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HOST SPECIFICITY TESTS OF *GRATIANA GRAMINEA* (COLEOPTERA: CHRYSOMELIDAE), A POTENTIAL BIOLOGICAL CONTROL AGENT OF TROPICAL SODA APPLE, *SOLANUM VIARUM* (SOLANACEAE)

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ABSTRACT

Multiple-choice and no-choice tests were conducted at the Department of Agriculture, Division of Plant Industry Quarantine facility in Gainesville, FL to determine the specificity of the Brazilian leaf-beetle *Gratiana graminea* Klug, a candidate for biological control of *Solanum viarum*, tropical soda apple. One hundred fifteen plant species in 32 families were included in the feeding-oviposition multiple-choice tests including the target weed and the 5 major cultivated Solanaceae *Capsicum annuum* L., *Lycopersicon esculentum* Mill., *Nicotiana tabacum* L., *Solanum melongena* L., and *Solanum tuberosum* L. Eight to 12 plant species, including always the main target weed, growing in 1-gallon pots were simultaneously exposed to 20 *G. graminea* adults (10 males and 10 females that most of the time had recently emerged from pupae) in an aluminum cage (60 × 60 × 60 cm). At the beginning of each test the insects were placed at the bottom center of each cage to allow them to orient by themselves to the tested plants. Plant species in each test were replicated 3-4 times (one replication of tested plants in each separate cage). Plants tested were exposed to *G. graminea* adults from 3-6 weeks. Observation of oviposition and feeding were made during almost all the weekdays. No-choice host specificity tests were conducted with *G. graminea* adults on potted plants in cages made of clear-plastic cylinders and with *G. graminea* larvae placed on cluster of leaves of each individual plant tested. Ten *G. graminea* adults were exposed to 29 plant species individually tested during 3 to 5 weeks, and 10 neonate larvae were exposed to 31 plant species. Plant species in each test were replicated 3-4 times. Results indicated that *G. graminea* fed and developed only on the target weed. The tests indicated that a host range expansion of *G. graminea* to any of the major cultivated Solanaceae species is highly unlikely. A petition for field release in Florida was submitted to the Technical Advisory Group for Biological Control Agents of Weeds (TAG) in Sep 2008.

Key Words: weed biological control, host-specificity tests, *Gratiana graminea*, *Solanum viarum*, tropical soda apple, Solanaceae

RESUMEN

Pruebas de ovoposición y alimentación (con y sin elección), se realizaron para evaluar la especificidad del escarabajo defoliador, de origen Brasileiro, *Gratiana graminea* Klug como agente potencial para el control biológico de tropical soda apple, *Solanum viarum* Dunal en los Estados Unidos. Las pruebas se efectuaron en la cuarentena del Departamento de Agricultura de la Florida, División de Industria de Plantas en Gainesville. Ciento quince especies de plantas, en 32 familias, fueron incluidas en las pruebas de especificidad de elección multiple, incluyendo la maleza objetivo y las cinco plantas cultivadas pertenecientes a la familia Solanaceae más importantes: *Capsicum annuum* L., *Lycopersicon esculentum* Mill., *Nicotiana tabacum* L., *Solanum melongena* L., y *Solanum tuberosum* L. En cada prueba se utilizaron de ocho a doce plantas, incluyendo siempre la maleza objetivo, creciendo en macetas de un galón las cuales fueron expuestas a 20 adultos de *G. graminea* (10 machos y 10 hembras recién emergidos de pupa) durante 3 a 6 semanas. Registros de alimentación y ovoposición fueron realizados casi todos los días de la semana. Pruebas de alimentación/ovoposición sin elección fueron también realizadas usando plantas creciendo en macetas y jaulas cilíndricas hechas de plástico claro transparente. Diez adultos de *G. graminea* fueron expuestos a 29 especies de plantas en forma individual durante 3 a 5 semanas. Diez larvas recién nacidas fueron también expuestas a 31 especies de plantas en forma individual. Cada prueba tuvo 3-4 repeticiones. Los resultados indicaron que *G. graminea* se alimentó, colocó posturas y completó su desarrollo únicamente en la maleza objetivo. Las pruebas de especificidad indicaron que la posibilidad de *G. graminea* de llegar a ser una plaga de las Solanaceas cultivadas es muy remota. La solicitud a TAG para liberar el escarabajo fue presentada en septiembre 2008.

Translation provided by the authors.

Tropical soda apple, *Solanum viarum* Dunal (Solanaceae), is a perennial weed, originally from northeast Argentina, southern Brazil, Paraguay, and Uruguay, that has been spreading throughout Florida at an alarming rate during the last two decades. The pasture-land infested in 1992 was estimated to be approximately 60,000 hectares (Mullahey et al. 1993), and increased to more than 300,000 hectares in 1995-96 (Mullahey et al. 1997). Currently, the infested area is estimated at more than 400,000 hectares (Medal et al. 2008). Tropical soda apple, first reported in the United States in Glades County, Florida in 1988 (Coile 1993; Mullahey & Colvin 1993), is also present in Alabama, Georgia, Mississippi, North Carolina, South Carolina, Texas, and Puerto Rico (Bryson & Byrd, Jr. 1996; Dowler 1996; Mullahey et al. 1997; Medal et al. 2003). The potential range of tropical soda apple in the United States may be extended even further based on studies of the effects of temperatures and photoperiods conducted by Patterson (1996) in controlled environmental chambers. This invasive exotic weed was placed on the Florida and Federal Noxious Weed Lists in 1995.

In addition to its invasion of pasture lands and reduction of cattle carrying capacity (Mullahey et al. 1993; Bredow et al. 2007), tropical soda apple is known to harbor at least 6 viruses that affect cultivated solanaceous crops such as tomato, tobacco, and pepper (McGovern et al. 1994a, 1994b, 1996). Tropical soda apple is also an alternative host for key pests such as the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), major defoliating insect pest of potato in North America; the tomato hornworm, *Manduca quinquemaculata* (Haworth) and the tobacco hornworm, *Manduca sexta* (L.), (Lepidoptera: Sphingidae), major pests of tomato and tobacco plants; the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae) one of the most troublesome insect pest worldwide of many field and vegetable crops; the tobacco budworm, *Heliothis virescens* (Fabr.) (Lepidoptera: Noctuidae) one of the most destructive pests of tobacco; the green peach aphid, *Myzus persicae* (Sulzer) an important pest of peach trees and vector of plant viruses to solanaceous plants and other food crops (Homoptera: Aphididae); the southern green stinkbug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae) an important pest of soybean and vegetable crops; and the suckfly, *Tupiocoris notatus* (Distant) (Hemiptera: Miridae) a pest of several crops including tobacco (Habeck et al. 1996; Medal et al. 1999a; Sudbrink et al. 1999). Although it is very difficult to estimate the real (direct and indirect) economic losses due to this invasive weed, the production loss to Florida ranchers by tropical soda apple was estimated from \$6.5-16 million annually (Thomas 2007).

Although tropical soda apple is able to spread vegetatively from the root system, the primary

method of dispersal is by seed dissemination (Bryson et al. 1995; Medal et al. 1999b), which occurs mainly by livestock and wildlife that feed on the fruits and scarify the seeds (Akanda et al. 1996; Brown et al. 1996). A single plant of tropical soda apple can produce up to 150 fruits per year, with each fruit containing on average 400 seeds. The estimated seed production is 60,000 seeds/plant/season with a viability of more than 75% (Mullahey & Colvin 1993; Pereira et al. 1997).

Currently recommended management practices for this invasive plant in southeastern United States include herbicide applications and mechanical techniques (mowing/tilling) (Mislevy et al. 1996; Mullahey et al. 1996; Sturgis & Colvin 1996; Akanda et al. 1997). These control tactics provide temporary weed suppression at an economic cost estimated at \$62 and \$47 per hectare in chemical and mechanical control methods, respectively (Thomas 2007). However, application of these control methods is often difficult to employ in remote and/or inaccessible areas.

A biological control project for tropical soda apple was started in Dec 1996 by the University of Florida in collaboration with the Universidade Estadual Paulista, Jabotical campus, Brazil, Universidade Federal do Parana in Curitiba, Brazil, Universidade Regional de Blumenau, Santa Catarina state, Brazil, Universidade Centro-Oeste in Irati, Parana state, Brazil, and the US-DAS-ARS, Biological Control Laboratory in Hurlingham, Buenos Aires Province, Argentina. The release of the Brazilian leaf-beetle *Graminea graminea* Klug (Coleoptera: Chrysomelidae) in Florida will complement the defoliation effects that *Gratiana boliviana* Spaeth has been making on tropical soda apple plants during the warm season in Florida since it was released in the summer 2003 (Medal et al. 2008; Overholt et al. 2008). Tropical soda apple defoliation by *G. boliviana* and *G. graminea*, in southern Brazil is causing a major suppressive effect on tropical soda apple density (Gandolfo et al. 2007; Medal et al. unpublished data). These two leaf-feeder beetles have a synergistic effect on tropical soda apple defoliation and occupy different niches in somewhat overlapped geographical regions in southern parts of Brazil.

In this paper we report the results of the host-specificity tests conducted at the Florida Department of Agriculture-Division of Plant Industry quarantine facility in Gainesville with the leaf-beetle *G. graminea* as a potential biological control agent of the non-native weed tropical soda apple.

MATERIALS AND METHODS

Host-Feeding Specificity Tests

Plant-host specificity tests with *Gratiana graminea* adults and first instars were conducted

from Sep 2000 to Aug 2004 at the Florida Department of Agriculture and Consumer Services-Division of Plant Industry quarantine facility in Gainesville, Florida. Additional feeding/oviposition tests with *G. graminea* adults were conducted at the Gainesville quarantine facility from May to Sep 2008. *Gratiana graminea* (all developmental stages) were collected on tropical soda apple plants in Rio Grande do Sul, Brazil, introduced into Florida-quarantine, placed on caged plants of tropical soda apple growing in 1-gallon pots and eggs were removed twice a week to provide the insects required for testing.

Multiple-Choice Feeding and Oviposition Tests

One hundred fifteen plant species in 32 families were included in the feeding and oviposition preference tests in quarantine (Table 1). The plants tested included 56 species in the family of the target weed (Solanaceae) of which 29 were from the genus *Solanum* and 27 from 15 other genera that include plants of agricultural or ecological importance. Ten species representing 5 families (Boraginaceae, Convolvulaceae, Ehretiaceae, Nolanaceae, Polemoniaceae) that are very closely related phylogenetically to the Solanaceae and in the same order Polemoniales (Heywood 1993) were included. Forty-nine plant species representing 26 families, most of them with an economically and/or environmental value in North America, were also tested. The major target weed (tropical soda apple), and 10 plant species in the Solanaceae were tested at least 3 times (Table 1). They included *Solanum donianum* Walpers that is in the list of Florida threatened plants (Coile 1998); 4 secondary target-weeds (*Solanum tampicense* Dunal, *Solanum torvum* Sw., *Solanum capsicoides* All., *Solanum elaeagnifolium* Cav.); and the 5 major cultivated *Solanaceae* (*Capsicum annuum* L., *Lycopersicon esculentum* Mill., *Nicotiana tabacum* L., *Solanum melongena* L., *Solanum tuberosum* L.). Eight to 12 plant species, including always the main target weed, growing in 1-gallon pots were simultaneously exposed to 20 *G. graminea* adults (10 males and 10 females which were newly emerged from pupae most of the time) in an aluminum cage (60 × 60 × 60 cm). At the beginning of each test the insects were placed at the bottom center of each cage to allow them to orient by themselves to the tested plants. Plant species in each test were replicated 3-4 times (1 replication of tested plants in each separate cage). Plants were exposed to *G. graminea* adults from 3-6 weeks. Observations of oviposition and feeding were made during most of the weekdays. Plants consumed were replaced as needed. Plants were checked for oviposition sites and eggs were removed and counted weekly. On the last day of each experiment, plants were checked for feeding and eggs

laid on them. Leaf area consumed was measured with a Portable Area Meter Model LI-3000 (Lambda Instrument Corporation) and the leaf-feeding area is reported on a scale from 0-5 (0 = no feeding, 1 = probing or <5% of leaf area consumed, 2 = light feeding or 5-20% of the area, 3 = moderate feeding or 21-40%, 4 = heavy feeding or 41-60%, and 5 = intense feeding or >60% of the leaf area consumed).

No-Choice Larval Feeding Tests

No-choice host specificity tests were conducted with *G. graminea* neonate larvae in an environmental chamber at a temperature of $22 \pm 2^\circ\text{C}$, relative humidity of 55-65%, and a photoperiod of 12:12 (L:D). Recently hatched non-fed larvae were exposed to 31 plant species including 30 species in the family of the target weed (Solanaceae) and 1 species in the family Convolvulaceae. The species tested included 7 genera of plants very closely related phylogenetically in the same family as the target weed, and with an economical and/or environmental value in North America (Table 2). Larvae were exposed to clusters of leaves of each individual plant tested by placing the clusters individually in a 30-mL plastic-cup containing water and fitted with a paper lid to avoid insect contact with the water. The leaf petiole was inserted through a hole (3-4 mm diameter) made in the middle of the paper-lid. The cup and plant cluster was placed inside a clear-plastic container covered with a plastic-lid having 6-7 small holes to allow air circulation. Moistened tissue paper was placed at the bottom of the plastic container and under the plastic-lid to provide moisture. The plants (treatments) were arranged in a completely randomized design. Three to 4 replications were used with 10 one-d- old larvae per replication. Each group of 10 *G. graminea* larvae was provided with only 1 plant species which they fed on or died. Daily observations of feeding were made and leaves were replaced as needed. Larval mortality counts were made 3 and 7 d after the experiment started.

No-Choice Adult Feeding Tests

No-choice host specificity tests were conducted with *G. graminea* adults at the Gainesville quarantine facility with potted plants (20-60 cm height) in cages. *Gratiana graminea* adults were exposed to 29 plant species including *S. donianum* in the list of Florida threatened plants, all major cultivated Solanaceae, and 7 exotic (Table 3). Five to 6 plant species were individually tested each time due to limitation in availability of cages. Ten *G. graminea* adults (5 males, 5 females) per replication (3-4 replications) were exposed to plants during 21 to 35 d. Cages were made of clear plastic cylinders (15 cm diameter,

TABLE 1. HOST RANGE ADULT FEEDING AND OVIPOSITION TESTS WITH *GRATIANA GRAMINEA* IN FLORIDA.

Plant Family Species	Common Names (*indicates native <i>Solanum</i> species)	No. of Tests	No. of Insects	Feeding Score ¹	Eggs Laid per Female
Category 1. Genetic types of the target weed species found in North America					
SOLANACEAE					
Tribe Solaneae					
Genus <i>Solanum</i>					
Subgenus <i>Leptostemonum</i>					
Section Acantophora					
<i>Solanum viarum</i> Dunal	Tropical soda apple	9	600	4-5	58-124
Category 2. Species in the same genus as the target weed, divided by subgenera (if applicable)					
Tribe Solaneae					
Genus <i>Solanum</i>					
Subgenus <i>Leptostemonum</i>					
<i>Solanum capsicoides</i> All.	Red soda apple	3	220	1	0
<i>Solanum mammosum</i> L.	Nipplefruit	2	120	1	0
Section Lasiocarpum					
<i>Solanum quitoense</i> Lam.	Naranjilla	2	120	0	0
<i>Solanum pseudolulo</i> Heise	Falso lulo	2	120	0	0
<i>Solanum sessiliflorum</i> Dunal	Nightshade	2	120	0	0
Section Micracantha					
<i>Solanum tampicense</i> Dunal	Wetland nightshade	5	220	1	0
<i>Solanum jamaicense</i> Mill.	Jamaican nightshade	2	120	0	0
Section Melongena					
Subsection Lathyrocarpum					
<i>Solanum carolinense</i> L.	Horse nettle*	2	140	0	0
<i>Solanum dimidiatum</i> Raf.	Western horsenettle*	2	140	0	0
Section Persicariae					
<i>Solanum bahamense</i>	Bahama nightshade	2	120	0	0
<i>Solanum torvum</i> Sw.	Turkeyberry	6	420	1-3	0
<i>Solanum verbascifolium</i> L.	Mullein nightshade*	2	140	0	0
Subgenus <i>Solanum</i>					
<i>Solanum americanum</i> Mill.	American nightshade*	2	140	0	0
<i>Solanum diphyllum</i> L.	Two-leaf nightshade*	2	140	0	0
<i>Solanum erianthum</i> Don.	Potato tree*	2	140	0	0
<i>Solanum jasminoides</i> Paxt.	White potato vine	2	140	0	0
<i>Solanum mauritianum</i> Scop.	Earleaf nightshade	2	140	0	0
<i>Solanum nigrescens</i> Mart. & Gal	Divine nightshade*	2	140	0	0
<i>Solanum nigrum</i> L.	Black nightshade*	2	160	0	0
<i>Solanum parishii</i> heller	Parish nightshade*	2	140	0	0
<i>Solanum ptycanthum</i> Dunal	Wonder berry*	2	140	0	0
<i>Solanum seforthianum</i> Andr.	Brazilian nightshade	2	140	0	0
<i>Solanum tuberosum</i> L.	Potato	6	420	0	0
Category 3. Species in other genera in the same family as the target weed, divided by subfamily (if applicable)					
Genus <i>Acnistus</i>					
<i>Acnistus australe</i> (Griseb.) Griseb.	Acnistus	2	140	0	0
Genus <i>Capsicum</i>					
<i>Capsicum annuum</i> L.	Bell pepper	6	420	0	0
<i>Capsicum frutescens</i> L.	Chile	2	140	0	0
Genus <i>Iochroma</i>					
<i>Iochroma</i> sp.	Iochroma	2	140	0	0
Genus <i>Physalis</i>					
<i>Physalis angulata</i> L.	Cutleaf Ground-Cherry	2	140	0	0
<i>Physalis arenicola</i> Kearney	Cypresshead	2	140	0	0

Each test included 3-4 replications with 20 adults (10 males, 10 females) per replication.
¹0 = no feeding, 1 = probing (<5% of leaf area), 2 = light feeding (5-20%), 3 = moderate feeding (21-40%), 4 = heavy feeding (41-60%), 5 = intense (60% of leaf-area).

TABLE 1. (CONTINUED) HOST RANGE ADULT FEEDING AND OVIPOSITION TESTS WITH *GRATIANA GRAMINEA* IN FLORIDA.

Plant Family Species	Common Names (*indicates native <i>Solanum</i> species)	No. of Tests	No. of Insects	Feeding Score ¹	Eggs Laid per Female
<i>Physalis crassifolia</i> Benth	Ground-Cherry	2	140	0	0
<i>Physalis gigantea</i> L.	Ground-cherry	2	140	0	0
<i>Physalis ixocarpa</i> Brot.	Tomatillo	2	140	0	0
<i>Physalis pubescens</i> L.	Strawberry tomato	2	120	0	0
<i>Physalis walteri</i> Nutt.	Ground-cherry	2	140	0	0
Tribe Daturae					
Genus <i>Brugmansia</i>					
<i>Brugmansia sanguinea</i> (Ruiz & Pav.) Don	Red floripontio	2	140	0	0
Genus <i>Datura</i>					
<i>Datura discolor</i> Bernh	Angels'trumpet	2	140	0	0
<i>Datura metel</i> L.	Downy thorn apple	2	140	0	0
<i>Datura meteloides</i> D.	Datura	2	140	0	0
<i>Datura stramonium</i> L.	Jimson weed	2	140	0	0
Tribe Lycieae					
Genus <i>Lycium</i>					
<i>Lycium carolinianum</i> Walt.	Christmas berry	2	140	0	0
<i>Lycium fremontii</i> Gray.	Lycium	2	140	0	0
Genus <i>Lycopersicon</i>					
<i>Lycopersicon esculentum</i> Mill.	Tomato	6	420	0	0
Tribe: Nicandreae					
Genus: Nicandra					
<i>Nicandra physaloides</i> (L.) Gaertn.	Apple of Peru	2	140	0	0
Tribe Nicotianae					
Genus <i>Nicotiana</i>					
<i>Nicotiana tabacum</i> L.	Tobacco	6	420	0	0
<i>Nicotiana rustica</i> L.	Wild tobacco	2	160	0	0
<i>Nicotiana sylvestris</i> Speg. & Comes	Tobacco	2	140	0	0
Genus <i>Nierembergia</i>					
<i>Nierembergia scoparia</i> Sendtri	Cupflower	2	140	0	0
Genus <i>Petunia</i>					
<i>Petunia</i> × <i>hybrida</i>	Garden-petunia	2	140	0	0
Tribe Salpiglossidae					
Genus <i>Salpiglossis</i>					
<i>Salpiglossis sinuata</i> Ruiz & Pav	Painted tongue	2	140	0	0
Genus <i>Schizanthus</i>					
<i>Schizanthus</i> spp.	Butterfly flower	2	140	0	0
Tribe Solandaeae					
Genus <i>Solandra</i>					
<i>Solandra glandiflora</i> Swartz	Chalice vine	2	140	0	0
Category 4. Threatened and endangered species in the same family as the target weed divided by subgenus, genus, and subfamily					
Section Torva					
<i>Solanum donianum</i> Walpers	Mullein nightshade*	4	300	0	0
Category 5. Species in other families in the same order that have some phylogenetic, morphological, or biochemical similarities to the target weed					
BORAGINACEAE					
<i>Heliotrope</i> sp.	Heliotrope	1	80	0	0
<i>Myosotis alpestris</i> Schmidt	Forget-Me-Not	1	80	0	0
CONVOLVULACEAE					
<i>Convolvulus purpurea</i> L.	Convolvulus	1	80	0	0

Each test included 3-4 replications with 20 adults (10 males, 10 females) per replication.

¹0 = no feeding, 1 = probing (<5% of leaf area), 2 = light feeding (5-20%), 3 = moderate feeding (21-40%), 4 = heavy feeding (41-60%), 5 = intense (60% of leaf-area).

TABLE 1. (CONTINUED) HOST RANGE ADULT FEEDING AND OVIPOSITION TESTS WITH *GRATIANA GRAMINEA* IN FLORIDA.

Plant Family Species	Common Names (*indicates native <i>Solanum</i> species)	No. of Tests	No. of Insects	Feeding Score ¹	Eggs Laid per Female
<i>Ipomoea batata</i> (L.) Lam.	Sweet-potato	2	160	0	0
<i>Evolvulus muttallianus</i>	Evolvulus	1	80	0	0
EHRETIACEAE					
<i>Cordia sebestena</i> L.	Geiger tree	1	80	0	0
NOLANACEAE					
<i>Nolana paradoxa</i> Lindl.	Chilean bellflower	1	80	0	0
POLEMONIACEAE					
<i>Cobaea scandens</i> Cav.	Cobaea	1	80	0	0
<i>Gilia tricolor</i> Benth	Bird's-eyes	1	80	0	0
<i>Phlox paniculata</i> L.	Phlox	1	80	0	0
Category 6. Species in other orders that have some morphological or biochemical similarities to the target weed or that share the same habitat					
ACERACEAE					
<i>Acer rubrum</i> L.	Red maple	1	80	0	0
ACTINIDIACEAE					
<i>Actinidia deliciosa</i> Liang & Fergusson	Kiwi vine	1	80	0	0
ANACARDIACEAE					
<i>Anacardium occidentale</i> L.	Cashew	1	80	0	0
<i>Mangifera indica</i> L.	Mango	1	80	0	0
<i>Pistacia vera</i> L.	Cultivated pistacho	1	80	0	0
APIACEAE					
<i>Daucus carota</i> L.	Carrot	1	80	0	0
ASTERACEAE					
<i>Helianthus annuus</i> L.	Annual sunflower	1	80	0	0
<i>Lactuca sativa</i> L.	Lettuce	1	80	0	0
CAMPANULACEAE					
<i>Campanula persicifolia</i> L	Bell flower	1	80	0	0
CARICACEAE					
<i>Carica papaya</i> L.	Papaya	1	80	0	0
CHENOPODIACEAE					
<i>Beta vulgaris</i> L.	Beet	1	80	0	0
CRUCIFERAE					
<i>Brassica oleracea</i> L. var. <i>botrytis</i>	Broccoli/Cauliflower	2	160	0	0
<i>Brassica oleracea</i> L. var. <i>capitata</i>	Cabbage	1	80	0	0
<i>Raphanus sativus</i> L.	Radish	1	80	0	0
CUCURBITACEAE					
<i>Citrullus lanatus</i> (Thumb)	Watermelon	1	80	0	0
<i>Cucumis melo</i> L.	Cantaloupe	1	80	0	0
<i>Cucurbita pepo</i> (L.) Alef.	Pumpkin/Squash	1	80	0	0
<i>Cucurbita sativus</i> L.	Cucumber	1	80	0	0
EBENACEAE					
<i>Diospyros virginiana</i>	Persimmon	1	80	0	0
ERICACEAE					
<i>Vaccinium ashei</i> Rende.	Rabbit-eye blueberry	1	80	0	0
FABACEAE					
<i>Arachis hypogaea</i> L	Peanut	1	80	0	0
<i>Glycine max</i> (L.) Merrill	Soybean	1	80	0	0
<i>Phaseolus vulgaris</i> L.	Pinto bean	1	80	0	0
<i>Phaseolus lunatus</i> L.	Snowpea	1	80	0	0
<i>Pisum sativum</i> L.	Cowpea	1	80	0	0
<i>Vigna unguiculata</i> (L.) Walp.	Lima bean	1	80	0	0

Each test included 3-4 replications with 20 adults (10 males, 10 females) per replication.
¹0 = no feeding, 1 = probing (<5% of leaf area), 2 = light feeding (5-20%), 3 = moderate feeding (21-40%), 4 = heavy feeding (41-60%), 5 = intense (60% of leaf-area).

TABLE 1. (CONTINUED) HOST RANGE ADULT FEEDING AND OVIPOSITION TESTS WITH *GRATIANA GRAMINEA* IN FLORIDA.

Plant Family Species	Common Names (*indicates native <i>Solanum</i> species)	No. of Tests	No. of Insects	Feeding Score ¹	Eggs Laid per Female
LAURACEAE					
<i>Persea americana</i> Mill.	Avocado	1	80	0	0
LOBELIACEAE					
<i>Lobelia cardinalis</i> L.	Cardinal flower	1	80	0	0
LOGANIACEAE					
<i>Buddleia davidii</i> Franch	Butterfly bush	1	80	0	0
MALVACEAE					
<i>Abelmoschus esculentus</i> (L.)	Okra	1	80	0	0
<i>Gossypium hirsutum</i> L.	Cotton	1	80	0	0
<i>Hibiscus</i> spp.	Rose mallow	1	80	0	0
MORACEAE					
<i>Ficus aurea</i> Nutt	Fig	1	80	0	0
MUSACEAE					
<i>Musa acuminata</i> Colla.	Banana	1	80	0	0
MYRTACEAE					
<i>Psidium guajaba</i> L.	Tropical guava	1	80	0	0
PASSIFLORACEAE					
<i>Passiflora edulis</i> Sims	Passion fruit	1	80	0	0
POACEAE					
<i>Oryza sativa</i> L.	Rice	1	80	0	0
<i>Saccharum officinarum</i> L.	sugarcane	1	80	0	0
<i>Zea mays</i> L.	Sweet corn	1	80	0	0
PUNICACEAE					
<i>Punica granatum</i> L.	Pomegranate	1	80	0	0
ROSACEAE					
<i>Malus pumilla</i> Mill.	Apple	1	80	0	0
<i>Prunus americana</i> Marsh.	American plum	1	80	0	0
<i>Rosa</i> sp.	Miniature rose	1	80	0	0
<i>Rubus betulifolius</i> Small	Blackberry	1	80	0	0
RUTACEAE					
<i>Citrus sinensis</i> (L.) Osbeck	Sweet orange	1	80	0	0
<i>Citrus limon</i> (L.) Burm.	Lemon	1	80	0	0
<i>Citrus paradise</i> Mcfady	Grapefruit	1	80	0	0
SCROPHULARIACEAE					
<i>Antirrhinum majus</i> L.	Common snapdragon	1	80	0	0
<i>Nemensia strumosa</i> Benth.	Nemensia	1	80	0	0
Category 7. Any plant on which close relatives of the biological control agent (within the same genus) have been found or recorded to feed/ or reproduce					
SOLANACEAE					
Section Melongena					
Subsection Lathyrocarpum					
<i>Solanum elaeagnifolium</i> Cav.	Silverleaf nightshade*	4	300	0	0
Subsection Melongena					
<i>Solanum melongena</i> L.	Eggplant	4	300	0-1	0-2
Subsection Cryptocarpum					
<i>Solanum sisymbriifolium</i> Lam.	Sticky nightshade	2	120	0	0

Each test included 3-4 replications with 20 adults (10 males, 10 females) per replication.

¹0 = no feeding, 1 = probing (<5% of leaf area), 2 = light feeding (5-20%), 3 = moderate feeding (21-40%), 4 = heavy feeding (41-60%), 5 = intense (60% of leaf-area).

50-60 cm height), with a mesh screen at the top and covering 6 circular holes (6 cm diameter) located in pairs at the bottom, middle, and upper part of the cylinder to allow for air circulation.

Adults tested originated from F2- F3 generations reared in quarantine from larvae and adults collected on tropical soda apple plants in southern Brazil. The adults had either recently emerged

TABLE 2. HOST RANGE OF *GRATIANA GRAMINEA* FIRST INSTAR IN NO-CHOICE FEEDING TESTS IN FLORIDA QUARANTINE.

Plant family Species	Common names	No.of Tests	No. of Insects	Feeding Score ¹	Mortality (%)
CONVOLVULACEAE					
<i>Ipomoea batatas</i>	Sweetpotato	1	30	0	100
SOLANACEAE					
<i>Capsicum annuum</i>	Bell pepper	2	70	0	100
<i>Datura discolor</i>	Angels' trumpet	1	30	0	100
<i>Datura stramonium</i>	Jimson weed	1	30	0	100
<i>Lycium carolinianum</i>	Christmas berry	1	30	0	100
<i>Lycopersicon esculentum</i>	Tomato	2	70	0	100
<i>Nierembergia scoparia</i>	Cupflower	1	30	0	100
<i>Nicotiana tabacum</i>	Tobacco	2	60	0	100
<i>Physalis angulata</i>	Cutleaf Ground-Cherry	1	40	0	100
<i>Physalis pubescens</i>	Strawberry tomato	1	30	0	100
<i>Solanum americanum</i>	American nightshade	1	30	0	100
<i>Solanum capsicoides</i>	Red soda apple	2	70	2	100
<i>Solanum carolinense</i>	Horsenettle	1	30	0	100
<i>Solanum citrullifolium</i>	Watermelon nightshade	1	30	0	100
<i>Solanum dimidiatum</i>	Western horsnettle	1	30	0	100
<i>Solanum donianum</i>	Mullein nightshade	2	70	0	100
<i>Solanum elaeagnifolium</i>	Silverleaf nightshade	1	30	0	100
<i>Solanum heterodoxum</i>	Melonleaf nightshade	1	30	0	100
<i>Solanum jamaicense</i>	Jamaican nightshade	1	30	0	100
<i>Solanum jasminoides</i>	White potato vine	1	30	0	100
<i>Solanum melongena</i>	Eggplant	2	70	1	100
<i>Solanum nigrescens</i>	Divine nightshade	1	30	0	100
<i>Solanum ptycanthum</i>	Wonder berry	1	30	0	100
<i>Solanum pumillum</i>	Rock-outcrop Solanum	1	30	0	100
<i>Solanum quitoense</i>	Naranjilla	1	30	0	100
<i>Solanum retroflexum</i>	Sunberry	1	30	0	100
<i>Solanum scabrum</i>	Garden huckleberry	1	30	0	100
<i>Solanum tampicense</i>	Wetland nightshade	2	70	0	100
<i>Solanum torvum</i>	Turkeyberry	2	70	1	100
<i>Solanum tuberosum</i>	Potato	2	70	0	100
<i>Solanum viarum</i>	Tropical soda apple	2	70	5	26

Each test included 3-4 replications with 10 neonate larvae per replication.
¹= no feeding, = probing (<5% of leaf area), 2 = light feeding (5-20%), = moderate feeding (21-40%), 4 = heavy feeding (41-60%), 5 = intense feeding (>60% of leaf area).

from pupae or were still young (1-2 week old) and showing the intense green color that differentiate them from pale-yellow older adults. Eggs laid on plants, if any, were removed weekly and plants replaced as needed. At the end of the testing periods, feeding and adult mortality were recorded.

RESULTS AND DISCUSSION

Multiple-Choice Feeding-Oviposition Tests

In the quarantine multiple-choice tests (Table 1), *Gratiana graminea* adults fed heavily to intensively (41-100% of the leaf area offered) on the major target weed tropical soda apple. It fed lightly to moderately (5-40% of the leaf area offered) on turkeyberry, *Solanum torvum* Sw. (na-

tive to west Africa and on the list of Federal noxious weeds and on Florida's invasive species list of the Florida Exotic Pest Plant Council; webpage: <http://www.fleppc.org/97list.htm>). Minor or exploratory feeding (<5% of the leaf area offered) was observed on the non-native red soda apple, *Solanum capsicoides* All. (prickly weed of South American origin also present and spreading in Florida), on eggplant, *Solanum melongena* L. (crop of economic importance), on the non-native *Solanum mammosum* L. (native from Central America, not growing in USA), on the non-native *Solanum tampicense* Dunal (weed of Mexico, Central America, and Caribbean origin and now established and expanding in south Florida; also on the Florida's invasive species list of the Florida Exotic Pest Plant Council). No feeding was ob-

TABLE 3. HOST RANGE OF *GRATIANA GRAMINEA* ADULTS IN NO-CHOICE FEEDING TESTS IN FLORIDA QUARANTINE.

Plant family Species	Common names	No. of tests	No. of Insects	Feeding Score ¹	Eggs/ female
CONVOLVULACEAE					
<i>Ipomoea batatas</i>	Sweetpotato	1	40	0	0
SOLANACEAE					
<i>Capsicum annuum</i>	Bell pepper	3	110	0	0
<i>Capsicum frutescens</i>	Chile	1	30	0	0
<i>Lycopersicon esculentum</i>	Tomato	3	110	0	0
<i>Nicotiana tabacum</i>	Tobacco	2	70	0	0
<i>Nierembergia scoparia</i>	Cupflower	1	30	0	0
<i>Physalis crassifolia</i>	Ground-cherry	1	30	0	0
<i>Solanum americanum</i>	American nightshade	1	40	0	0
<i>Solanum bahamense</i>	Bahama nightshade	1	30	0	0
<i>Solanum capcicoides</i>	Red soda apple	1	40	0	0
<i>Solanum carolinense</i>	Horse nettle	1	40	0	0
<i>Solanum citrullifolium</i>	Watermelon nightshade	1	30	0	0
<i>Solanum dimidiatum</i>	Western horsenettle	1	40	0	0
<i>Solanum diphillum</i>	Two-leaf nightshade	1	30	0	0
<i>Solanum donianum</i>	Mullein nightshade	2	70	0	0
<i>Solanum elaeagnifolium</i>	Silverleaf nightshade	2	70	1	0
<i>Solanum heterodoxum</i>	Melonleaf nightshade	1	30	0	0
<i>Solanum jamaicense</i>	Jamaican nightshade	1	40	0	0
<i>Solanum jasminoides</i>	White potato vine	1	40	0	0
<i>Solanum mammosum</i>	Nipplefruit	1	30	0-2	0
<i>Solanum melongena</i>	Eggplant				
cv Black Beauty					
cv Classic		4	140	0-1	0
cv Ichiban		2	60	0	0
cv Italian-Nadia		2	60	0	0
cv Market		2	60	0	0
cv Neon		2	60	0-1	0
cv Orient charm		1	60	0	0
cv Orient express		2	60	0	0
cv Thai		2	60	0	0
<i>Solanum nigrescens</i>	Divine nightshade	1	30	0	0
<i>Solanum pumilum</i>	Rock-outcrop	1	30	0	0
<i>Solanum ptycanthum</i>	Wonder berry	1	30	0	0
<i>Solanum retroflexum</i>	Sunberry	1	30	0	0
<i>Solanum scabrum</i>	Garden huckleberry	1	30	0	0
<i>Solanum seaforthianum</i>	Brazilian nightshade	1	30	0	0
<i>Solanum tampicense</i>	Wetland nightshade	2	70	1	0
<i>Solanum torvum</i>	Turkeyberry	2	70	2	0
<i>Solanum tuberosum</i>	Potato	3	80	0	0
<i>Solanum viarum</i>	Tropical soda apple	4	140	5	47-61

Each test included 3-4 replications with 10 adults (5 females, 5 males) per replication.

¹0 = no feeding, 1 = probing (<5% of leaf area), 2 = light feeding (5-20%), 3 = moderate feeding (21-40%), 4 = heavy feeding (41-60%), 5 = intense feeding (>60% of leaf area).

served on any of an additional 109 plant species in 32 families that were tested. The adults laid from 58-104 eggs (average: 86) on tropical soda apple, and from 0-2 (average: 0.1 eggs) on eggplant (Table 1). No eggs were deposited on any of the other 113 plant species tested, including the threatened *S. donianum*. Although some minor feeding on eggplant has occurred in quarantine,

this insect has never been recorded attacking eggplant in South America. Expanded host ranges of weed biocontrol candidates under confined quarantine laboratory conditions have been reported by South African researchers (Neser et al. 1989; Hill & Hulley 1995; Olckers et al. 1995; Hill & Hulley 1996; Olckers 1996). They indicated that almost all the agents that have been tested for

biocontrol of *Solanum* weeds have shown feeding on closely related plant species, but they are never attacked under natural conditions. For example, *Gratiana spadicea* (Klug) (Coleoptera: Chrysomelidae) as a biocontrol agent against *Solanum sisymbriifolium* Lam. in South-Africa (Hill & Hulley 1995), fed and was successfully reared on eggplant in laboratory tests. This insect was field released in South Africa in 1994 based mainly on the lack of records as a pest of eggplant in South America. This insect has become established on *S. sisymbriifolium* with no reports of attacks of eggplant fields in South Africa.

No-Choice Larval Feeding Tests

Larvae of *G. graminea* exposed to individual plants (31 species) in quarantine growth chambers (Table 2) completed development on the target weed tropical soda apple (74% reached the pupae stage, $n = 70$, 2 trials). Some feeding (5-20%) was observed on *Solanum capsicoides* (red soda apple), and also some probing or exploratory feeding (<5% of the leaf area offered) was observed on *Solanum melongena* (eggplant) and on *Solanum torvum* (turkeyberry), but larvae stopped feeding and died within a week after the experiment began. The rapid death of the *G. graminea* larvae with no feeding and no development on the 30 non-target plant species tested clearly indicated no risk of attack on these plants by this beetle.

No-Choice Adult Feeding Tests

Starvation tests (no-choice) with *G. graminea* adults exposed to individual potted plants (29 species) in cages at the quarantine facility (Table 3) indicated that the insect fed and laid eggs (47-61 eggs per female; average: 57 per female) only on tropical soda apple. Feeding on tropical soda apple was intense (>60% of the leaf area offered) compared to a probing or exploratory feeding (<5%) observed in *S. melongena* (eggplant cultivars Black Beauty and Market), on *S. tampicense*, and on *S. elaeagnifolium* (secondary target weeds). Although there was some feeding (5-20% of the leaf area offered) on *S. torvum* (secondary target weed), and on *S. mammosum* (non-native), the females did not lay eggs on these plants. No eggs were laid on any of the 28 non-target plant species tested including the 9 eggplant cultivars (Black Beauty, Classic, Ichiban, Italian-Nadia, Market, Neon, Orient Charm, Orient Express, and Thai).

The high specificity shown by this beetle in the host range feeding tests and development only on the target weed, indicated no adverse impacts would be expected on the 6 solanaceous species that were not tested and are listed as threatened or endangered in Hawaii and Puerto Rico. Indirect beneficial effects on wildlife populations as-

sociated with release and establishment of *G. graminea* may be expected due to recolonization by native plants that have been displaced by the rapidly growing and highly competitive tropical soda apple plants.

The host specificity tests in quarantine indicated that *G. graminea* is safe to release. Occasional temporary feeding might occur on the non-native weeds *S. torvum* and *S. tampicense* (in the Federal Noxious Weed list), and *S. capsicoides*, a prickly weed introduced from South America (Kissman & Groth 1995). Noticeable damage to eggplant is unlikely to occur based on our host tests. The lack of a record as a crop pest in the native range of the beetle support our findings on the specificity and safety of *G. graminea* as a biocontrol agent of tropical soda apple.

Based on the specificity of *G. graminea* feeding and developing only on the target weed, we consider this beetle safe for field release against tropical soda apple. Therefore, a petition to release the Brazilian leaf-beetle *G. graminea* for the control of tropical soda apple in the southeastern United States was submitted to the USDA-APHIS-PPQ Technical Advisory Group (TAG) members on Sep 2008.

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