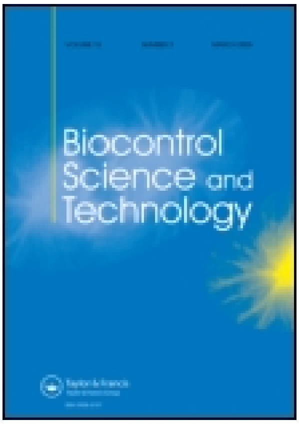

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Laboratory host range testing of Lilioceris sp. near impressa (Coleoptera: Chrysomelidae) - a potential biological control agent of air potato, Dioscorea bulbifera (Dioscoreaceae)

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RESEARCH ARTICLE

Laboratory host range testing of *Lilioceris* sp. near *impressa* (Coleoptera: Chrysomelidae) – a potential biological control agent of air potato, *Dioscorea bulbifera* (Dioscoreaceae)

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Air potato, *Dioscorea bulbifera*, is an invasive, herbaceous, climbing vine, which dominates invaded native vegetation in Florida. The fortuitous discovery of *Lilioceris* sp. near *impressa* defoliating *D. bulbifera* vines and feeding on the bulbils (aerial tubers) in the Katmandu Valley of Nepal initiated a project to assess the potential of this leaf beetle for biological control of air potato in Florida. Quarantine host specificity tests were conducted on 41 plant species in 24 families and 13 orders, with 26 species outside of the Dioscoreaceae and 15 species within the Dioscoreaceae. Adults test fed (nibbled) on 4/12 of tested *Dioscorea* species, but no larval feeding or development occurred on any plant other than the target, *D. bulbifera*. The larvae feed gregariously and quickly skeletonize offered leaves of air potato. Air potato bulbils that received any feeding damage to the primary meristematic region did not sprout. The ability of the beetle larvae and adults to feed on the bulbils is important because in Florida, the plant rarely flowers or produces fruit, so these aerial tubers are the primary means of persistence and spread. The adults can live for several months without food. This extremely specialized herbivore from part of the weed's native range appears to have great promise as a biological control of air potato.

Keywords: air potato; Chrysomelidae; Dioscoreaceae; host-specificity tests; weed biological control

Introduction

Air potato, *Dioscorea bulbifera* L. (Dioscoreaceae), is an invasive vine that is a serious pest plant in Florida (Schmitz, Simberloff, Hofstetter, Haller, and Sutton 1997; Langeland and Craddock Burks 1998; Gordon, Gann, Carter, and Thomas 1999). *Dioscorea bulbifera* is native to and widely distributed in tropical and subtropical Asia and Africa (Burkill 1960; Coursey 1967; Tindall 1993). In the Western Hemisphere, it is widely naturalized in the tropics and subtropics of the West Indies, Central, and South America (McVaugh 1989; Schultz 1993).

Dioscorea bulbifera is an herbaceous, perennial, twining vine, 20 m long or greater, which is capable of climbing and out-competing native vegetation (Schmitz et al. 1997; Langeland and Craddock Burks 1998; Gordon et al. 1999). It was introduced to Florida from tropical Asia or Africa in 1905 (Morton 1976), and it now constitutes one of the most aggressive weeds ever introduced to Florida

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(Hammer 1998). The noted horticulturalist Henry Nehrling was apparently the first to express concern for its invasiveness, 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 (Long and Lakela 1976) with recommendations to limit the planting of this ornamental species (Morton 1976; Ward 1977). By the 1980s, this vine was found growing in thickets, waste areas, and hedges or fencerows in southern and central Florida (Bell and Taylor 1982). By 1999, *D. bulbifera* was listed as a noxious weed by the Florida Department of Agriculture and Consumer Services, FDACS (Florida Exotic Pest Plant Committee Council, FLEPPC hereafter 2009). *Dioscorea bulbifera* is considered the most serious type of environmental threat, described as a Category I weed by the FLEPPC (Overholt et al. 2008; FLEPPC 2009), 'invasive exotics that are altering native plant communities by displacing native species, changing community structure or ecological functions, or hybridizing with natives'. Presently, *D. bulbifera* occurs in Florida from the northwestern panhandle, Escambia County, to the southern tip of the state and collections from herbaria and reports from state regional biologists have listed 29 of 67 Florida counties infested with it (Schmitz 1994; Gann, Bradley, and Woodmansee 2001; Wunderlin and Hansen 2003). This species has also been reported to be naturalized in Mississippi, Louisiana, Texas, and Hawaii (USDA-SCS 1982; Nesom and Brown 1998).

While flowers are produced in the plant's native range (Tindall 1993), they have only rarely been seen in Florida (Schultz 1993) and when they do appear, they are considered infertile (Gordon et al. 1999). The primary means of spreading is through human movement of bulbils (Schultz 1993).

Herbicidal spraying used to control air potato vines often injures non-target plants on which the vine grows. The weed is an appropriate target for biological control because it lacks regulating specialist natural enemies in Florida and has few close relatives in the state which might limit the selection of biological control agents.

Presently, studies are underway to identify the origin of the invasive variety of *D. bulbifera* in Florida through chloroplast DNA analysis (Overholt, Hughes, Wallace, and Morgan 2003). Preliminary molecular findings indicated Africa as the source of air potato in Florida, but ongoing and more recent work suggests Asia as the source (W. Overholt, personal communication). Possibly the best adapted biological control agents will be imported from the area of origin of the invasive *D. bulbifera* form but this depends on the breadth of the host range of the natural enemies and is truer for enemies known to have narrower host ranges, such as eriophyid mites and rust diseases.

This biological project against air potato was developed because of the fortuitous discovery of the *Lilioceris* beetle defoliating *D. bulbifera* vines and feeding on the bulbils in the Katmandu Valley of Nepal in 2002 by R. Pemberton and M. Rahamahji. *Lilioceris* sp. is impressive in the field, skeletonizing the plant's leaves and feeding on the aerial tubers as both adults and larvae. This high level of destruction was observed in the native area despite the occurrence of at least two pupal parasitoids. The fact that chrysomelid beetles have been outstanding biological control of weeds agents (Julien 1992) also influenced the decision to conduct research on this *Lilioceris* sp. to determine its suitability as a potential biological control agent of air potato. In this study we investigated the host specificity of this beetle.

Materials and methods

Target weed air potato (Dioscorea bulbifera)

Economic plants related to air potato

The *Dioscorea* genus includes several important species that have been used around the world for food (Martin 1974; Mabberley 1997). The most important species, the African *D. alata*, was introduced to Georgia (Raz 2002) and South Florida (Young 1923; Raz 2002), where some modest home garden use may still occur (Hammer 1998). However, like *D. bulbifera*, *D. alata* is widely naturalized in Florida where it has been reported from nine counties (Wunderlin and Hansen 2003), and is also considered a FLEPPC Category I weed species (FLEPPC 2009). Another exotic species, *D. polystachya*, as *D. oppositifolia*; sensu lato, is native to eastern Asia where it is cultivated for food (Al-Shehbaz and Schubert 1989). In the US, this species has become naturalized in many states (North Carolina, South Carolina, Georgia, Tennessee, Alabama, and Arkansas) where it was introduced as an ornamental (Al-Shehbaz and Schubert 1989; Raz 2002). To our knowledge, no *Dioscorea* species is cultivated commercially as food crop in Florida or the southeastern US. Species of *Dioscorea* are of greater economic importance in the West Indies, where several species are cultivated (Martin 1974; Perez et al. 2005). See Appendix 1 for a listing of the cultivated yams that were tested and their taxonomic sections, which are different than that of the target weed.

Native plants related to air potato

Dioscorea is the only native genus in the family Dioscoreaceae in North America. Two native species, *Dioscorea floridana* Barlett (Florida yam) and *D. villosa* L. (wild yam) occur in the US and Florida, and a third species, *D. quaternata* (four-leaf yam), has been listed by some authors to occur in Florida (Al-Shehbaz and Schubert 1989; USDA-NRCS 2002). However, more recent treatments of the North American species subsumed *D. quaternata* into *D. villosa* based upon significant phenotypic variability within the complex without any apparent natural gaps in the variation (Raz 2002; Wunderlin and Hansen 2003). *Dioscorea* species are divided by sections and *D. floridana* and *D. villosa* are assigned to the section *Macropoda* (Knuth 1924; Raz 2002). Traditionally *D. bulbifera* has been placed in the section *Opsophyton* along with other tropical Old World species (Knuth 1924; Huber 1998). Recent phylogenetic analysis based on sequence data from two plastid genes place *D. bulbifera* in the mostly Old World 'B' clade and within a lower level compound-leaf subclade (CL) (Wilkin et al. 2005). This analysis did not include the two native US *Dioscorea* species (Raz 2002). However, other results indicate that the two native species should be placed in the Stenophora subclade of Wilkin et al. 2005 (L. Raz, personal communication). *Dioscorea floridana* occurs in Florida, Georgia, and South Carolina (Raz 2002; USDA-NRCS 2002; Wunderlin and Hansen 2003). *Dioscorea villosa* occurs in Florida, north to Connecticut and west to Texas, and Minnesota (Al-Shehbaz and Schubert 1989; Raz 2002; USDA-NRCS 2002; Wunderlin and Hansen 2003). The West Indies has a greater native species diversity of *Dioscorea* than North America and also has the genus *Rajania*, which phylogenetic analysis based upon molecular sequence data suggests should be subsumed into the much larger *Dioscorea* genus (Caddick, Rudall, Wilkin, Hedderson, and Chase 2002a; Wilken et al. 2005). West Indian native *Dioscorea* species representing all of the *Dioscorea* sections occurring in the region and a *Rajania* species were tested (Appendix 1). None are closely related to the target weed or to the two North American *Dioscorea* species.

Plant species tested and rationale

The host range of *Lilioceris* sp. was determined by testing 41 plant species in 24 families and 13 orders; 26 species outside of the Dioscoreaceae and 15 species within the Dioscoreaceae. Test plants within the Dioscoreaceae were chosen to represent both the major taxonomic groups (sections) of the family that have representatives in the United States and West Indies, and species of economic importance. Batflower (*Tacca integrifolia*), an ornamental in Florida, was recently moved from the Taccaceae to the Dioscoreaceae, so it was also tested (Caddick, Wilkin, Rudall, Hedderson, and Chase 2002b). Representatives of the Burmanniaceae, a family that has been recently placed in the Dioscoreales (Judd, Campbell, Kellogg, Stevens, and Donoghue 2002) would have been considered for testing, especially representatives of the three *Burmannia* species and the single *Apteria* species that occur in Florida. However, these plants are tiny ephemeral mycoparasitic herbs with no leaves and thread like flower stalks that rise a few centimeters above the ground, so an insufficient above ground potential food resource is present. Also, by the time that some *B. biflora* L. plants were located, test data indicating extreme specificity of the *Lilioceris* within the genus *Dioscorea* had been obtained. Given this result, and the likely difficulties involved in attempting to cultivate and test *B. biflora* or other members of the Burmanniaceae, it was decided that testing members of this family was not necessary. Test plants outside the Dioscoreaceae were representatives of different orders of monocots with Florida natives and economic species (Appendix 1).

Full host range testing took place in the Fort Lauderdale Quarantine Laboratory with the following test plant design. Conceptually, the testing proceeds from the most closely related plants to progressively less related plants. This is a concentric ring approach, also called the Centrifugal Ring approach (Wapshire 1974), in which the target plant is in the center ring with progressively less related plants in rings beyond. First the closest native and economic relatives (*Dioscorea* species) in Florida and the West Indies were tested selecting species as representatives of the sections of *Dioscorea* that occur in the region. Then we tested the only other member of the family Dioscoreaceae outside the genus *Dioscorea* that occurs in Florida-the ornamental species (*Tacca integrifolia*). This was followed by a representative of another family within the order Dioscoreales, then representatives of the allied Liliales and other monocot orders. In addition, plant species or representatives of genera recorded as host plants of *Lilioceris impressa* in the literature were tested. With the exception of *D. alata*, these literature host plants were dicots (Appendix 1).

All *D. bulbifera* plants were grown from bulbils collected from Tree Tops Park, Davie, FL, USA (26.0° N, 80.14° W). Colonies of adult beetles were maintained on regularly cut *D. bulbifera*, also collected from Tree Tops Park. Native West Indian *Dioscorea* and *Rajania* species were collected in Puerto Rico. Other test plants were obtained in Florida from commercial sources, gardens, or nature.

***Lilioceris near impressa* – the test insect**

Native region

Lilioceris impressa is native to Asia and has been reported as *Crioceris impressa* from Nepal, (1524–3353 m elevation) (Bryant 1952; Takizawa 1989); Bangladesh (Das and Islam 1984); India (Sinba, Singh, and Yadav 1978); Malaysia, Burma, Sri Lanka (Srivastava and Bhagat 1967); throughout Southeast Asia (Kimoto and Gressitt 1979); and China (Yu 1993). In addition to discovering the beetle in the subtropical Katmandu Valley of Nepal at ca. 27° N, we have encountered the beetle feeding on *D. bulbifera* in the Chiang Mai area of northern Thailand and in subtropical Xishuanbanna in southern Yunnan Province of China at about 22° N.

Known host range

The only known host plant of the beetle is *D. bulbifera.* The literature, however, contains host records for the *Lilioceris impressa* complex that includes additional species, namely *D. alata*, *Ficus elastica* L. (Moraceae), *Holarrhena antidysenterica* Wall. (Apocynaceae), *Callicarpa macrophylla* Vahl. (Verbenaceae), *Santalum album* (Srivastava and Bhagat 1967), and *Cassia sophera* (Fabaceae) (Saha 1973). The non-*Dioscorea* host records are most likely due to morphologically similar species in the *L. impressa* complex rather than to the air potato feeding *Lilioceris.* We will provide data to demonstrate that the *L. impressa* associated with air potato is a narrow specialist, not able to complete its development on other *Dioscorea*, much less on species belonging to other families.

Source of populations tested

Adult *Lilioceris* beetles were collected from air potato plants in the Katmandu Valley, Nepal (27.5° N 85.2° E), and reared in a laboratory on *D. bulbifera* originating from bulbils from Florida plants, by the Division of Entomology, Nepalese Agricultural Research Council, Nepal. The colony was maintained at the Division of Entomology Laboratory. Beetles were reared each year and shipped to the USDA-ARS-SAA-IPRL quarantine facility in Ft. Lauderdale, Florida.

Determination and voucher specimens

A.S. Konstantinov, USDA/Agricultural Research Service, Systematic Entomology Laboratory, Beltsville, MD, determined the specimens collected from *D. bulbifera* in Nepal as *Lilioceris* sp. near *impressa*, and indicated that the *impressa* group is a complex of species. A study of this complex that will determine which taxon the Nepalese air potato feeding *Lilioceris* near *impressa* represents is underway (A. Konstantinov, personal communication).

Feeding and life cycle

Lilioceris larvae feed gregariously and skeletonize the leaves while moving across the undersides of the leaves, exuding frass onto their dorsal surfaces of their bodies. The larvae prefer tender, newly emerged air potato leaves, but can also eat older, toughened leaves. Early instars can consume air potato bulbils if there is an initial tear through its surface, but more mature larvae can eat intact bulbils without difficulty.

When *Lilioceris* larvae finish feeding in their fourth instar, they move from the vines to the soil, which they enter. The larvae secrete a white substance from their mouths and form a cocoon in which they pupate. These harden to a styrofoam-like texture and are surrounded loosely with soil. Pupation in the soil is done gregariously, often in clumps of six to eight individuals joined by the white foam-like substance. In nature they emerge and overwinter as adults. Two species of wasp parasitoids emerged from four of the five shipments of *Lilioceris* pupae shipped from Nepal. Parasitoids emerged from a total of 35 pupae; 31 in 2006 and four in 2007, inducing a total percent parasitism of 0.62%. One of the parasitoids is a yet to be determined braconid wasp, and the other is an ichneumonid wasp, *Callidora* sp. (Campopleginae).

Colony maintenance

Lilioceris sp. beetles were reared in 5.7-L plastic containers, and fed air potato leaves that had been bleach-sterilized and rinsed with water. During the first winter, *D. bulbifera* leaves were unavailable so we were unable to conduct testing. The beetles were overwintered in a walk-in environmental chamber set at 10°C and a 12-h photoperiod. During the six months in the environmental chamber, beetles readily moved when disturbed and shifted position in the chamber containers when undisturbed, indicating that they do not enter diapause. During the second and third years, we were able to conduct testing all year because of our ability to grow the deciduous host plant *D. bulbifera* in the quarantine glasshouse during the winter. The beetles for these tests were reared in a Percival environmental chamber with a daytime temperature of 27°C, a night time temperature 26°C and a 14-h photoperiod; humidity was maintained consistently above 90%. Due to limited space in the Percival environmental chamber, some beetles were reared in the laboratory in 5.7-L containers at ambient conditions. The adults are long-lived, living without food three months or more in the laboratory and six months in an environmental chamber set at 10°C.

Testing

In the quarantine facility, adult beetles prefer to lay eggs on the undersides of leaves of the target plant *D. bulbifera* but will often lay indiscriminately on all offered plants and even on container walls. Therefore oviposition tests were not deemed meaningful to conduct. Host range testing of adult beetles first focused on adult feeding on foliage because adults of beetles may have broader host ranges than larvae (Blossey, Schroeder, Hight, and Malecki 1994). If any feeding on test plants occurred during the adult feeding tests, then larval feeding trials were conducted on these plants using mature egg transfers. In addition, adult beetles were caged with the two Florida native *Dioscorea* species to optimize the opportunity for the insects to accept these important Florida congeners of the target weed.

No choice adult feeding test on bulbils' sprouting success

No choice adult feeding tests were conducted to examine the effect of adult feeding on the sprouting success of *D. bulbifera* bulbils. Five beetles were placed in a 5.7-L rearing bin on a 3-cm deep soil substrate with two bulbils, each with a diameter of 5 cm. This test was replicated five times and kept at 26°C, 50% relative humidity and ambient photoperiod in a glasshouse. After 2 weeks, bulbils were quartered and percent damage of bulbils was estimated. It was also noted if there was damage to the primary meristematic region, the small area from which arise all stems and roots of the plant. Bulbils were then potted and monitored for sprouting success.

Multi-choice (choice minus control) adult feeding tests on cut material

Adult feeding tests were conducted on 26 species in 23 families outside of the order Dioscoreales, using multiple choice minus control *D. bulbifera*. In each test, a single or multiple cut leaves of each of three test plant species were placed into 0.6-L plastic Glad™ brand sandwich box containers. The petiole or leaf stalks were wrapped in wet cotton and covered with parafilm to inhibit desiccation of the leaves. Attempts were made to offer similar amounts of material of each test plant, which meant using a variety of leaf material, including whole leaves, sections of single large leaves, and clusters of small leaves. Cut material of *D. bulbifera*, usually one leaf, was similarly set up as controls. Two beetles were placed in each test container and monitored for one month. Old leaves were replaced with fresh leaves weekly. The tests were replicated five times using leaves from different plant individuals. Leaves showing feeding damage were pressed and scanned to quantify the amount of feeding using Sigma ScanPro in this and subsequent leaf feeding tests. During the first year of testing, the duration of the tests was three months while that of subsequent years was one month. The change was due to our understanding that feeding on test plants occurred within the first month.

No choice adult feeding tests on cut material

No choice tests to assess adult feeding were conducted on species in the genus *Dioscorea*. The tests were conducted in 0.6-L plastic Glad™ brand sandwich boxes as described above but with leaf material from a single test plant species. Control boxes containing the target *D. bulbifera* leaves were set up simultaneously. All tests were replicated five times and the duration of the tests was one month.

No choice larval development tests – egg transferral on cut material

For the *Dioscorea* species that received adult feeding (only nibbling occurred), the Florida natives *D. floridana* and *D. villosa*, and *D. altissima* and *D. polystachya* (weedy), larval development tests were conducted. Mature eggs were transferred instead of larvae because transferring the delicate larvae easily injured them. For each test, a single cut leaf of each plant species was placed into 0.6-L plastic Glad™ brand sandwich boxes. Because larval fecal matter tends to increase rotting of test leaves, the solid tops of the boxes were fitted with mesh to allow air flow. The petioles were wrapped in wet cotton and covered with parafilm to inhibit desiccation. Five *Lilioceris* eggs were placed on each leaf in each test container, and monitored daily for hatch.

Only three larvae were allowed to remain in each bin to ensure adequate amounts of food material. Leaves were replaced frequently, often every other day, to present fresh food and to reduce leaf decay related to feeding. Leaves showing feeding damage during the first week when most larval development occurs, were pressed for subsequent scanning to measure consumption. No larval feeding occurred on any plant other than the control, so only larvae in the control were reared for complete development. They were moved to 5.7-L plastic rearing bins containing soil and fed cut *D. bulbifera* until the fully developed larvae moved into the soil to pupate. The number of emerging adults was counted to determine the number of beetles fully developing. Each test and the control *D. bulbifera* were replicated five times.

No choice larval development tests – egg transferral on whole plants

For the native species of *Dioscorea* receiving adult nibbling, the Florida native *D. floridana* and *D. villosa*, larval development tests were also conducted on whole plants. For each test plant and control, we placed black mesh sleeve cages over entire staked vines of potted plants. The top of the sleeve cage was suspended from a bamboo stake above the plant and the base was secured with a twist-tie around the vine stem. Five *Lilioceris* eggs were transferred to the underside of a newly emerged leaf. Larval feeding and potential development were monitored with a hand lens daily. Because no larval feeding occurred on any plant other than the control, only larvae in the controls were reared for complete development using the methods described in the preceding section. Each test and the control *D. bulbifera* were replicated five times.

No choice oviposition and development tests – adults on whole plants

Because of attempted feeding by the adult beetle on both Florida native *D. floridana* and *D. villosa* in the first two replicates of no choice adult feeding trials, no choice oviposition and development tests on whole plants were conducted. Test plants were set up with the black mesh sleeve cages, as described in the previous section. Two adult beetles were placed into each sleeve through the zippered top. Leaves that were fed upon were removed weekly and pressed to quantify feeding. Although this beetle has demonstrated indiscriminate oviposition, numbers of eggs were counted. The duration of each test was one month and they were replicated five times. Egg masses were removed and allowed to complete development in 5.7-L plastic rearing bins. Because one plant would provide an insufficient amount of plant material to support complete development, all surviving larvae were moved to 5.7-L plastic rearing bins with cut material for feeding and development. Due to concerns about the quality of some plants and because of the importance of potential use of these native plants, all five replicates were retested four times using different beetles.

Qualitative efficacy tests

Two qualitative tests of efficacy were conducted to assess damage to *D. bulbifera* by this *Lilioceris* beetle. In each test, a one half meter tall plant growing in a three gallon pot was pruned so the plant had 25 fully expanded leaves and placed inside a fine mesh black sleeve cage. In the first test, two egg masses, one producing 27 larvae and the other 23 larvae, were transferred onto two of the leaves of a *D. bulbifera* plant and neonate larvae were allowed to feed for 1 week. In the second test, three mating pairs of adult beetles were placed on a *D. bulbifera* plant and allowed to feed for 1 week. All 25 leaves of each test plant were then removed to estimate percentage of feeding on each leaf. Feeding was estimated between 0 and 100% in 10% increments to calculate mean feeding per leaf.

Statistical methods

Relative feeding by *Lilioceris* sp. on *Dioscorea bulbifera* and *D. floridana* in two of the replicates in which feeding occurred on *D. floridana* were compared using the non-parametric Mann–Whitney *U*-test. The extreme difference in the feeding on the test plants and target weed and the clarity of the results (several thousand fold higher feeding and complete development on the target weed vs. no feeding or test feeding-nibbling by adults on a few *Dioscorea* species, but no larval feeding or development) limited the need for statistic analysis.

Results

No choice adult feeding test on bulbils' sprouting success

Ninety percent of bulbils were fed upon by *Lilioceris* adults with feeding percentages of damage ranging from 5 to 75%. Although 60% of the bulbils were able to sprout successfully, all bulbils with meristematic damage failed to sprout (Table 1).

Multi-choice (choice minus control) adult feeding tests on cut material

No feeding was observed on any of the test plants other than the control *D. bulbifera*. The discrepancy in feeding values on the control are due to the fact that tests A, B and C were conducted for three months rather than one (Table 2).

No choice adult feeding tests on cut material

Modest nibbling was observed on four of the 12 species tested, representing three of seven sections of the genus *Dioscorea*. The total amount of leaf eaten on *D. altissima* was one

Table 1. No choice adult feeding test on bulbils' sprouting success.

Bulbil #	Area of feeding	Shoot damage[1]	Sprouting success
1	5%	No	Yes
2	10%	No	Yes
3	0%	No	Yes
4	75%	Yes	No
5	5%	Yes	No
6	10%	No	Yes
7	25%	Yes	No
8	10%	No	Yes
9	30%	Yes	No
10	10%	No	Yes

[1]Feeding by adult beetles on the primary meristematic region of the air potato bulbil.

Table 2. Multi-choice (choice minus control) adult feeding tests on cut material.

Test	Species[1]	Leaf area eaten (cm²) Total	Mean ± SE
A[2]	*Dioscorea bulbifera*; air potato (Section Opsophyton)	1226.52	306.63 ± 63.81
A	***Callicarpa americana***; beauty berry	0.00	0.0 ± 00.00
A	***Cassia ligustrina***; senna	0.00	0.0 ± 00.00
A	*Tacca integrifolia*; bat flower	0.00	0.0 ± 00.00
B&C[2,3]	*Dioscorea bulbifera*; air potato (Section Opsophyton)	1366.88	273.38 ± 102.58
B	***Crinum americanum***; FL swamp lily	0.00	0.0 ± 00.00
B	***Pontederia cordata***; pickerel weed	0.00	0.0 ± 00.00
B	***Sagittaria latifolia***; broadleaf arrowhead	0.00	0.0 ± 00.00
B	***Thalia geniculata***; arrowroot	0.00	0.0 ± 00.00
C	*Ficus elastica*; Indian rubber tree	0.00	0.0 ± 00.00
C	*Pandanus spiralis*; screw palm	0.00	0.0 ± 00.00
C	***Sabal palmetto***; sabal palm	0.00	0.0 ± 00.00
C	*Saccharum officinarum*; sugarcane	0.00	0.0 ± 00.00
D	*Dioscorea bulbifera*; air potato (Section Opsophyton)	374.91	74.98 ± 20.07
D	*Musa acuminata*; banana	0.00	0.0 ± 00.00
D	***Tradescantia ohiensis***; Ohio spiderwort	0.00	0.0 ± 00.00
E	*Dioscorea bulbifera*; air potato (Section Opsophyton)	379.63	75.93 ± 18.48
E	*Costus woodsonii*; red button ginger	0.00	0.0 ± 00.00
E	*Hedychium coronarium*; butterfly ginger	0.00	0.0 ± 00.00
E	***Juncus effusus***; soft rush	0.00	0.0 ± 00.00
F&G[3]	*Dioscorea bulbifera*; air potato (Section Opsophyton)	606.23	121.25 ± 56.98
F	***Cladium jamaicense***; sawgrass	0.00	0.0 ± 00.00
F	***Iris virginica***; blue flag iris	0.00	0.0 ± 00.00
F	*Lilium* sp (ornamental); lily	0.00	0.0 ± 00.00
G	***Aletris farinosa***; colicroot	0.00	0.0 ± 00.00
G	*Canna indica*; Indian shot	0.00	0.0 ± 00.00
G	*Heliconia caribeae*	0.00	0.0 ± 00.00
H	*Dioscorea bulbifera*; air potato (Section Opsophyton)	323.39	64.68 ± 9.69
H	***Zephyranthes grandiflorum***; rose pink zephyr lily	0.00	0.0 ± 00.00
H	***Canna flaccida***; bandana of the Everglades	0.00	0.0 ± 00.00
I	*Dioscorea bulbifera*; air potato (Section Opsophyton)	369.58	73.92 ± 10.94
I	*Asparagus officinalis*; garden asparagus	0.00	0.0 ± 00.00
I	*Asparagus sprengeri*; asparagus fern	0.00	0.0 ± 00.00

[1]Plant species in bold type are native to Florida.
[2]Tests conducted for 2 months longer than others.
[3]Tests conducted concurrently.

tiny nibble on three of the five replicates. The feeding on the weedy *D. polystachya* was one tiny nibble in one replicate. The amount of feeding on *D. villosa* comprises eight very tiny bites in one replicate and two bites in another. The amount of feeding on *D. floridana* comprised four tiny bites in each of two replicates (Table 3). These extremely minute values are interpreted as test feeding. Relative feeding was measured on *D. floridana* and the control, *D. bulbifera* (Figure 1). The amount of feeding on the control *D. bulbifera* was several thousand times greater than on the test species. The amount of feeding on the control *D. bulbifera* typically consisted of over 85% of the control leaves throughout the month-long experiments. It is important to note that in four subsequent experiments on whole plants of *D. floridana* and *D. villosa,* there was no adult feeding.

No choice larval development tests – egg transfers on cut material

All larvae successfully hatched from the egg transfers in all replicates. All first instar larvae died without feeding on all test plants except the control *D. bulbifera*. Newly emerged larvae on the control *D. bulbifera* fed immediately and began to grow. Feeding measurements reflect 1 week of larval feeding tests because all of the larvae on the four test species died without feeding (Table 4). Of the 15 larvae on the control, 13 completed their development to adulthood.

Table 3. No choice adult feeding tests on cut material.

		Leaf area eaten (cm^2)	
Test	Plant species[1]; common name (Section)	Total	Mean ± SE
A&B[2,3]	*Dioscorea bulbifera*; air potato (Opsophyton)	1621.49	324.3 ± 62.18
A	***Dioscorea villosa***; wild yam (Macropoda)	0.67	0.07 ± 00.05
B	***Dioscorea floridana***; Florida yam (Macropoda)	0.46	0.05 ± 00.07
C[2]	*Dioscorea bulbifera*; air potato (Opsophyton)	1226.522	306.63 ± 63.81
C	*Dioscorea alata*; water yam (Enantiophyllum)	0.00	0.0 ± 00.00
D[2]	*Dioscorea bulbifera*; air potato (Opsophyton)	1216.062	243.21 ± 13.98
D	***Smilax laurifolia***; bamboo vine	0.00	0.0 ± 00.00
E	*Dioscorea bulbifera*; air potato (Opsophyton)	637.53	127.51 ± 38.47
E	*Dioscorea altissima*; dunguey (Chondrocarpa)	0.17	0.06 ± 00.01
E	*Dioscorea cayenensis*; yellow guinea yam (Enantiophyllum)	0.00	0.0 ± 00.00
E	*Rajania cordata*; himber (Rajania)	0.00	0.0 ± 00.00
E	*Dioscorea pilosiuscula*; bulbous yam (Lynchonostemon)	0.00	0.0 ± 00.00
E	*Dioscorea polygonoides*; mata gallina (Dematoste-mon)	0.00	0.0 ± 00.00
F	*Dioscorea bulbifera*; air potato (Opsophyton)	615.67	123.13 ± 30.27
F	*Dioscorea alata*; water yam (Enantiophyllum)	0.00	0.0 ± 00.00
F	*Dioscorea polystachya* (cult.); Chinese yam (Enantiophyllum)	0.00	0.0 ± 00.00
F	*Dioscorea polystachya* (weedy); Chinese yam (Enantiophyllum)	0.05	0.05 ± 00.00
F	*Dioscorea rotundata*; guinea yam (Enantiophyllum)	0.00	0.0 ± 00.00
G	*Dioscorea bulbifera*; air potato (Opsophyton)	310.57	62.11 ± 24.36
G	*Dioscorea sansibarensis*; Zanzibar yam (Opsophyton)	0.00	0.0 ± 00.00
G	*Disocorea trifida*; Indian yam (Macrogynodium)	0.00	0.0 ± 00.00

[1]Plant species in bold type are native to Florida.
[2]Tests conducted for 2 months longer than others.
[3]Tests conducted concurrently.

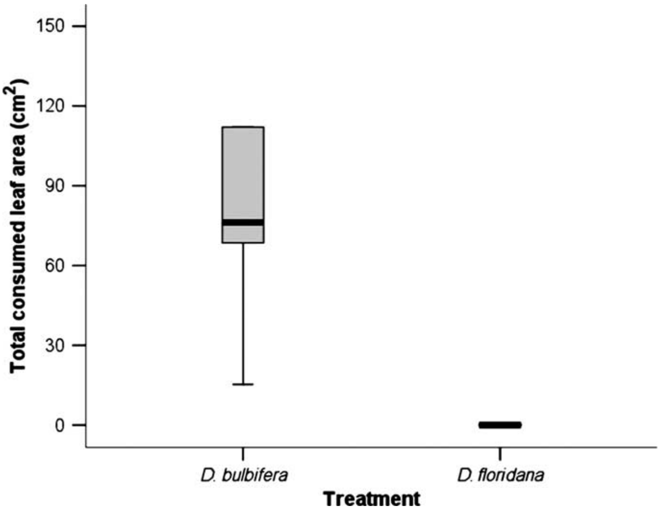

Figure 1. Relative feeding by *Lilioceris* sp. on *Dioscorea bulbifera* and *Dioscorea floridana* in the two of five replicates in which feeding occurred on *D. floridana*. Box plot presents maximum, upper quartile, median, lower quartile and minimum feeding values. Non-parametric Mann–Whitney *U*-test, $Z = -2.619$, $P = 0.008$.

No choice larval development tests – egg transfers on whole plants

All of the test larvae successfully hatched from the egg transfers in all replicates. All first instar larvae died without feeding on all test plants except the control *D. bulbifera*. Larvae on the control *D. bulbifera* fed immediately and began to grow. Feeding measurement reflects 1 week of larval feeding (Table 5). Of the 25 larvae on the control, 22 completed their development to adulthood.

No choice oviposition and development tests – adults on whole plants

In this test, adult feeding occurred only on the control *D. bulbifera*. No adult feeding was documented on the two test plants. Egg masses were laid on the two test species, *Dioscorea villosa* and *Dioscorea floridana*, and the control, *D. bulbifera.* Oviposition was consistently lower on test plants than on the control, which could be attributed to the lack of feeding by adults. The numbers of eggs laid on *D. villosa* in the four

Table 4. No choice larval development tests – egg transfers on cut material.

Plant species[1]	Leaf area eaten Mean ± SE (cm²)
Dioscorea bulbifera; air potato (Section Opsophyton)	18.98 ± 7.54
Dioscorea altissima; dunguey (Section Chondrocarpa)	0.0 ± 0.00
Dioscorea floridana; Florida yam (Section Macropoda)	0.0 ± 0.00
Dioscorea polystachya (weedy); Chinese yam (Section Enantiophyllum)	0.0 ± 0.00
Dioscorea villosa; wild yam (Section Macropoda)	0.0 ± 0.00

[1]Plant species in bold type are native to Florida.

Table 5. No choice larval development tests – egg transfers on whole plants.

Plant species[1]	Leaf area eaten Mean ± SE (cm^2)
Dioscorea bulbifera; air potato (Section Opsophyton)	15.81 ± 06.55
Dioscorea altissima; dunguey (Section Chondrocarpa)	0.0 ± 00.00
Dioscorea floridana; Florida yam (Section Macropoda)	0.0 ± 00.00
Dioscorea polystachya (weedy); Chinese yam (Section Enantiophyllum)	0.0 ± 00.00
Dioscorea villosa; wild yam (Section Macropoda)	0.0 ± 00.00

tests were 25, 40, 35 and 45, respectively. The numbers laid on *D. floridana* were 31, 21, 11 and 38, while the numbers of eggs on the control *D. bulbifera* were 124, 143, 201 and 182. There was no larval feeding, nor adult feeding on the test plants (Table 6).

Summary of all host range tests

A compilation of all of these host specificity tests is shown in Table 7. The only acceptable plant was the target weed air potato.

Qualitative efficacy tests

In the first qualitative test to estimate the damage of this *Lilioceris* sp. to *D. bulbifera* plants, 50 larvae consumed an average of 80–90% of each of 25 leaves. In the second test, three pairs of adult beetles consumed and average of 60–70% of each of 25 leaves.

Discussion

This tropical/subtropical Nepalese beetle, *Lilioceris* sp. near *impressa*, is host specific to the target weed *Dioscorea bulbifera*. Eggs laid on some test plants could be due to either inappropriate oviposition, or to the indiscriminate oviposition frequently observed by this beetle. Feeding of larvae that hatched from eggs laid on test plants was insignificant. Tiny amounts of adult test feeding was observed on the two North American and Florida native yams (*D. floridana* and *D. villosa*), a naturalized weed (*D. polystachya*), and a Brazilian species naturalized in Puerto Rico (*D. altissima*). Larvae were unable to feed on the leaves of any of these *Dioscorea* species. There was no adult feeding on any of the cultivated *Dioscorea* species, or on any of the tested plant representatives of the monocot orders. No feeding occurred on plants (*Cassia* or *Ficus* species) recorded in the literature to be used by *Lilioceris impressa* except for the target *D. bulbifera*. These results suggest that it is possible to have similar adult test nibbling on these few *Dioscorea* species in the field, but significant feeding should not occur, and because the larvae can not develop on these plants, they will not become hosts.

The amount of leaf consumption by this beetle is very large. During the development of a single larva, it consumes almost a square meter of leaf tissue, and

Table 6. No choice oviposition and development tests – adults on whole plants.

Test	Plant species[1]	Leaf area eaten (cm^2)[2]		Oviposition & development[3]		
		Total	Mean ± SE	Eggs	Adults	% to adult
A	*D. bulbifera*; air potato (Opsophyton)	523.79	104.76 ± 19.90	124	118	95.16
A	***D. floridana***; Florida yam (Macropoda)	0.00	0.0 ± 00.00	31	0	0.00
A	***D. villosa***; wild yam (Macropoda)	0.00	0.0 ± 00.00	25	0	0.00
B	*D. bulbifera*; air potato (Opsophyton)	574.81	114.96 ± 07.31	143	129	90.21
B	***D. floridana***; Florida yam (Macropoda)	0.00	0.0 ± 00.00	21	0	0.00
B	***D. villosa***; wild yam (Macropoda)	0.00	0.0 ± 00.00	40	0	0.00
C	*D. bulbifera*; air potato (Opsophyton)	348.21	69.64 ± 17.38	201	189	94.03
C	***D. floridana***; Florida yam (Macropoda)	0.00	0.0 ± 00.00	11	0	0.00
C	***D. villosa***; wild yam (Macropoda)	0.00	0.0 ± 00.00	35	0	0.00
D	*D. bulbifera*; air potato (Opsophyton)	483.00	96.6 ± 34.80	182	167	91.76
D	***D. floridana***; Florida yam (Macropoda)	0.00	0.0 ± 00.00	38	0	0.00
D	***D. villosa***; wild yam (Macropoda)	0.00	0.0 ± 00.00	45	0	0.00

[1]Plant species in bold type are native to Florida.
[2]Adult feeding.
[3]Eggs laid on test plants, number of adults produced on each plant, and the percent of larvae developing into adults.

Table 7. Summary of results of host range testing of *Lilioceris* near *impressa*.

Order; Family-(Section)	Species[1]	Tests on cut material: Adult feeding	Tests on cut material: Larval feeding	Tests on whole plants: Larval feeding	Tests on whole plants: Adult feeding
Dioscoreales; Dioscoreaceae-(Chondrocarpa)	*Dioscorea altissima*	MIN	NO	NO	n/a[2]
Dioscoreales; Dioscoreaceae-(Enantiphyllum)	*Dioscorea polystachya* (weedy)	MIN	NO	NO	n/a[2]
Dioscoreales; Dioscoreaceae-(Macropoda)	***Dioscorea floridana***	MIN	NO	NO	NO
Dioscoreales; Dioscoreaceae-(Macropoda)	***Dioscorea villosa***	MIN	NO	NO	NO
Dioscoreales; Dioscoreaceae-(Opsophyton)	*Dioscorea bulbifera*	YES	YES	YES	YES

Larval feeding and whole plant tests were conducted only on plants with feeding in adult feeding tests with cut material. 'MIN' indicates a minimal amount of test feeding which occurred on four *Dioscorea* species.
[1]Plant species in bold type are native to Florida.
[2]Not tested due to lack of suitable plant material.

the adults, who can live three months or more, can eat another two square meters of leaf (Sanjay Bista and R. Pemberton, unpublished data). The adult beetles proved to be vigorous and long-lived feeders in the quarantine lab.

Both the adults and larvae consume the leaves and aerial bulbils of air potato. The larvae feed gregariously and quickly skeletonize offered leaves. Bulbils that had any feeding damage to the primary meristematic region do not sprout. The ability of the beetle larvae and adults to feed on the bulbils is also important because the weed in the US rarely flowers or produces fruit, so the bulbils are the primary means of persistence and spread. The source beetles in the Katmandu Valley of Nepal population were quite damaging to the *D. bulbifera* plants on which they were found. This high level of destruction was observed in the native area despite the occurrence of pupal parasitoids. Without specialized natural enemies in Florida, the beetle should develop larger populations than it does in its native Nepal.

In Nepal, the long-lived adult beetle appears to be well synchronized with air potato's phenology, becoming quiescent during the cool winter season when the plants lose their leaves. Air potato also looses its leaves in all areas of Florida but winter temperature varies considerably from frost free Miami to northern areas where hard freezes regularly occur (Table 8). Northern Florida is most similar to Nepal with regard to winter low temperatures (Table 8), so we expect that the beetles released in northern Florida may behave as they do in Nepal. We do not know how the beetles will behave in central and southern Florida during the winter. The beetles could remain more active as they do during the winter in our quarantine laboratory in southern Florida when given leaves or bulbils. If the beetles remain active in central and southern Florida after leaf drop, they could sustain themselves on fallen bulbils on the ground, feeding that could damage the bulbils and prevent their sprouting. In addition, adult beetles have an ability to survive without food for an

Table 8. Mean low temperatures through winter months in Nepal and Florida.

	Nov	Dec	Jan	Feb	Mean
Katmandu; Nepal[1]	7°	3°	2°	4°	3°
Gainesville; FL[2]	12°	8°	7°	8°	8°
Orlando; FL	15°	12°	10°	11°	11°
Ft. Lauderdale; FL	19°	17°	15°	16°	15°

[1]Climate data for Nepal from the BBC; http://www.bbc.co.uk.com
[2]Climate data for Nepal from the Weather Channel; http://www.weather.com

extended time period, as demonstrated in our initial feeding trials in which the beetles lived three months without feeding.

Successful establishment of *Lilioceris* sp. on air potato could reduce its dominance, allowing the recovery of native species, and possibly help limit the spread of this invasive weed in Florida and the American South. Chrysomelid beetles have been among the best biological control agents for weeds including: Klamath-weed, *Hypericum perfoliatum* L.; alligatorweed, *Alternanthera philoxeroides* (Mart.) Griseb.; ragweed, *Ambrosa* spp. (Julien 1992); and more recently: leafy spurge, *Euphorbia esula* L. (Butler, Parker, and Murphy 2006); and tamarisk, *Tamarix* spp. (Milbrath and DeLoach 2006).

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Appendix 1. Plants used in host specificity testing for *Lilioceris* near *impressa*.

Plant taxonomy & testing category[1]	Common name	Nativity and distribution
The Target Weed:		
DIOSCOREALES; DIOSCOREACEAE		
Section Opsophyton		
Dioscorea bulbifera	Air potato	Native Asia & Africa; Cultivated West Indies; Invasive MS, LA, TX, HI &FL
North American Species in the Same Genus as the Target Weed:		
Section Macropoda		
Dioscorea floridana	Florida yam	Native FL, GA and SC
Dioscorea villosa	Wild yam	Native FL north to CN & MN, west to TX
West Indian Species in the Same Genus as the Target Weed:		
Section Chondrocarpa		
Dioscorea altissima	Dunguey	PR; exotic-native to Brazil
Section Dematostemon		
Dioscorea polygonoides	Mata gallina	Hispaniola; Puerto Rico; Cuba; Jamaica
Section Lynchonostemon		
Dioscorea pilosiuscula	Bulbous yam	Hispaniola; Puerto Rico; VI; Jamaica
Section Macrogynodium		
Dioscorea trifida	Cush cush yam	West Indies; S. American, cultivated
Section Rajania		
Dioscorea cordata	Himber	Cuba; Jamaica; Puerto Rico
Weedy and/or Cultivated Species in the Same Genus as the Target Weed:		
Section Enantiophyllum		
Dioscorea alata	White yam	Native Africa Eastern US PR, VI, Invasive in FL
Dioscorea cayenensis	Chinese yam	Introduced FL, to VT
Dioscorea polystachya (cult.)	Yellow guinea yam	Cultivated WI; African
Dioscorea polystachya (weedy)	Cultivated chinese yam	E. USA weed; Cultivation limited; Indochina
Dioscorea rotundata	Guinea yam	Cultivated WI; African
Section Macrogynodium		
Dioscorea trifida	Cush cush yam	West Indies; S. American, cultivated
Section Opsophyton		
Dioscorea sansibarensis	Zanzibar yam	Native East Africa; Exterminated from FL
Species in Other Genera in the Same Family, Dioscoreaceae, as Target Weed:		
Tacca integrifolia	Bat flower	Native Indochina, Malaysia; Ornamental FL
Species in Other Orders than the Target Weed:		
ARECALES; ARECACEAE		
Sabal palmetto	Cabbage palmetto	Native SE USA, Bahamas & Cuba

Appendix 1. (*Continued*).

Plant taxonomy & testing category[1]	Common name	Nativity and distribution
ALISMATALES; ALISMATACEAE		
Sagittaria latifolia	Broadleaf arrowhead	Native N. America
ASPARAGALES; IRIDACEAE		
Crinum americum	Seven sisters	Native Southeast USA; Invasive PR
Iris virginica	Virginia iris	Native E. N. America; widely cultivated
COMMELINALES; COMMELINACEAE		
Tradescantia ohiensis	Ohio spiderwort	Native NE USA
COMMELINALES; PONTEDERIACEAE		
Pontederia cordata	Pickerelweed	Native NE USA
FABALES; FABACEAE		
Cassia (Senna) ligustrina	Privet wild Sensitive plant	Native Africa & Asia; Introd. Neotropics; VI
LILIALES; LILIACEAE		
Lilium sp.	Lily	Ornamental non-native
Zephyranthes grandiflora	Rosepink zephyr lily	Rare; Native FL & Mexico
LILIALES; NARTHECIACEAE		
Aletris farinosa	White colicroot	Native Central N America & E. Canada
LILIALES; SMILICACEAE		
Smilax laurifolia	Laurel greenbrier	Native Central N America, Bahamas & Cuba
LAMIALES; VERBENACEAE		
Callicarpa americana	American beauty berry	Florida, Southeastern US
PANDALES; PANDANACEAE		
Pandanus spiralis	Screw pine	Native Australia
POALES; CYPERACEAE		
Cladium jamaicense	Jamaica swamp sawgrass	Native Australia, Southern N. America; HI & PR
POALES; JUNCACEAE		
Juncus effusus	Soft rush	Cosmopolitan; temperate wetlands worldwide
POALES; POACEAE		
Saccharum officinarum	Sugarcane	Native Asia; Cultivated FL to TX
ZINGIBERALES; CANNACEAE		
Canna flaccida	Bandana of the Everglades	Native Neotropics; Widely cultivated
Canna indica	Indian shot	Native to Tropics; Exotic
ZINGIBERALES; COSTACEAE		
Costus woodsonii	Red button ginger	Native Asia & PR; Cultivated widely in tropics
ZINGIBERALES; HELICONIACEAE		
Heliconia caribaea	Lobsterclaw	Native Caribbean; Cultivated exotic

Appendix 1. (*Continued*).

Plant taxonomy & testing category[1]	Common name	Nativity and distribution
ZINGIBERALES; MARANTACEAE		
Thalia geniculata	Alligatorflag	Native Africa, N. and S Americas; Invasive FL
ZINGIBERALES; MUSACEAE		
Musa acuminata	Edible banana	Native Asia & Africa; Cultivated widely in tropics
ZINGIBERALES; ZINGIBERACEAE		
Hedychium coronarium	Butterfly ginger	Native Asia; Exotic FL GA LA HI & PR
Species in the Same Genus as Those on which Target Agent is Found:		
DIOSCOREALES; DIOSCOREACEAE		
Dioscorea alata	White yam	Native Africa. Introd. GA, LA, PR, VI. Invasive in FL
URTICALES; MORACEAE		
Ficus elastica	Rubber tree	Native Asia; Introd. FL & PR; widely cultivated
FABALES; FABACEAE		
Cassia (Senna) ligustrina[2]	Privet wild sensitive plant	Native Africa & Asia; Introd. Neotropics; VI
LAMIALES; VERBENACEAE		
Callicarpa americana[2]	American beauty berry	Native SE USA and Caribbean
Species on which Close Relative is Found:		
ASPARAGALES; ASPARAGACEAE		
Asparagus officinalis	Garden asparagus	Native N Africa Europe & Asia; Widely cultivated
Asparagus densiflorus sprengeri	Asparagus fern	Native S. Africa; Introduced AUS NZ WI, FL CA & HI
LILIALES; LILIACEAE		
Lilium sp	Lily	Ornamental non-native, native species occur in FL

[1]Species native to Florida are in bold font.
[2] Target agent found on *Cassia sophera* and *Callicarpa macrophylla*.