HOST SPECIFICITY OF *ANTHONOMUS TENEBROSUS* (COLEOPTERA: CURCULIONIDAE), A POTENTIAL BIOLOGICAL CONTROL AGENT OF TROPICAL SODA APPLE (SOLANACEAE) IN FLORIDA

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ABSTRACT

Multiple-choice and no-choice tests were conducted at the Florida Department of Agriculture quarantine facility to determine the host specificity of the South American flower bud weevil, *Anthonomus tenebrosus* Boheman, intended for biological control of the exotic weed tropical soda apple (TSA), *Solanum viarum* Dunal in Florida, USA. Ninety-one plant species in 21 families were included in multiple-choice feeding and oviposition experiments, including the target weed and the 6 major cultivated Solanaceae: bell pepper (*Capsicum annuum* L.), chili pepper (*C. frutescens* L.), tomato (*Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum* L.), eggplant (*Solanum melongena* L.), and potato (*Solanum tuberosum* L.). Plant bouquets with flower-buds of 8 to 10 randomly selected plant species, always including TSA (*S. viarum*) were exposed to 10-20 *A. tenebrosus* adults for 1 to 2 weeks. Oviposition and feeding were observed twice a week. No-choice host-specificity tests were also conducted with *A. tenebrosus* adults using potted flowering plants. Ten adults were exposed to 29 plant species individually tested for 1 to 2 weeks. Plant species in each test were replicated 3 or 4 times. All tests showed that *A. tenebrosus* fed and laid eggs only on the target weed. No eggs were deposited on any of the other of the 91 plant species tested. Host-specificity tests indicated that a host range expansion of *A. tenebrosus* to include any of the crops, and native Solanaceae, and non-solanaceous plants tested is highly unlikely. A petition for field release in the USA was submitted to the Technical Advisory Group for Biological Control Agents of Weeds (TAG) in Oct 2007.

Key Words: host-specificity tests, weed biological control, *Solanum viarum*, Solanaceae

RESUMEN

Pruebas de ovoposición y alimentación (con y sin elección), se realizaron para evaluar la especificidad del picudo del botón floral, de origen suramericano, *Anthonomus tenebrosus* Boheman, como agente potencial para control biológico de bola de gato, *Solanum viarum* Dunal en los Estados Unidos. Las pruebas se efectuaron en la cuarentena del Departamento de Agricultura de la Florida. Noventa y una especies de plantas, en 21 familias, fueron incluidas en las pruebas de especificidad de múltiples elección, incluyendo la maleza objetivo y las seis plantas cultivadas pertenecientes a la familia Solanaceae más importantes: chile dulce (*Capsicum annuum* L.), chile (*Capsicum frutescens* L.), tomate (*Lycopersicon esculentum* Mill.), tabaco (*Nicotiana tabacum* L.), berenjena (*Solanum melongena* L.), y papa (*Solanum tuberosum* L.). En cada prueba se utilizaron racimos florales de ocho a diez plantas escogidas al azar incluyendo siempre la planta objetivo las cuales fueron expuestas a 10-20 adultos de *A. tenebrosus* por una a dos semanas. Registros de alimentación y ovoposición fueron realizados dos veces por semana. Pruebas de alimentación/ovoposición sin elección fueron también realizadas usando plantas en floración. Diez adultos fueron expuestos a 29 especies de plantas en forma individual por una a dos semanas. Cada prueba tuvo tres o cuatro repeticiones. Las pruebas mostraron que *A. tenebrosus* se alimentó y colocó posturas solo en bola de gato. Ninguna postura fué depositada en las otras 90 especies de plantas evaluadas. Las pruebas indicaron que la posibilidad de *A. tenebrosus* de llegar a ser una plaga de las Solanaceae cultivadas es muy remota. La solicitud al comité TAG para liberar el picudo en los Estados Unidos fue presentada en octubre 2007.

Tropical soda apple (TSA), *Solanum viarum* Dunal (Solanaceae), is an invasive weed native to southeastern Brazil, northeastern Argentina, Paraguay, and Uruguay that has invaded Florida grasslands and natural ecosystems. In 1988, TSA was first reported in the USA in Glades County, Florida (Coile 1993; Mullahey & Colvin 1993); the introduction pathway is unknown. In 1993, a survey of beef cattle operations in south Florida estimated 157,145 ha of infested pasture land, twice the infestation present in 1992 (Mullahey et al. 1994). The infested area increased to more than

303,000 ha in 1995-96 (Mullahey et al. 1998). Currently, more than 404,000 ha are believed to be infested in Florida (Medal et al. 2010b). Due, at least in part, to favorable environmental conditions, the lack of natural enemies (herbivores and pathogens), and seed dispersal by wildlife and cattle feeding on the fruits. TSA has been spreading rapidly and has been observed in the majority of the counties in Florida and also in Alabama, Georgia, Louisiana, Mississippi, North Carolina, Pennsylvania, South Carolina, Tennessee, Texas, and Puerto Rico (Bryson & Byrd Jr. 1996; Dowler 1996; Mullahey et al. 1993, 1998; Medal et al. 2003, 2010a). Although TSA has been reported in Pennsylvania and Tennessee, it is highly probable that does not overwinter in these states. Patterson (1996) studied the effects of temperatures and photoperiods on TSA in controlled environmental chambers and speculated that the range of TSA could expand northward into the midwestern US. *S. viarum* was placed on the Florida and Federal Noxious Weed Lists in 1995.

TSA typically invades improved pastures, where it reduces livestock carrying capacity. Foliage and stems are unpalatable to cattle; dense stands of the prickly shrub prevent access of cattle to shaded areas, which results in summer heat stress (Mullahey et al. 1998). TSA control costs for Florida ranchers were estimated at $6.5 to 16 million annually (Thomas 2007), and economic losses from cattle heat stress alone were estimated at $2 million (Mullahey et al. 1998). TSA is a reservoir for at least 6 crop viruses (potato leafroll virus, potato virus Y, tomato mosaic virus, tomato mottle virus, tobacco etch virus, and cucumber mosaic virus) and the early blight of potato and tomato fungus, *Alternaria solani* Sorauer (McGovern et al. 1994a, 1994b; McGovern et al. 1996). In addition, major insect pests utilize TSA as an alternate host; including Colorado potato beetle, *Leptinotarsa decemlineata (Say);* tomato hornworm *Manduca quinquemaculata* (Haworth); tobacco hornworm, *M. sexta* (L.); tobacco budworm, *Helicoverpa virescens* (Fabricius); tomato pinworm, *Keiferia lycopersicella* (Walsingham); green peach aphid, *Myzuz persicae* (Sulzer); silverleaf whitefly biotype B of *Bemisia tabaci* (Gennadius); soybean looper, *Pseudoplusia includens* (Walker); and the southern green stink bug, *Nezara viridula* (L.) (Habeck et al. 1996; Medal et al. 1999; Sudbrink et al. 2000). TSA also reduces biodiversity in natural areas, ditch banks, and roadsides by displacing native vegetation (Langeland & Burks 1998). TSA interferes with restoration efforts in Florida by invading areas that are reclaimed following phosphate mining operations (Albin 1994).

TSA Management practices in Florida pastures primarily involve herbicide applications and mowing (Sturgis & Colvin 1996; Mislevy et al. 1996, 1997; Akanda et al. 1997). Herbicides or mowing provide temporary weed suppression at an estimated cost of $61 and $47 per ha, respectively (Thomas 2007). However, application of these control methods is not always feasible in rough terrain or inaccessible areas.

In June 1994, the first exploration for TSA natural enemies in South America was conducted by University of Florida and Brazilian researchers (Medal et al. 1996). Sixteen species of insects were found attacking the weed during this 2-week survey. Host specificity tests were initiated in 1997 by J. Medal (University of Florida) in collaboration with the Universidade Estadual Paulista, Jaboticabal campus, Brazil, and the USDA Biological Control Laboratory in Hurlingham, Buenos Aires province, Argentina, and in Stoneville, MS. The South American leaf-feeder *Gratiana boliviana* (Chrysomelidae) was approved for field release in Florida in summer 2003. In total, at least 230,000 beetles have been released in 39 Florida counties since the summer 2003. The beetles established at almost all the release sites in central/south Florida and they are having extensive defoliations and reducing the weed fruit production on TSA plants (Medal & Cuda 2010; Medal et al. 2010a; Overholt et al. 2009, 2010).

A second potential TSA biocontrol agent is the flower-bud weevil *Anthonomus tenebrosus* Boheman (Coleoptera: Curculionidae). This insect was collected on TSA in Rio Grande do Sul, Brazil (29.66465°S, 50.80171°W) by the late Daniel Gandolfo and Julio Medal in April 2000. The identity of *A. tenebrosus* was confirmed by Drs. Wayne Clark (Auburn University, AL) and Germano Rosado Neto (Universidade Federal do Paraná in Curitiba, Brazil). Voucher specimens of *A. tenebrosus* are deposited at Auburn University, Alabama, at the Universidade Federal do Paraná - Curitiba campus, Brazil, and at the Florida State Collection of Arthropods, Division of Plant Industry in Gainesville, Florida. This species does not have a common name in South America. The only known *A. tenebrosus* host plants in South America are *S. viarum* and *S. acculeatisimum*.

The biology of *A. tenebrosus* was studied by Davis (2007) at the quarantine facility in Gainesville, Florida. Eggs are inserted individually into TSA flower-buds, and hatch in 3-5 days. Larvae are cream-colored with a yellowish brown head capsule. They feed on the contents of the flower-bud, and this feeding prevents the flower-bud from opening. There is typically 1 larva, but occasionally 2 larvae in a single flower-bud. As larval feeding progresses, the flower-bud senesces and drop from the plant. Three larval stadia are completed in 7-13 days. The pupal stage is completed in 3-7 days inside the fallen flower bud. Pupae resemble the adult in form; they are cream-colored but darken shortly before eclosion. Emerging adults chew their way out of the flower-bud. Development from egg to adult stage lasts 11-69 days. Longer developmental times are apparently not associated with seasonal differences as they occurred throughout the year. Adults can live up to 210 days under laboratory conditions. Adult size appears to be related to food abundance during development rather than beetle sex. Copulation has been observed a few hours after adult emergence and throughout the oviposition period. At least 7-8 generations per year can occur under laboratory conditions (temperature 24° ± 3C, relative humidity 50-70%) conditions.

Materials and Methods

Host Specificity Tests

Laboratory host specificity tests with *A. tenebrosus* adults were conducted from May 2000 to January 2003 at the Florida Department of Agriculture and Consumer Services-Division of Plant Industry quarantine facility in Gainesville, Florida. Open field host-specificity tests were conducted at the Universidade Federal do Paraná Agricultural Experiment Station in Paraná state, Brazil from Oct 2005 to Mar 2007. For Florida tests, *A. tenebrosus* adults were collected from TSA plants in Rio Grande do Sul, Brazil and introduced onto caged plants of TSA plants growing in 1-gallon pots to establish a laboratory colony in quarantine.

In this article we report the results of various host-specificity tests with the flower-bud weevil *A. tenebrosus,* to assess its possible use as biological control agent of the non-native weed tropical soda apple.

Multiple-Choice Feeding and Oviposition Tests

Ninety-one plant species in 21 families were included in the feeding oviposition preference tests at the Gainesville quarantine (Table 1). Tested plants included 53 species in the family of the target weed (Solanaceae), 26 of which were from the genus *Solanum* and 27 from 14 other genera that include plants of agricultural or ecological importance. Ten species represented 5 families (Boraginaceae, Convolvulaceae, Ehretiaceae, Nolanaceae, Polemoniaceae) very close related to Solanaceae within the order Polemoniales (Heywood 1993) were also included. Twenty-eight plant species representing 15 families, most of them with economic and/or environment value in North America, were also tested. The target weed (*S. viarum*), and other 9 plant species in Solanaceae were tested at least 3 times (Table 1). These included the natives *Solanum donianum* Walpers, listed as a threatened plant in Florida (Coile 1998), and *S. americanum* Mill, 2 non native-weeds (*S. tampicense* Dunal, *S. torvum* Sw.), and the 5 major cultivated *Solanaceae* (bell pepper, *Capsicum annuum* L., tomato, *Lycopersicon esculentum* Mill., tobacco, *Nicotiana tabacum* L., eggplant, *Solanum melongena* L., and potato, *Solanum tuberosum* L.). Bouquets of leaves and flower-buds of 8 to 10 plant species, always including TSA were simultaneously exposed to 10-20 *A. tenebrosus* adults (approximately 50% males and 50% females) in clear plastic round containers (26 cm diameter by 9 cm height, with four 4-7 cm diameter vents drilled along the sides of the container to allow for air circulation). At the beginning of each test, the insects were placed at the bottom center of each container to allow them to choose any tested plants. Plant species in each test were replicated 3-4 times (1 replication of tested plants in each separate container). Bouquets were exposed to *A. tenebrosus* adults for 1 to 2 weeks. Observations of oviposition and feeding were made twice a week, and consumed bouquets were replaced as needed. Flower-buds were checked for oviposition and eggs were counted weekly. On the last day of each experiment, flower-buds were scored for feeding damage, and eggs laid on them were counted. Leaf and flower bud area consumed was visually estimated using a scale from 0 to 5 (0 = no feeding, 1 = probing or <5% of 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 area consumed).

No-Choice Adult Feeding and Oviposition Tests

No-choice host specificity tests were also conducted with *A. tenebrosus* adults at the Gainesville-quarantine facility using potted plants (20-60 cm height) in cages. Cages were made of clear-plastic cylinders (15 cm diam, 50-60 cm height), with a mesh screen at the top and covering 6 circular holes (6 cm diam) located in pairs at the bottom, middle, and upper part of the cylinder to allow for air circulation. *A. tenebrosus* adults were exposed to 29 plant species in 3 families, including the native *S. donianum*, and all major cultivated Solanaceae (Table 2). Five to 7 plant species with flower-buds were individually tested each time due to limited cage numbers. Plants were exposed to 10 *A. tenebrosus* adults (5 males, 5 females) for 1 to 2 weeks; each test plant was replicated 3 or 4 times. Adults were F_2 or F_3 progeny from adults originally collected in southern Brazil and reared in quarantine on TSA. Adults had either recently eclosed from pupae or were still young less than 1 week old. Plants were replaced as needed. At the end of the testing periods, feeding and oviposition were recorded.

First Field Experiment in Brazil

A multiple-choice, open field experiment was conducted at the Universidade Federal do Paraná, Agriculture Experimental Farm 'Canguiri'. *A.*

TABLE 1. *ANTHONOMUS TENEBROSUS* ADULT FEEDING AND OVIPOSITION ON SELECTED PLANTS IN QUARANTINE MULTIPLE-CHOICE TESTS.

Plant Family Species	Common Names *indicates native species	No. of Plants	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 *Solanum*					
Subgenus *Leptostemonum*					
Section Acantophora					
Solanum viarum Dunal	Tropical soda apple	40	430	3-5	5-9
Category 2. Species in the same genus as the target weed, divided by subgenera (if applicable).					
Tribe Solaneae					
Genus *Solanum*					
Subgenus *Leptostemonum*					
Section Acantophora					
Solanum capsicoides All.	Red soda apple	12	160	0	0
Section Lasiocarpum					
Solanum quitoense Lam.	Naranjilla	4	40	0	0
Solanum pseudolulo Heise	Falso lulo	4	40	0	0
Solanum sessiliflorum Dunal	Cocona nightshade	4	40	0	0
Section Micracantha					
Solanum jamaicense Mill.	Jamaican nightshade	3	30	0	0
Solanum tampicense Dunal	Wetland nightshade	10	110	1	0
Section Melongena					
Subsection Lathyrocarpum					
Solanum carolinense L.	Horse nettle*	4	40	0	0
Solanum dimidiatum Raf.	Western horsenettle*	3	30	0	0
Solanum elaeagnifolium Cav.	Silverleaf nightshade*	4	40	0	0
Subsection Melongena					
Solanum melongena L.	Eggplant				
Cv. 'Black Beauty'		7	70	0	0
Cv. 'Classic'		4	40	0	0
Cv. 'Market'		7	70	0	0
Cv. 'Asian Long Purple'		3	35	0-1	0
Section Persicariae					

*0 = No feeding, 1 = Probing (<5% of flower bud/leaf area), 2 = Light (5-20%), 3 = Moderate (21-40%), 4 = Heavy (41-60%), 5 = Intense (>60% area). Solanaceous taxonomic categories were taken from the Radboud University of Nijmegen, Netherland website (www.bagard.sci.kun.nl). Most of the plant common names are from: http:/www.plants.usda.gov.

TABLE 1. (*CONTINUED*) *ANTHONOMUS TENEBROSUS* ADULT FEEDING AND OVIPOSITION ON SELECTED PLANTS IN QUARANTINE MULTIPLE-CHOICE TESTS.

Plant Family Species	Common Names *indicates native species	No. of Plants	No. of Insects	Feeding Score[1]	Eggs Laid per Female
Subgenus Leptostemonum					
Solanum bahamense	Bahama nightshade	7	75	0	0
Section Torva					
Solanum torvum Sw.	Turkey berry	12	140	0	0
Solanum verbascifolium L.	Mullein nightshade*	3	30	0	0
Subgenus *Solanum*					
Solanum americanum Mill.	American nightshade*	10	100	0	0
Solanum diphyllum L.	2-leaf nightshade*	3	30	0	0
Solanum erianthum Don.	Potato tree*	3	30	0	0
Solanum jasminoides Paxt.	White potato vine	7	75	1	0
Solanum mauritianum Scop.	Earleaf nightshade	4	40	0	0
Solanum nigrescesns Mart. & Gal	Divine nightshade*	3	30	0	0
Solanum nigrum L.	Black nightshade*	4	50	0	0
Solanum pumillum Dunal	Rock outcrop Solanum*	3	30	0	0
Solanum seaforthianum Scop.	Brazilian nightshade	3	30	0	0
Solanum tuberosum	L. Potato	8	95	0	0
Category 3. Species in other genera in the same family as the target weed, divided by subfamily (if applicable).					
Genus *Acnistus*					
Acnistus australe (Griseb.) Griseb.	Acnistus	3	30	0	0
Genus *Iochroma*					
Iochroma sp.	Iochroma	3	30	0	0
Genus *Physalis*					
Physalis angulata L.	Cutleaf groundcherry	3	30	0	0
Physalis arenicola Kearney	Cypresshead*	3	30	0	0
Physalis crassifolia Benth	Yellow groundcherry*	3	30	0	0
Physalis gigantea L.	Strawberry groundcherry	3	30	0	0
Physalis ixocarpa Brot.	Tomatillo	3	30	0	0
Physalis pubescens L	Husk tomato*	3	30	0	0
Physalis walteri Nutt.	Walter's groundcherry*	3	30	0	0
Tribe Daturae					
Genus *Brugmansia*					
Brugmansia sanguinea (Ruiz & Pav.) Don	Angel's trumpet	3	30	0	0
Genus *Datura*					

*0 = No feeding, 1 = Probing (<5% of flower bud/leaf area), 2 = Light (5-20%), 3 = Moderate (21-40%), 4 = Heavy (41-60%), 5 = Intense (>60% area). Solanaceous taxonomic categories were taken from the Radboud University of Nijmegen, Netherland website (www.bagard.sci.kun.nl). Most of the plant common names are from: http:/www.plants.usda.gov.

TABLE 1. (*CONTINUED*) *ANTHONOMUS TENEBROSUS* ADULT FEEDING AND OVIPOSITION ON SELECTED PLANTS IN QUARANTINE MULTIPLE-CHOICE TESTS.

Plant Family Species	Common Names *indicates native species	No. of Plants	No. of Insects	Feeding Score[1]	Eggs Laid per Female
Datura discolor Bernh	Desert thorn-apple*	3	30	0	0
Datura metel L.	Devil's trumpet	3	30	0	0
Datura meteloides D.	Devil's weed*	3	30	0	0
Datura stramonium L.	Jimson weed*	3	30	0	0
Tribe Lycieae					
Genus *Lycium*					
Lycium carolinianum Walter	Carolina desert-thorn*	3	30	0	0
Lycium fremontii Gray.	Fremont desert thorn*	3	30	0	0
Genus *Lycopersicon*					
Lycopersicon esculentum Mill.	Tomato	12	130	0	0
Tribe: Nicandreae					
Genus: *Nicandra*					
Nicandra physaloides (L.) Gaertn.	Apple of Peru	3	30	0	0
Tribe Nicotianae					
Genus *Nicotiana*					
Nicotiana tabacum L.	Tobacco	8	100	0	0
Nicotiana rustica L.	Aztec tobacco	7	75	0	0
Nicotiana sylvestris Speg. & Comes	Woodland tobacco	3	30	0	0
Genus *Nierembergia*					
Nierembergia scoparia Sendtri	Broom cupflower	3	30	0	0
Tribe Salpiglossidae					
Genus *Salpiglossis*					
Salpiglossis sinuata Ruiz & Pav	Painted tongue	3	30	0	0
Genus *Schizanthus*					
Schizanthus spp.	Butterfly flower	3	30	0	0
Tribe Solandeae					
Genus *Solandra*					
Solandra glandiflora Swartz	Showy chalicevine	3	30	0	0
Category 4. Threatened and endangered species in the same family as the target weed divided by subgenus, genus, and subfamily.					
Section Torva					
Solanum donianum Walpers	Mullein nightshade*	9	90	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					

*0 = No feeding, 1 = Probing (<5% of flower bud/leaf area), 2 = Light (5-20%), 3 = Moderate (21-40%), 4 = Heavy (41-60%), 5 = Intense (>60% area). Solanaceous taxonomic categories were taken from the Radboud University of Nijmegen, Netherland website (www.bagard.sci.kun.nl). Most of the plant common names are from: http:/www.plants.usda.gov.

TABLE 1. (*CONTINUED*) *ANTHONOMUS TENEBROSUS* ADULT FEEDING AND OVIPOSITION ON SELECTED PLANTS IN QUARANTINE MULTIPLE-CHOICE TESTS.

Plant Family Species	Common Names *indicates native species	No. of Plants	No. of Insects	Feeding Score[1]	Eggs Laid per Female
Heliotrope sp.	Heliotrope	3	30	0	0
Myosotis alpestris Schmidt	Forget-Me-Not*	3	30	0	0
Convolvulus purpurea L.	Morning-glory*	3	30	0	0
Ipomoea batata (L.) Lam.	Sweet-potato	3	30	0	0
Evolvulus muttallianus	Shaggy dwarf morning-glory*	3	30	0	0
EHRETIACEAE					
Cordia sebestena L.	Largeleaf geigertree*	3	30	0	0
NOLANACEAE					
Nolana paradoxa Lindl.	Chilean bellflower	3	30	0	0
POLEMONIACEAE					
Cobaea scandens Cav.	Catedral bells	3	30	0	0
Gilia tricolor Benth	Bird's-eye gilia*	3	30	0	0
Phlox panuculata L.	Fall phlox*	3	30	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.					
ANACARDIACEAE					
Mangifera indica L.	Mango	3	30	0	0
Pistacia vera L.	Cultivated pistachio	3	30	0	0
APIACEAE					
Daucus carota L.	Carrot	3	30	0	0
ASTERACEAE					
Helianthus annuus L.	Common sunflower*	3	30	0	0
Lactuca sativa L.	Lettuce	3	30	0	0
CAMPANULACEAE					
Campanula persicifolia L	Peachleaf bellflower*	3	30	0	0
CRUCIFERAE					
Brassica oleracea L. var. botrytis	Broccoli	3	30	0	0
CUCURBITACEAE					
Citrullus lanatus (Thumb)	Watermelon	3	30	0	0
Cucurbita sativus L.	Cucumber*	3	30	0	0
ERICACEAE					
Vaccinium ashei Rende	Rabbiteye blueberry*	3	30	0	0
FABACEAE					
Glycine max (L.) Merrill	Soybean	3	30	0	0

*0 = No feeding, 1 = Probing (<5% of flower bud/leaf area), 2 = Light (5-20%), 3 = Moderate (21-40%), 4 = Heavy (41-60%), 5 = Intense (>60% area). Solanaceous taxonomic categories were taken from the Radboud University of Nijmegen, Netherland website (www.bagard.sci.kun.nl). Most of the plant common names are from: http:/www.plants.usda.gov.

TABLE 1. *(CONTINUED) ANTHONOMUS TENEBROSUS* ADULT FEEDING AND OVIPOSITION ON SELECTED PLANTS IN QUARANTINE MULTIPLE-CHOICE TESTS.

Plant Family Species	Common Names *indicates native species	No. of Plants	No. of Insects	Feeding Score[1]	Eggs Laid per Female
Phaseolus vulgaris L.	Kidney bean	3	30	0	0
LOBELIACEAE					
Lobelia cardinalis L.	Cardinalflower*	3	30	0	0
LOGANIACEAE					
Buddleia davidii Franch	Butterfly bush	3	30	0	0
POACEAE					
Oryza sativa L.	Rice	3	30	0	0
Saccharum officinarum L.	Sugarcane	3	30	0	0
Zea mays L.	Corn*	3	30	0	0
ROSACEAE					
Fragariax ananassa Duchesne	Garden strawberry	3	30	0	0
Malus pumilla Mill.	Paradise apple*	3	30	0	0
Rosa sp.	Miniature rose	3	30	0	0
Rubus betulifolius Small	Blackberry*	3	30	0	0
RUTACEAE					
Citrus sinensis (L.) Osbeck	Sweet orange	3	30	0	0
Citrus limon (L.) Burm.	Lemon	3	30	0	0
Citrus paradise Mcfady	Grapefruit	3	30	0	0
Murraya paniculata (L.) Jacq.	Orange Jasmine	6	60	0	0
SCROPHULARIACEAE					
Antirrhinum majus L.	Garden snapdragon	3	30	0	0
Nemensia strumosa Benth	Capejewels	3	30	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.					
MALVACEAE					
Gossypium hirsutum L.	Cotton	10	100	0	0
SOLANACEAE					
Genus *Capsicum*					
Capsicum annuum L.	Bell pepper	8	80	0	0
Capsicum frutescens L.	Chili pepper	4	40	0	0
Genus Solanum					
Solanum sisymbriifolium Lam.	Sticky nightshade	3	30	1	0

*0 = No feeding, 1 = Probing (<5% of flower bud/leaf area), 2 = Light (5-20%), 3 = Moderate (21-40%), 4 = Heavy (41-60%), 5 = Intense (>60% area). Solanaceous taxonomic categories were taken from the Radboud University of Nijmegen, Netherland website (www.bagard.sci.kun.nl). Most of the plant common names are from: http:/www.plants.usda.gov.

TABLE 2. ANTHONOMUS TENEBROSUS ADULT FEEDING AND OVIPOSITION ON SELECTED PLANTS IN QUARANTINE NO-CHOICE TESTS.

Plant family Species	Common names (*indicates native Species)	No. of Plants	No. of Insects	Feeding Score*	Eggs/female
SOLANACEAE					
Capsicum annuum	Bell pepper	9	90	0	0
Capsicum frutescens	Chili pepper	7	70	0	0
Lycopersicon esculentum	Tomato	9	90	0	0
Nicotiana tabacum	Tobacco	7	70	0	0
Nierembergia scoparia	Broom cupflower	3	30	0	0
Physalis crassifolia	Yellow groundcherry*	3	30	0	0
Solanum americanum	American nightshade*	3	30	0	0
Solanum capsicoides	Red soda apple	3	30	0	0
Solanum carolinense	Horse nettle*	3	30	0	0
Solanum citrullifolium	Watermelon nightshade*	3	30	0	0
Solanum dimidiatum	Western horsenettle*	3	30	0	0
Solanum diphillum	2-leaf nightshade	3	30	0	0
Solanum donianum	Mullein nightshade	7	70	0	0
Solanum elaeagnifolium	Silverleaf nightshade*	3	30	1	0
Solanum heterodoxum	Melonleaf nightshade*	3	30	0	0
Solanum jamaicense	Jamaican nightshade	3	30	0	0
Solanum jasminoides	White potato vine*	3	30	0	0
Solanum melongena	Eggplant				
cv. Black Beauty		3	30	0	0
cv. Classic		3	30	0	0
cv. Market		3	30	0	0
cv. Asian Long Purple		9	90	0	0
Solanum nigrescens	Divine nightshade*	3	30	0	0
Solanum pumilum	Rock-outcrop Solanum*	3	30	0	0
Solanum ptycanthum	Wonder berry*	3	30	0	0
Solanum retroflexum	Sunberry*	3	30	0	0
Solanum scabrum	Garden huckleberry*	3	30	0	0
Solanum tampicense	Wetland nightshade	7	70	1	0
Solanum torvum	Turkeyberry	7	70	1	0
Solanum tuberosum	Potato	9	90	0	0
Solanum viarum	Tropical soda apple	9	90	3-5	4-11
MALVACEAE					
Gossypium hirsutum L.	Cotton	9	90	0	0
CONVOLVULACEAE					
Ipomoea batata (L.) Lam.	Sweet-potato	9	90	0	0

*0=No feeding, 1 = Probing (<5% of flower bud/leaf area), 2 = Light (5-20%), 3 = Moderate (21-40%), 4 = Heavy (41-60%), 5 = Intense (>60% area). Most of the plant common names are from: http:/www.plants.usda.gov.

tenebrosus adults were collected in Rio Grande do Sul state in Dec 2005. These insects were reared in screened cages (0.6 × 0.6 × 0.9 m) at the Neotropical Biological Control Laboratory in Curitiba on TSA plants growing in 1-2 gallon pots to provide progeny weevils for the experiment. One hundred *A. tenebrosus* adults recently emerged from pupae were released in the field (30 × 20m^2) with 5 plant species (TSA, eggplant cv. 'Black Beauty', bell-pepper, potato, and tomato). Seven plants of each species tested (35 plants/plot, 1m between plants, 35m^2/plot, 4 plots, 10m between plots) were randomly assigned in each of the experimental plots following a Complete Block Randomized Experimental Design with 4 replications. Test plants (n = 140) were transplanted in Oct 2005, and insects were released when plants were flowering during the last week of Dec 2005 on the ground approximately 1m from any plant. All plants were thoroughly examined weekly from 22 Dec 2005 to 31 Mar 2006, and number of adults, feeding, and number of egg on the plants were recorded.

Second Field Experiment in Brazil

Another multiple-choice, open field experiment exposing *A. tenebrosus* adults to flowering eggplant cv.' Black Beauty', tomato, potato, and bell pepper, but not TSA, was conducted at the Universidade Federal do Paraná, Agriculture Experimental Farm 'Canguiri', Brazil from Dec 2006 to Mar 2007. Control plots with flowering TSA plants alone, were also established at the Neotropical Biological Control Laboratory in Curitiba located approximately 45 km from the cultivated crop plots to prevent plant species interference. Distance between plants was similar to the first experiment. Field collected *A. tenebrosus* weevils were released into crop and TSA plots (80 and 76 adults, respectively). Beetles were randomly released in groups (6-10) on the ground but not on any test plant. Evaluations (visual estimation of number of insects and feeding) were made weekly checking thoroughly each of the plants tested.

Results and Discussion

Multiple-Choice Feeding-Oviposition Tests

In the quarantine multiple-choice tests, *A. tenebrosus* adults fed moderately to intensively (>20% of the area offered) on *S. viarum,* the target weed (Table 1). Weevils did some probing or exploratory feeding (<5% of the area offered) on *S. tampicense* Dunal (an exotic weed of Mexico-Central America-Caribbean origin and established and expanding in central-south Florida), on *S. sisymbriifolium* Lam., and *S. jasminoides* Paxt. (weeds of South-American origin also present in Florida), and on eggplant cv. 'Asian Long Purple' (crop of economic importance). No feeding was observed on any of the other 86 plant species in 21 families that were tested. *A. tenebrosus* adults lay from 5 to 9 eggs inside TSA flower-buds during the 1-2 week period of the test (Table 1). No eggs were deposited on any of the other 90 plant species tested, including the threatened *S. donianum*. Although minor *A. tenebrosus* feeding occurred on eggplant in quarantine, this insect has never been recorded attacking eggplant in South America. Expanded host ranges of weed-biocontrol insects under confined laboratory conditions have been reported by South African researchers (Neser et al. 1988; Hill & Hulley 1995; Olckers et al. 1995; Hill & Hulley 1996; Olckers 1996). They indicated that almost all agents tested for biocontrol of *Solanum* weeds have fed on closely related plant species that are never attacked under natural conditions. For example, *Gratiana spadicea* (Klug) (Coleoptera: Chrysomelidae) screened as a potential biocontrol agent of *S. sisymbrifolium* in South-Africa (Hill & Hulley 1995), fed and completed development on eggplant in the laboratory. In 1994, this insect was released in South-Africa based mainly on the lack of records as an eggplant pest in South America. *Gratiana spadicea* is established on *S. sisymbriifolium* with no reports of attacks in South African eggplant fields. Multiple choice tests conducted at the USDA-South American Biological Control Laboratory in Hurlingham, Argentina with *Anthonomus sisymbrii* Hustache by late Daniel Gandolfo, showed this weevil fed and lay eggs on eggplant and potato, although the number of eggs lay on these crops was significantly lower than on TSA. He also reported that 75% of the eggs on potato and 25% on eggplant were abnormally oviposited outside the flower-buds. Gandolfo indicated that *A. sisymbrii* could use eggplant, and possibly other *Solanum*, at least for feeding purposes that may result in an economic impact (Gandolfo et al. 2004). The only known natural hosts of *A. sisymbrii* are *S. sisymbrifolium*, *S. viarum*, and *S. aculeatissimum* (Medal unpublished data). To corroborate the specificity and safety of *A. tenebrosus*, a weevil related to *A. sisymbrii,* 2 open field experiments (discussed later) were conducted in Brazil, which indicated that *A. tenebrosus* did not represent a threat to eggplant and other economic crops tested under natural conditions and it is safe as a biological control agent of TSA.

No-Choice Adult Feeding Tests

Starvation (no-choice) tests with *A. tenebrosus* adults exposed to individual potted plants (29 species in 3 families) in quarantine cages indicated that this insect fed and laid eggs (range: 4-11; average, 8 eggs per female) only on TSA (Table 2). Feeding on TSA was moderate to intense (>21% of the area offered) compared to a probing or exploratory feeding (<5%) observed on *S. elaeagnifolium*, *S. tampicense*, and on *S. torvum*. No eggs were laid on any of the 28 non-target plant species tested including 4 eggplant cultivars ('Black Beauty', 'Classic', 'Market', 'Asian Long Purple').

First Field Experiments in Brazil

In the open-field planted with TSA, bell-pepper, tomato, potato, and eggplant, *A. tenebrosus* adults (100) fed and laid eggs, and larvae developed only on TSA. A total of 83 eggs, 21 larvae, and 51 adults of *A. tenebrosus* were recorded on TSA plants. Feeding on TSA flower-buds was moderate to heavy (21 to 50% of the area). No feeding was observed on any of the Solanaceous crops tested. This field test confirms that *A. tenebrosus* feeds and develops only on TSA and does not represent a threat to eggplant, tomato, potato, or bell-pepper.

Second Field Experiment in Brazil

The field test exposing *A. tenebrosus* adults to eggplant, tomato, potato, and bell-pepper, showed that no adults or immature stages were found on these crops tested when TSA plants were not present. In a separate plot, *A. tenebrosus* feeding on TSA flower buds was moderate (21-30%), contrary to no-feeding on the crops tested. A total of 124 eggs, 72 larvae, and 45 adults of *A. tenebrosus* were recorded on TSA plants. This test showed that *A. tenebrosus* adults fed and laid eggs, and larvae developed only on TSA, with no utilization of eggplant, potato, tomato, and bell pepper when TSA is not present.

The laboratory and open-field experiments indicated that no *A. tenebrosus* feeding damage and reproduction on the native solanaceous plants and crops tested are likely to occur. It is expected that this weevil will complement the TSA damage by *G. boliviana* in south and central Florida.

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