1967; Center et al., 2013; Overholt et al., 2014, 2016; Rayamajhi et al., 2016, 2019, 2021). New shoots emerge from those bulbils and perennial subterranean tubers during the following spring.

Nature of the Problem

Dioscorea bulbifera is aggressive, forming impenetrable mats of vines that can blanket trees, shrubs, and surrounding vegetation (Nehrling, 1944; Morisawa, 1999; Air potato Task Force, 2008; Rayamajhi et al., 2019) (see **Fig. 1**). Recent research shows that the growth rate of *D. bulbifera* vines depends on propagule size; vines generated by larger bulbils grow faster than those from smaller bulbils (Rayamajhi et al., 2016). This weed is prolific, and in southern Florida bulbils weighing 1.0–372.6 g (<0.035–13.1 oz) can produce vines measuring 23–51 m (75–167 ft) long, growing 0.2–25 cm (0.08–9.8 in) per day, and bearing 0–365 bulbils in the leaf axils (Rayamajhi et al., 2016). *Dioscorea bulbifera* vines can be confused with those of edible yam species. However, *D. bulbifera* is easily differentiated from edible species by their cylindrical stems (Overholt et al., 2016) and bitter-tasting foliage and bulbils (Bhandari and Kawabata, 2005).

In North America, *D. bulbifera* spreads mainly through bulbils disseminated by anthropogenic means (Schultz, 1993; Rayamajhi et al., 2016), although hurricane winds can also disperse bulbils (Horvitz et al., 1998). *Dioscorea bulbifera* vines exploit and colonize disturbed sites, including hurricane-damaged natural areas, and such invasions interfere with recovery by native vegetation (Gordon, 1998; Horvitz et al., 1998; Horvitz and Koop, 2001). Vines in older infestations form solid canopies that significantly reduce light penetration, weigh down and collapse supporting vegetation, and ultimately kill plants underneath mats

(Schmitz et al., 1997; Center et al., 2013). *Dioscorea bulbifera* infestations that begin with a single vine can eventually develop into a contiguous patch of innumerable vines covering several hectares (Rayamajhi et al., 2019). Currently, *D. bulbifera* infestations occur in all 67 Florida counties and in southern parts of other Gulf Coast states (Croxton et al., 2011; Overholt et al., 2016; Rayamajhi et al., 2021).

WHY CONTROL THIS INVASIVE?

Air potato is capable of causing both ecological and economic damage in an invaded ecosystem. Unchecked rapid growth by this invader, with its ability to smother canopy and understory vegetation, can convert large species-rich expanses of natural areas into monotypic stands that continue to expand through bulbil production (Rayamajhi et al., 2016). Equipped with these invasive attributes, *D. bulbifera* has become a transformer species that diminishes plant communities by displacing native flora and altering community structure, ultimately disrupting ecological processes and functions (Gordon, 1998; Overholt et al., 2014), as predicted by Nehrling (1944). Introduced primarily during the early 1900s, by the 1980s *D. bulbifera* vines had already smothered hedges, ornamental plants, fences, and vacant properties (Bell and Taylor, 1982), and covered electric poles and transformers (M. Rayamajhi, pers. obs.).

Traditionally, land managers and private property owners used various cultural, mechanical, or herbicidal control methods to temporarily suppress air potato patches on their properties (Overholt et al., 2014). However, treating air potato vines with herbicides is relatively ineffective and can harm non-target plants (Overholt et al., 2014). With or without herbicide treatment, air potato foliage dies back at the end of each growing season, but the perennial subterranean tubers and seasonally produced bulbils, which are unaffected by herbicide treatments, remain to resprout the following year (Overholt et al., 2014; Rayamajhi et al., 2016, 2019). Mechanical methods involving the complete removal of *D. bulbifera*, including extraction of subterranean tubers, may cost several thousand dollars per hectare (Miami Dade County Natural Areas Management, pers. comm.). It is difficult to get an accurate estimate of the cost of air potato eradication because contractors may remove a range of other invasive species during a control operation without differentiating the proportions each species contributed to the overall job. For example, complete removal of several exotic invasive plants including *D. bulbifera* from an 0.809 ha (2 acre) patch in the Everglades National Park was estimated to cost $30,000 (Overholt et al., 2014), and in Fern Forest (Broward County, Florida), the cost of removing *D. bulbifera* and other invasive plants was estimated at $1,750/ha/yr ($709/acre/yr) (Wheeler et al., 2007).

One of the most popular cultural methods of reducing the spread of *D. bulbifera* was through annual bulbil collection and disposal events, popularly known as "air potato roundups" (Weaver, 2008), conducted by students and volunteer groups. These activities intensified public attention on damage from *D. bulbifera* infestations in Florida and helped remove potential sources of new (or re-) infestations (Overholt et al., 2014). Three practical reasons have been cited for the popularity of these programs: 1) *D. bulbifera*'s high prevalence and distinctiveness helped volunteers easily recognize the plant, which was described as a menace to natural areas, public parks, and private lands; 2) picking up bulbils resembling potatoes required little training regardless of volunteer ages and abilities, and required no specialized tools (only buckets); and 3) events could be scheduled during winter, when most of the bulbils have fallen to the ground, and those that have not dropped are easy to see on the dead vines.

However, these methods alone are inadequate and cost-inefficient for long-term suppression of this weedy vine (Wheeler et al., 2007). Rayamajhi et. al. (2016) argued that only self-sustaining control methods that could reduce growth rate and propagule (bulbil) production would mitigate the further spread and smothering impact of *D. bulbifera* vines on native plants.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Air potato in the United States has only a few close relatives and no known specialist natural enemies, and was therefore considered an appropriate target for biological control (Pemberton and Witkus, 2010). Initially, its native range was thought to be on the African continent, and surveys for biological control agents were begun in Ghana and Uganda in June of 2003 (Overholt et al., 2003; Wheeler et al., 2007). However, no host-specific agents were discovered during these surveys (Overholt et al., 2014). In 2002, a chrysomelid beetle was discovered by USDA scientists R. Pemberton and M. Rayamajhi while conducting a survey in and around the Kathmandu Valley for biological control agents of different invasive weeds (*Lygodium microphyllum* and *Paederia foetida*). This beetle's adults and larvae were observed skeletonizing leaves and scraping young bulbils on a few vines that were climbing on native shrubs (Pemberton and Witkus, 2010). These beetles were initially identified as *Lilioceris* sp. nr. *impressa* (Pemberton and Witkus, 2010).

Chrysomelid beetles that have been used as biocontrol agents on other systems have generally been effective (Van Driesche et al., 2008). Consequently, the Nepalese beetles were imported into the USDA-ARS Invasive Plant Research Laboratory (IPRL) in Fort Lauderdale, Florida in 2004 for further study, to potentially initiate an air potato biological control program (Pemberton and Witkus, 2010). These beetles were later identified as *Lilioceris cheni* (**Fig. 3**) based on morphological and molecular studies (Tishechkin et al., 2011; Center et al., 2013).

An extensive host testing process was done of the beetle's feeding behavior, reproductive potential, and overall life cycle on various *Dioscorea* species from the Caribbean, Meso-America and North America, as well as 41 other plant species of economic or environmental importance. These studies demonstrated that *L. cheni* (hereafter, the air potato foliage-feeding beetle or APFB) from Nepal had a very narrow host range, feeding and completing its life cycle almost exclusively on *D. bulbifera* foliage (Pemberton and Witkus, 2010). Later, the beetles were occasionally observed feeding on tender bulbils of this vine at the end of the growing season when green foliage in the field was scarce and the adults were about to enter a quiescent winter period (Center et al., 2013; Rayamajhi et al., 2019). Permission for field releases of the Nepalese biotype of APFB was obtained from the USDA Animal and Plant Health Inspection Services (APHIS) in 2009, but unfortunately by that time the quarantine colonies had died out.

Surveys in southwestern China during 2010–11 located a Chinese biotype of APFB with red elytra (in contrast to the orange Nepalese APFB; **Fig. 3d**). During the 2011 surveys, a second chrysomelid beetle was discovered by F.A. Dray and G. Witkus that seemed to feed in the bulbils. This beetle, later identified as *Lilioceris egena* (Center et al., 2013), and the APFB were imported into IPRL for further testing. Studies on APFB confirmed that the Chinese beetles were the same species and had the same host specificity

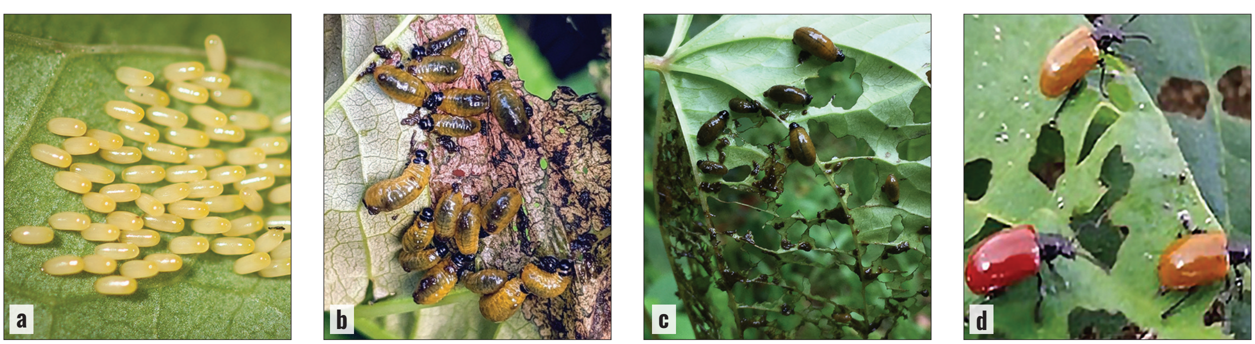

Figure 3. *Lilioceris cheni* (a) egg mass; (b,c) aggregated larvae gregariously feeding on the undersurface of succulent *D. bulbifera* leaves, (b)1st and 2nd-instar larvae feed from lower surface and skeletonize leaves by leaving upper epidermis intact while (c) 3rd and 4th-instar feed through and perforate leaves like the adults do; (d) Chinese (red) and Nepalese (orange) biotypes also feeding and creating holes on the lower surface of *D. bulbifera* leaves. (a: Melissa Smith, USDA-ARS; b: Irvin Louque, iNaturalist.org CC BY-NC-ND 4.0; c: Carrie Seltzer, iNaturalist.org CC BY-NC 4.0; d: Jenna Owens, USDA-ARS)

(based on 10 *Dioscorea* spp.) as the Nepalese beetles, so field tests began in November 2011 (Center et al., 2013). Additional shipments of originally approved Nepalese APFB adults were imported during 2012 and incorporated into the field-release program.

Samples of both the Nepalese and Chinese APFB biotypes were shipped to collaborators at the Florida Department of Agriculture and Consumer Services (FDACS) in Gainesville, Florida, and the University of Florida (UF), Indian River Research and Education Center in Fort Pierce as part of genomic technology transfer activities in 2012–2013. The USDA and collaborating agencies subsequently reared and field released beetles in nearly all 67 counties in Florida (Rayamajhi et al., 2019, 2021; Kraus et al., 2022). Later, a few hundred beetles of both biotypes were shipped to Drs. Rodrigo Diaz (Louisiana State University) and Veronica Manrique (Southern University and Agriculture and Mechanical Engineering College) in Baton Rouge, Louisiana. Drs. Diaz and Manrique, along with FDACS scientists, have released additional beetles at various locations in the southern parts of Alabama, Louisiana, and Texas.

Mass rearing of APFB required abundant, healthy (insect pest- and disease-free) *D. bulbifera* plants, which was most easily achieved by starting with the vegetative propagules (bulbils). Each year, from November through February, our laboratory acquired a few hundred kg (>100 lb) of bulbils from the previous growing season, both via our own direct collections and donations from various parks and volunteer organizations. These bulbils were stored in the dark at 10°C (50°F) to inhibit sprouting until they were needed to produce vines for foliage to feed APFB. We maintained over 1,000 potted plants on benches in screenhouses that provided partial shade and daily watering via overhead sprinklers. These plants were routinely checked for unwanted pests and pathogens and were promptly subject to remedial actions as appropriate.

We initially reared APFB using a slight modification of the techniques developed by Pemberton and Witkus (2010) for maintaining colonies in the IPRL biocontainment. Several adult beetles were placed in 81.5 x 39.5 x 39.5 cm (32 x 15.5 x 15.5 in) acrylic boxes, each holding 1 to 2 small plants in 3.8 L pots. Adults were allowed to oviposit for several days, and then moved to another cage, thereby allowing the eggs (**Fig. 3a**) to hatch and larvae to develop in the original cages. However, these methods were best suited to produce the relatively small numbers of insects needed for host range trials, and so we adapted our approach to facilitate production of larger numbers of insects for a mass-rearing program. We reared APFB in 1.8 x 1.8 x 1.8 m (6 x 6 x 6 ft) Lumite® mesh cages, each containing six air potato plants trellised over bamboo hoops inserted in 11.4 L pots (Center et al., 2013; Halbritter et al., 2021). One side of each cage was fitted with a 1.8 m (6 ft) long metallic zipper to allow for closing the cage to keep beetles from escaping, and opening to allow people to walk in and out to inoculate plants with beetles and hand water plants as needed. Each cage was inoculated with 5–7-day-old adults, including gravid females that oviposited on the lower surfaces of the young tender leaves. Eggs hatched within 5 to 7 days, and larvae (**Fig. 3b**) gregariously fed on leaves and growing tips, becoming 3rd or 4th instars (**Fig. 3c**) within a week. These larvae were removed from the caged plants and transferred onto fresh *D. bulbifera* leaves placed on a ca 2.5-cm (1 in) layer of slightly moist, inert commercial media (vermiculite) in a plastic bin with ventilated cover. After feeding briefly, the larvae burrowed into the media, developed cocoons, and emerged as adults (**Fig. 3d**) in 14–16 days. In most cases, this beetle completed its life cycle in 28–35 days. New adults were allowed to mate for ca 5–7 days and used for experimental purposes or field releases.

Usually, *L. cheni* beetles overwinter from December through early March in southern and central Florida (Rayamajhi et al., 2021); this overwintering period may be slightly longer in the more temperate climates of southern Georgia, Alabama, Mississippi, Louisiana, and Texas. Factors that may affect the length of the beetle's overwintering period include the availability of green vines (e.g., frost-protected areas could have foliage available later in the growing season) as well as soil organic matter content and moisture. Knowledge of these factors permitted us to produce *D. bulbifera* plants year-round by growing under controlled indoor conditions with desired day lengths. This method allowed us to prevent beetle colonies from entering diapause (the inactive period during winter) and reproduce as if in summer. Maintaining active indoor colonies during winter permitted us to have beetles ready for field-release as soon as *D. bulbifera* plants in the field began to sprout and grow.

Dispersal techniques varied somewhat among agencies. USDA scientists packed 10–100 adults into sandwich- to shoebox-sized plastic containers with freshly collected air potato leaves to allow feeding while adults were transported to release sites. Property owners and land managers were contacted before release events, beetles were delivered in person, and questions or concerns about the *D. bulbifera* biological control program were discussed and answered at the time of the releases. The approaches used by FDACS and UF were similar, except that boxes of beetles were shipped to landowners and extension agents upon request, relying upon them to conduct the actual releases (Kraus et al., 2022). Over the first six years of this project, over 600,000 beetles (both biotypes combined) were released (USDA: 343,326; FDACS: 246,747; and UF: 41,224) at more than 6,000 sites throughout Florida. Our collaborators, especially FDACS and LSU scientists, have continued to spread beetles to new locations since 2018, both in Florida and other states (see Kraus et al., 2022 for further information on more recent Florida releases).

While APFB was being dispersed throughout the southeastern United States, the second beetle (*L. egena*) was undergoing host range testing. A total of 82 plant species (including 15 species of *Dioscorea*) were used in the vetting process, but only *D. bulbifera* proved to be an acceptable developmental host (Dray, 2017). These studies also confirmed that this beetle can complete development on air potato leaves but prefers the bulbils. Larvae from a single 12–18 egg cohort can devastate a bulbil before pupating. Results of the host trials were submitted to federal and state regulatory agencies (Dray, 2017), and in March 2021 the USDA-APHIS issued a permit for release of *L. egena* in the United States. Initial releases were conducted in cages at IPRL during August of 2021 as part of a study examining whether the beetles would attack bulbils on the vine as well as those on the ground (F.A. Dray and M. Rayamajhi, unpub. data). Field releases began in October 2021 at two county parks in Broward County, Florida and are continuing.

HOW WELL DID IT WORK?

The ability of *D. bulbifera* to exploit natural and disturbed habitats in rural and urban landscapes made air potato a threat to diverse ecosystems across invaded areas. In its adventive range, the major invasive attribute of *D. bulbifera* is its high growth rate, which in the United States is second only to kudzu (*Pueraria montana* var. *lobata*). Air potato's ability to grow both vertically and horizontally by climbing up and over shrubs and trees to form dense mats, and its prolific vegetative reproduction, made this vine a high-impact invader in both native vegetation and urban landscapes (Rayamajhi et al., 2016). Trees as tall as 5–10 m (16–33 ft) in our experimental plots were observed to be smothered and killed within four years by *D. bulbifera* vines.

In our test plots, *L. cheni* was multivoltine, completing its life cycle in 28–40 days, depending upon food quality and environmental conditions (Center, 2013; Manrique et al., 2017). The APFB proved to be relatively easy to rear and was found to reproduce and spread quickly in the field, provided there were plenty of *D. bulbifera* vines with tender foliage for oviposition and larval feeding at the release site. Both biotypes of APFB are well adapted across the latitude of *D. bulbifera*'s known distribution range in the United States, but the Nepalese biotype may be better suited to the more northern part of the weed's invaded U.S. range due to high fecundity early in the growing season as compared to the Chinese biotype, whose fecundity is more evenly distributed throughout the growing season (Smith et al., 2018). As few as ten beetles can establish a self-sustaining population, but larger release sizes of 50 or more accelerates *D. bulbifera* vine damage and promotes quicker establishment at the field sites (Lake et al., 2018).

Successful biological control of an invasive plant in its adventive range is possible only when the phenology of the target host and biological control agent remain in synchrony. In the air potato-APFB system, the weed and its biological control agent have retained synchrony after being reunited in Florida, despite the vine having experienced more than a century of freedom from suppressive agents (Rayamajhi et al., 2021). The APFB is environmentally plastic in Florida (Smith et al., 2018), and this attribute, coupled with its broad distribution both latitudinally and altitudinally in Asia, suggests APFB appears to have the ability to keep up with its host if *D. bulbifera* extends its U.S. range northward in response to climate changes.

Dioscorea bulbifera vines in Florida sprout during March from perennial underground tubers and the aerial bulbils produced during the previous growing season; new bulbils begin to develop around June in Florida (Rayamajhi et al., 2021). This general pattern varies by up to a month depending upon differences in microenvironment and the latitude of the infestation. There appears to be a 3-month delay between vine appearance and bulbil development in Florida. Similarly, *L. cheni* beetles exit their winter diapause (inactive period) and become more active (and thus detectable) by April in southern Florida and by May in the north-central part of the state. Although APFB feeding reduces bulbil production (see the following discussion) it does not eliminate these vegetative propagules entirely. To address this problem, USDA has recently initiated releases of another biocontrol agent, the bulbil-feeding beetle *L. egena*. However, it is too early to evaluate how successful this agent will be at reducing bulbil production and viability.

To evaluate the impact of the first agent released against air potato, a 5-year project was carried out to measure APFB's ability to reduce bulbil production by *D. bulbifera* in the field (Rayamajhi et al., 2019). At release sites, APFB demonstrated a J-shape (accelerating) population growth curve when foliage was abundant at the time of release (Rayamajhi et al., 2019). At field research sites located in Alachua, Manatee, Broward, and Miami Dade counties, APFB damage lowered mean *D. bulbifera* coverage from 73% in 2012 to less than 23% in 2016 (Rayamajhi et al., 2019). Vines died within a month or so once 40% or more of the vine area had been damaged by skeletonization or perforation of leaves by larval and adult feeding (**Fig. 4**), which eliminated further bulbil development and growth. Lower levels of feeding damage resulted in smaller bulbils and longer times to plant death (M. Rayamajhi, pers. obs.). Reductions in vine cover

Figure 4. Typical *Lilioceris cheni* adult damage on *D. bulbifera*: (a) leaves of all ages with numerous feeding holes which stopped the vines from branching and blanketing trees and forming new bulbils; (b) expanding tender leaves colonized and gregariously fed upon and partially skeletonized by 2nd and 3rd-instar larvae; (c) three adults of the Chinese biotype feeding and creating holes on mature leaves; (d) severe defoliation of the *D. bulbifera* vines engulfing the bottom portion of a large tree; note emergence of new growth of *Vitis* species following severe defoliation of air potato by beetle larvae and adults. (a; Dale Halbritter; b,c: Jenna Owens; d: M. Rayamajhi; a–d: USDA-ARS)

released smothered vegetation from air potato competition, allowed better growth of previously suppressed perennial plants, and improved species richness (Rayamajhi et al., unpub. data).

Propagule densities in research plots (measured as the number of bulbils on the ground after vine senescence at the end of the growing season) were reduced from 20–42/m^2 (2–4/ft^2) at the onset of the experiment in 2012 to <1/m^2 (<0.09/ft^2) in 2016 in the beetle-treatment plots vs. from 12–56/m^2 (1–5/ft^2) in 2012 to 1–5/m^2 (0.09–0.5/ft^2) in 2016 in control plots (which were treated with granular systemic insecticide at 4-month intervals); both plots were located in the same general area (Rayamajhi et al., 2019). APFB damage to the foliage in control areas became quite high, causing early vine death. As the massive numbers of beetles attempted to feed on pesticide-poisoned vines in the control plots (when healthy foliage at the site became scarce) towards the last quarter of each growing season during the 5-year experimental period, they died within hours after feeding; nevertheless beetles damaged the vines (Rayamajhi et al., 2019). Bulbil biomass in control plots, however, remained unchanged over time because plots were generally free of beetles for three quarters of the growing season, by which time most bulbils had already developed to their mature size. Overall reductions in bulbil density and size at APFB-treated sites explains why many parks and recreational areas in Florida (e.g., Alachua County) stopped annual bulbil roundup programs several years into the biological control program (Jester, 2015; Overholt et. al., 2016).

APFB impacts, in terms of beetle population increase and subsequent reduction of *D. bulbifera* vines, varied between natural areas and urban or suburban areas where mosquito control programs were carried out against malaria and the Zika virus vectors. In areas sprayed for mosquitoes, all life stages of the beetle (egg clutches, larvae, and adults) would be detectable for 2–6 weeks following an APFB release, and vine damage often appeared impressive at first. Soon thereafter, however, all traces of the beetle's population would disappear. This prompted us to test the acute toxicity of two commonly used mosquito-control pesticides (naled and permethrin). Both pesticides caused mortality to the beetle, which was especially sensitive to permethrin (Wheeler et al., 2020). The APFB will likely remain relatively less effective in areas with active mosquito control programs. It is hoped that the second agent, the bulbil-feeding beetle, will be less susceptible to mosquito-control pesticides because the larvae feed inside the bulbils and seal their external openings to their feeding chambers with their own frass (F.A. Dray, pers. obs.).

In non-sprayed natural areas, APFB populations were observed to frequently and quickly infest and damage large swaths of *D. bulbifera* vines by the middle of the growing season, resulting in heavy damage (**Fig. 4**) and large adult and larval populations. Adults then dispersed from such populations to other sites in search of food to produce another generation of beetles. This cycle of destruction of air potato plants, followed by beetle dispersal, was repeated until the end of the growing season (October–December) when vines begin to senesce naturally, forcing adults to overwinter at their last location. The life span of adult beetles in the field varies from three to six months (M. Rayamajhi, pers. obs.), although the average laboratory lifespan was five months (Pemberton and Witkus, 2010). Many adults die while overwintering. Young adults that emerge at the end of the growing season successfully overwinter in dried-up *D. bulbifera* leaves that remain on the vines, under tree bark, or in the duff on the ground. These adults emerge the following growing season with depleted fat reserves and may take over a month of feeding on freshly regenerated *D. bulbifera* foliage before they mate, reproduce, and become abundant enough to be readily detectable by the general public. During this lag period between winter beetle quiescence and high population abundance, many land managers and property owners become impatient because, during the same time, vines are growing rapidly and the beetles that were present and so devastating the previous year seem absent. This spring lag can induce some landowners to turn to herbicides, further delaying APFB build-up on their properties. Through various forestry and agriculture extension programs and *D. bulbifera* working groups, we (USDA, FDACS, UF) have been working to provide clients and stakeholders with a proper understanding of the APFB's population ecology and life-stage synchrony with its host air potato (Rayamajhi et al., 2021) to reduce concerns about the delayed arrival at their property.

BENEFITS OF BIOLOGICAL CONTROL OF AIR POTATO

In summary, *L. cheni* has shown dramatic population increases, caused highly visible, substantial damage to *D. bulbifera* infestations, and reduced this weed's impact on native vegetation in Florida and beyond. Indirect but positive side effects include (1) increased plant diversity due to recovery of native species in places once occupied by *D. bulbifera*; (2) reduced use of herbicides against air potato and associated damage on non-target vegetation; and (3) reduced need for *D. bulbifera* roundup events (Weaver, 2008), permitting environment enthusiasts and students to redirect their attention to control activities for other invasive plants (Jester, 2015, Overholt et al., 2016). Certain hemipteran predators and spiders have occasionally been observed preying upon APFB larvae and adults. However, an increase of predators from the addition of a new food resource (APFB) has not been observed.

UNFINISHED BUSINESS WITH AIR POTATO BIOLOGICAL CONTROL

As mentioned earlier, damage by APFB significantly reduces the size and density of bulbils produced in *D. bulbifera* infestations. However, the bulbils produced during a growing season in areas with either mosquito pesticide sprays or delayed arrival of the leaf-feeding beetle contribute substantially to replenishing infestation levels in subsequent years. Thus, the USDA initiated a project investigating the bulbil-feeding beetle, *L. egena*, as a potential addition to the air potato biological control program. Laboratory studies show that damage by this beetle (the air potato bulbil beetle, or APBB) can prevent bulbils from sprouting. Early field trials show that the APBB attacks young bulbils on the vine as well as mature bulbils on the ground (Dray & Rayamajhi, unpub.). It is too early to predict the outcome of these releases, but by attacking different plant parts, APFB and APBB together should remove the competitive advantages that air potato vines have had in Florida's landscape, promoting natural restoration of native species.

ACKNOWLEDGMENTS

The South Florida Water Management District, the United States Army Corp of Engineers, Miami-Dade County DERM, and the Florida Fish and Wildlife Conservation Commission provided financial support for this project. We thank scientists and technicians of FDACS, UF-IRREC, and the Miami-Dade County Natural Area Management Division (NAM) for assistance/collaboration in different phases of field research on the air potato biological control program. Special thanks are extended to IPRL technicians Jorge Leidi, Reynaldo Moscat, Jorge Cedeno, Jenna Owens, Scott Goldstein, Anwar Abdel-Kader, Anyelina Mangru, and many student interns from Broward and Miami-Dade Colleges for technical assistance in various aspects of our field and laboratory research.

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