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Source: Florida Entomologist, 92(3) : 458-469

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.092.0307

HOST SPECIFICITY OF *ANTHONOMUS ELUTUS* (COLEOPTERA: CURCULIONIDAE), A POTENTIAL BIOLOGICAL CONTROL AGENT OF WETLAND NIGHTSHADE (SOLANACEAE) IN FLORIDA

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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 to determine the specificity of the Mexican/Central-American flower-bud weevil *Anthonomus elutus* Clark, a candidate for biological control of *Solanum tampicense* Dunal (wetland-nightshade) in Florida. Eighty-seven plant species in 17 families were included in the feeding-oviposition multiple-choice tests including the target weed and the 6 major cultivated Solanaceae *Capsicum annuum* L. *Capsicum frutescens* L., *Lycopersicon esculentum* Mill., *Nicotiana tabacum* L., *Solanum melongena* L., and *Solanum tuberosum* L. Plant bouquets with flower-buds of 8 to 10 plant species randomly selected, including always *S. tampicense,* were simultaneously exposed to 20-26 *A. elutus* adults during approximately 2 weeks. Observation of oviposition and feeding were made twice a week. No-choice host-specificity tests were conducted with *A. elutus* adults on potted plants in cages made of clear-plastic cylinders. Ten *A. elutus* adults were exposed to 30 plant species individually tested during 2 weeks. Plant species in each test were replicated 3-4 times. Results indicated that *A. tenebrosus* fed and laid eggs only on the target weed. No eggs were deposited on any of the other 86 plant species tested. The host-specificity tests indicated that a host range expansion of *A.elutus* to include 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 Dec 2008.

Key Words: host-specificity tests, weed biological control, wetland-nightshade, Solanaceae

RESUMEN

Pruebas de ovoposición y alimentación (con y sin elección), se realizaron para evaluar la especificidad del picudo del botón floral, de origen Mexicano-Centroamericano, *Anthonomus elutus* Clark, como agente potencial para el control biológico de *Solanum tampicense* Dunal en Florida, USA. Las pruebas se efectuaron en la cuarentena del Departamento de Agricultura de la Florida-División de Industria de Plantas en Gainesville. Ochenta y siete especies de plantas, en 17 familias, fueron incluidas en las pruebas de especificidad de elección multiple, incluyendo la maleza objetivo y las seis plantas cultivadas pertenecientes a la familia Solanaceae más importantes: *Capsicum annuum* L. *Capsicum frutescens* L., *Lycopersicon esculentum* Mill., *Nicotiana tabacum* L., *Solanum melongena* L., y *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 simultáneamente a 20-26 adultos de *A. elutus* durante aproximadamente dos semanas. Registros de alimentación y ovoposición fueron realizados dos veces por 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 *A. elutus* fueron expuestos a 30 especies de plantas en forma individual durante dos semanas. Cada prueba tuvo 3-4 repeticiones. Los resultados indicaron que *A. elutus* se alimentó y colocó posturas únicamente en la maleza objetivo wetland-nightshade. Ninguna postura fué depositada en las otras 86 especies de plantas evaluadas. Las pruebas de especificidad indicaron que la posibilidad de *A. elutus* de llegar a ser una plaga de las Solanaceae cultivadas es muy remota. La solicitud a TAG para liberar el picudo en Florida fue hecha en Diciembre 2008.

Translation by the authors.

Wetland-nightshade (also known as aquatic soda apple), *Solanum tampicence* Dunal (Solanaceae) is a prickly perennial shrub that has the potential to become a serious weed in Florida wetland habitats (Fox & Bryson 1998; Coile 1993). A synonym that has been used in the past is *Solanum houstonii* Dunal but the currently accepted name is *Solanum tampicense*. It was first recorded in the Dry Tortugas (Monroe County), Florida in 1974, and found 9 years later (1983) in mainland southwest Florida (Charlotte County). Two years later, it was reported in Highlands County (at Fisheating Creek Wildlife Refuge), and it was detected at Glades, De Soto, and Lee counties in the early 1990s. How this plant arrived in Florida in the 1970s is not known but it is spreading rapidly, forming moderate to dense stands replacing native vegetation mainly in wetland areas, open marsh, and in shaded woody areas (oak hammocks) at the edge of rivers. Wetland-nightshade infests a significant portion of the Peace River system. Currently, this weed is spreading into adjacent areas in at least 2 locations. The invaded wetland areas in Florida have been estimated at 200-300 ha and along at least 100 km of river (Fox & Bryson 1998; Coile 1993). This exotic weed was placed on the Florida Noxious Weed List, and on the Federal Noxious Weed List in 1998/99.

Wetland-nightshade is native to southern Mexico, Guatemala, Belize (Gentry & Standley 1974), the Caribbean region (Sauget & Liogier 1957), Nicaragua, Costa Rica (Medal personal observation), and probably has also spread into other regions including the northern part of South America. This plant has enormous reproductive potential through vegetative parts (sections of stems) as well as seed production. A single plant growing in an open sunny area can produce up to 8,620 seeds during an annual growing season that occurs from early spring to fall in southern Florida (Fox & Wigginton 1996b; Langeland & Burks 1998). Dispersion of the seeds may occur through wildlife feeding on the fruits as is common with other *Solanum* species (Medal et al. 2002; Medal & Cuda 2000). Since the mid 1990s, some initial research efforts involving state agencies have focused mainly on chemical weed control along with a few studies on the biology and ecology of the weed (Fox & Wigginton 1996a, b).

Currently, recommended management practices for wetland-nightshade in Florida wetlands are based on herbicide applications (Langeland & Stocker 1997). Herbicide treatment of this species is labor intensive, expensive, and may result in damage to non-target species. Many of the infested areas are not accessible by boat or other equipment, which makes herbicide applications even more difficult. Biological control research efforts with insects and nematodes against wetland-nightshade were initiated by University of Florida researchers who included this plant in the host-specificity tests against tropical soda apple, *Solanum viarum* Dunal, a highly invasive terrestrial congener of wetland-nightshade native to the southern part of South America (Cuda et al. 1998, 2002a; Medal et al. 1999). Several leaf-feeding insects were tested, including 3 chrysomelid beetles (*Metriona elatior* Klug, *Gratiana boliviana* Spaeth, and *Platyphora* sp.) that were found feeding on tropical soda apple in South America (Medal et al. 1996, 2003, 2007). Quarantine-laboratory screening tests with these insects indicated only minor feeding damage and no larval development on wetland-nightshade (Medal et al. 1999, 2002; Medal et al. unpublished data). Host-feeding tests were also conducted from 1995 to 1997 with a foliar and stem-galling nematode *Ditylenchus phyllobius* (Thorne) Filipje, and two leaf-beetles *Leptinotarsa texana* Schaeffer and *Leptinotarsa defecta* Stål from Texas (Cuda et al. 1998, 2002b). Although the nematode and the 2 *Leptinotarsa* leaf-beetles caused significant damage to silverleaf nightshade *Solanum elaeagnifolium* Cav. (their preferred host plant), they were unable to survive on wetland-nightshade in starvation tests. We initiated a classical biological control project against this exotic weed in the fall of 2005 because importation of specialized natural enemies that attack wetland-nightshade in its native range, Mexico-Central America-Caribbean region, may prevent or reduce the spread of this weed. Field surveys of potential biocontrol agents in the native range of wetland-nightshade, and host-specificity tests with the selected agents were conducted in southern Mexico and Central America, and at the Gainesville quarantine facility during a 3-year period.

Wetland-nightshade can grow up to 5 m tall sometimes forming dense stands mainly in swamps/wetland areas or along river margins. Stems and leaf veins are densely covered with short (0.5 cm long) recurved or straight spines. Leaves alternate in pairs or individually with a petiole up to 3 cm long. Leaf blades are longer (5.5-16 cm) than wide (2.2-5.5 cm) with lobed or indented margins. The corolla is white with anthers up to 4 mm long. Fruits are relatively small (up to 1 cm in diameter), and rounded, forming small clusters. Fruits are initially green, turning orange and finally red when ripe. Each fruit contains 10-60 yellowish, flat round seeds 2-2.5 mm long. Seeds can be viable for at least 12 months. Wetland-nightshade can reproduce by forming new stems from the stem base or regrowing from crowns. It is shade tolerant but will grow in full sun (Fox & Wigginton 1996b; Gentry & Standley 1974; Langeland & Burks 1998).

The flower-bud weevil, *Anthonomus elutus* Clark (Coleoptera: Curculionidae) was tested as a biological control agent of wetland nightshade in Florida. This insect was first found and collected

on wetland nightshade in Monterrico, Departamento Santa Rosa, Guatemala (N:13°54' 50.7", W:90°32' 26.1") in Feb 2006. The identity of *A. elutus* was confirmed by Drs. Wayne Clark (Auburn University, AL) and Germano Rosado Neto (Universidade Federal do Paraná in Curitiba, Brazil). Voucher specimens of *A. elutus* are deposited at Auburn University, Alabama, at the Universidade Federal do Paraná- Curitiba campus, Brazil, at the Colegio de la Frontera Sur, Tapachula, Chiapas, México, at the Ministerio de Agricultura in San Jose, Costa Rica, and at the Florida State Collection of Arthropods, Division of Plant Industry in Gainesville, Florida. This species does not have a common name in Central America. The only known host plant of *A. elutus* in Central America is *S. tampicense*. The distribution of *A. elutus* in southern Mexico and Central America is not well known.

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 flower-bud weevil *A. elutus* as a potential biological control agent of the non-native weed wetland-nightshade.

Materials and Methods

Host-specificity tests with *A. elutus* adults were conducted from Feb 2006 to Nov 2008 at the quarantine facility in Gainesville, Florida. Adults were first collected Feb 2006 on *S. tampicense* plants in Alajuela Province, Costa Rica, and Santa Rosa, Guatemala, and introduced into quarantine in Florida where they were placed with *S. tampicense* clusters of leaves/flower-buds in screened plastic containers. These field-collected adults and their offspring born in quarantine were used for host-specificity testing.

Multiple-Choice Feeding and Oviposition Tests

Eighty-seven plant species in 17 families were included in the feeding and oviposition preference tests in Gainesville-quarantine (Table 1). The plants tested included 62 species in the family of the target weed (Solanaceae) of which 33 were from the genus *Solanum* and 29 from 15 other genera that include plants of agricultural and/or ecological importance. Eight species representing 3 families (Boraginaceae, Convolvulaceae, Polemoniaceae) related phyllogenetically very closely to the Solanaceae, in the same order Polemoniales (according to Heywood 1993), or in the Solanales (based on the most recent classification made by Chase 2003) were also included. Twenty-five plant species representing 16 families, most of them with an economic and/or environmental value in North America, were tested. The major target weed (*S. tampicense*) and other 9 plant species in the Solanaceae were tested at least 3-times. They include *Solanum donianum* Walpers that is in the list of Florida threatened plants (Coile 1998); 2 non-native invasive weeds (*Solanum viarum* Dunal, *Solanum torvum* Sw.); and the 6 major cultivated Solanaceae *Capsicum annuum* L. (bell-pepper), *Capsicum frutescens* L. (chile), *Lycopersicon esculentum* Mill. (tomato), *Nicotiana tabacum* L. (tobacco), *Solanum melongena* L. (eggplant), and *Solanum tuberosum* L. (potato).

The plant species tested were obtained from local nurseries, from fields were they grew naturally, or were grown from seeds obtained from a commercial nursery. All plants were grown before testing in 1-gallon pots with a mixture of 2/3 parts top soil and 1/3 part sand. The plants were maintained out-of-door in a screened area (50% shade), provided with water as needed, and fertilized every 2-3 months. Cut branches or bouquets (10-15 cm) with foliage/flower buds from the potted plants were healthy, not fed upon by herbivorous, and had approximately similar amounts of foliage and numbers of flower buds (5-10) for each plant species in a given test. The cut branches or bouquets of the test plants were placed in 30-mL clear plastic cups filled with water. The top of the cup was covered with a plastic lid that had a small hole punched in the middle to insert the bouquet. The water cups with bouquets were placed individually on each cell of an egg-carton to keep the plant bouquet erect. Eight to 10 plant species randomly selected, including always the target weed, were simultaneously exposed to 20-26 *A. elutus* (approximately 50% male, 50% female) 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 observe their orientation to the tested plants. Plant species in each test were replicated 3-4 times (1 replication of tested plants in each separate container). Plants tested were exposed to *A. elutus* adults over a period of 10 to 14 d. Observations of oviposition and feeding were made twice a week. Plant bouquets containing darkened or loosened flower-buds were replaced as needed, and dissected to observe possible oviposition. The flower-bud and leaf area consumed was visually estimated based upon 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, and 5 = intense feeding or >60 of the area consumed).

No-Choice Adult Feeding Tests

No-choice host specificity tests were also conducted with *A. elutus* adults at the Gainesville-

TABLE 1. HOST RANGE, ADULT FEEDING, AND OVIPOSITION TEST WITH *ANTHONOMUS ELUTUS* IN FLORIDA.

Plant Family Species	Common Names (*indicates native *Solanum* 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 *Solanum*					
Subgenus *Leptostemonum*					
Section Micracantha					
Solanum tampicense Dunal	Wetland nightshade	7	420	4-5	3-10
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	2	120	1	0
Solanum mammosum L.	Nipplefruit	1	80	1	0
Solanum viarum Dunal	Tropical soda apple	3	180	0	0
Section Lasiocarpum					
Solanum quitoense Lam.	Naranjilla	2	140	0	0
Solanum pseudolulo Heise	Falso lulo	2	140	0	0
Solanum sessiliflorum Dunal	Nightshade	2	140	0	0
Section Micracantha					
Solanum jamaicense Mill.	Jamaican nightshade	2	140	0	0
Section Melongena					
Subsection Androceras					
Solanum citrullifolium A. Braun	Watermelon nightshade	1	80	0	0
Solanum heterodoxum Dunal	Melonleaf nightshade	1	80	0	0
Solanum rostratrum Dunal	Buffalobur nightshade	1	80	0	0
Solanum sisymbriifolium Lam.	Sticky nightshade	2	140	1	0

Each test 3-4 replications with 20-26 adults (50% males, 50% females) per rep.
[1]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).

TABLE 1. (CONTINUED) HOST RANGE, ADULT FEEDING, AND OVIPOSITION TEST WITH *ANTHONOMUS ELUTUS* IN FLORIDA.

Plant Family Species	Common Names (*indicates native *Solanum* species)	No. of Tests	No. of Insects	Feeding Score[1]	Eggs Laid per Female
Subsection Lathyrocarpum					
Solanum carolinense L.	Horse nettle*	2	140	0	0
Solanum dimidiatum Raf.	Western horsenettle*	2	140	0	0
Solanum elaeagnifolium Cav.	Silverleaf nightshade	2	140	0	0
Subsection Melongena					
Solanum bahamense	Bahama nightshade	1	80	0	0
Solanum melongena L.	Eggplant	5	360	0	
Solanum torvum Sw.	Turkeyberry	3	200	1	0
Solanum verbascifolium L.	Mullein nightshade*	2	120	0	0
Subgenus *Solanum*					
Solanum americanum Mill.	American nightshade*	2	140	0	0
Solanum diphyllum L.	Two-leaf nightshade*	2	140	0	0
Solanum erianthum Don.	Potato tree*	2	140	0	0
Solanum jasminoides Paxt.	White potato vine	2	140	0	0
Solanum mauritianum Scop.	Earleaf nightshade	2	140	0	0
Solanum nigrescesns Mart. & Gal	Divine nightshade*	2	160	0	0
Solanum nigrum L.	Black nightshade*	2	140	0	0
Solanum parishii heller	Parish nightshade*	2	160	0	0
Solanum ptycanthum Dunal	Wonder berry*	2	160	0	0
Solanum pumillum Dunal	Rock outcrop Solanum	1	80	0	0
Solanum retroflexum Dunal	Wonderberry	1	80	0	0
Solanum scabrum Mill.	Garden huckleberry	1	80	0	0
Solanum seaforthianum Andr.	Brazilian nightshade	2	160	0	0
Solanum tuberosum L.	Potato	5	400	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	2	160	0	0
Genus *Iochroma*					
Iochroma sp.	Iochroma	2	120	0	0

Each test 3-4 replications with 20-26 adults (50% males, 50% females) per rep.
*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).

TABLE 1. (CONTINUED) HOST RANGE, ADULT FEEDING, AND OVIPOSITION TEST WITH *ANTHONOMUS ELUTUS* IN FLORIDA.

Plant Family Species	Common Names (*indicates native *Solanum* species)	No. of Tests	No. of Insects	Feeding Score[1]	Eggs Laid per Female
Genus *Physalis*					
Physalis angulata L.	Cutleaf Ground-Cherry	2	120	0	0
Physalis arenicola Kearney	Cypresshead	2	120	0	0
Physalis crassifolia Benth	Ground-Cherry	2	120	0	0
Physalis gigantea L.	Ground-cherry	2	120	0	0
Physalis ixocarpa Brot.	Tomatillo	2	140	0	0
Physalis pubescens L	Strawberry tomato	2	120	0	0
Tribe Daturae					
Genus *Brugmansia*					
Brugmansia sanguinea (Ruiz & Pav.) Don	Red floripontio	2	120	0	0
Genus *Datura Datura iscolour* Bernh	Angels'trumpet	2	120	0	0
Datura metel L.	Downy thorn apple	2	120	0	0
Datura meteloides D.	Datura	2	120	0	0
Datura stramonium L.	Jimson weed	2	120	0	0
Tribe Lycieae					
Genus *Lycium*					
Lycium carolinianum Walt.	Christmas berry	2	120	0	0
Lycium fremontii Gray.	Lycium	2	120	0	0
Genus Lycopersicon					
Lycopersicon esculentum Mill.	Tomato	5	400	0	0
Tribe: Nicandreae					
Genus: *Nicandra*					
Nicandra physaloides (L.) Gaertn.	Apple of Peru	2	120	0	0
Tribe Nicotianae					
Genus *Nicotiana*					
Nicotiana tabacum L.	Tobacco	5	400	0	0
Nicotiana rustica L.	Wild tobacco	2	140	0	0
Nicotiana sylvestris Speg. & Comes	Tobacco	2	120	0	0

Each test 3-4 replications with 20-26 adults (50% males, 50% females) per rep.
[1]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).

TABLE 1. (CONTINUED) HOST RANGE, ADULT FEEDING, AND OVIPOSITION TEST WITH *ANTHONOMUS ELUTUS* IN FLORIDA.

Plant Family Species	Common Names (*indicates native *Solanum* species)	No. of Tests	No. of Insects	Feeding Score[1]	Eggs Laid per Female
Genus *Nierembergia*					
Nierembergia scoparia Sendtri	Cupflower	2	120	0	0
Genus *Petunia*					
Petunia × *hybrida*	Garden-petunia	2	120	0	0
Tribe Salpiglossidae					
Genus *Salpiglossis*					
Salpiglossis sinuata Ruiz & Pav	Painted tongue	2	120	0	0
Genus *Schizanthus*					
Schizanthus spp.	Butterfly flower	2	120	0	0
Tribe Solandeae					
Genus *Solandra*					
Solandra glandiflora Swartz	Chalice vine	2	120	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*	5	400	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					
Heliotrope sp.	Heliotrope	1	60	0	0
Myosotis alpestris Schmidt	Forget-Me-Not	1	60	0	0
CONVOLVULACEAE					
Convolvulus purpurea L.	Convolvulus	2	120	0	0
Ipomoea batata (L.) Lam.	Sweet-potato	2	120	0	0
Evolvulus muttallianus	Evolvulus	2	120	0	0
EHRETIACEAE					
Cordia sebestena L.	Geiger tree	1	60	0	0

Each test 3-4 replications with 20-26 adults (50% males, 50% females) per rep.
*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).

TABLE 1. (CONTINUED) HOST RANGE, ADULT FEEDING, AND OVIPOSITION TEST WITH *ANTHONOMUS ELUTUS* IN FLORIDA.

Plant Family Species	Common Names (*indicates native *Solanum* species)	No. of Tests	No. of Insects	Feeding Score[1]	Eggs Laid per Female
NOLANACEAE					
Nolana paradoxa Lindl.	Chilean bellflower	1	60	0	0
POLEMONIACEAE					
Cobaea scandens Cav.	Cobaea	1	60	0	0
Gilia tricolor Benth	Bird's-eyes	1	60	0	0
Phlox panuculata L.	Phlox	1	60	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					
Anacardium occidentale L.	Cashew	1	60	0	0
Mangifera indica L.	Mango	1	60	0	0
Pistacia vera L.	Cultivated pistachio	1	60	0	0
APIACEAE					
Daucus carota L.	Carrot	1	60	0	0
ASTERACEAE					
Helianthus annuus L.	Annual sunflower	1	60	0	0
Lactuca sativa L.	Lettuce	1	60	0	0
CAMPANULACEAE					
Campanula persicifolia L	Bell flower	1	60	0	0
CARICACEAE					
Carica papaya L.	Papaya	1	60	0	0
CRUCIFERAE					
Brassica oleracea L. var. Botrytis	Broccoli	1	60	0	0
MALVACEAE					
Abelmoschus esculentus (L.) Moench	Okra	1	60	0	0

Each test 3-4 replications with 20-26 adults (50% males, 50% females) per rep.
[1]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).

TABLE 1. (CONTINUED) HOST RANGE, ADULT FEEDING, AND OVIPOSITION TEST WITH *ANTHONOMUS ELUTUS* IN FLORIDA.

Plant Family Species	Common Names (*indicates native *Solanum* species)	No. of Tests	No. of Insects	Feeding Score[1]	Eggs Laid per Female
ROSACEAE					
Fragaria × *ananassa* Duchesne	Strawberry	1	60	0	0
RUTACEAE					
Citrus sinensis (L.) Osbeck	Sweet orange	1	60	0	0
Citrus paradise Mcfady	Grapefruit	1	60	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	3	200	0	0
SOLANACEAE					
Genus *Capsicum*					
Capsicum annuum L.	Bell pepper	5	400	0	0
Capsicum frutescens L.	Chile	5	380	0	0

Each test 3-4 replications with 20-26 adults (50% males, 50% females) per rep.
[†]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).

quarantine facility with potted plants (20-60 cm height) in cages. *Anthonomus elutus* adults were exposed to 30 plant species in 3 families including *S. donianum* in the list of Florida threatened plants, and all major cultivated Solanaceae (Table 2). Five to 6 plant species were individually tested each time due to limitation in cage numbers. Ten *A. elutus* adults (5 males, 5 females) per replication (3 replications) were exposed to plants for 2 weeks. Cages were made of clear-plastic cylinders (15 cm diameter, 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 adults collected on wetland-nightshade plants in Costa Rica and Guatemala. The adults tested were young (2-3-week old). Plants were replaced as needed. At the end of the testing periods, feeding and oviposition were recorded.

Results and Discussion

Multiple-Choice Feeding and Oviposition Tests

In the Florida-quarantine multiple-choice tests (Table 1), *Anthonomus elutus* adults fed heavily to intensively (41-100% of the area offered) on the target weed wetland-nightshade. A minor or exploratory feeding (<5% of the area offered) was observed on the non-natives *Solanum capsicoides, Solanum mammosum*, *Solanum sisymbriifolium*, and *Solanum torvum*. No feeding was observed on any of the other 82 plant species that were tested. *Anthonomus elutus* laid from 3 to 10 eggs per female inside the wetland-nightshade flower-buds during the duration of the test (Table 1). No eggs were deposited on any of the other 86 plant species tested. Results indicated that this potential biocontrol agent fed and laid eggs only on the target weed wetland-nightshade.

Table 2. Host range of *Anthonomus elutus* adults in no-choice feeding tests in Florida quarantine.

Plant family / Species	Common names	Feeding Score*	Eggs/Female
SOLANACEAE			
Capsicum annuum	Bell pepper	0	0
Capsicum frutescens	Chile	0	0
Lycopersicon esculentum	Tomato	0	0
Nicotiana tabacum	Tobacco	0	0
Nierembergia scoparia	Cupflower	0	0
Physalis crassifolia	Ground-cherry	0	0
Physalis pubescens L.	Strawberry tomato	0	0
Solanum americanum	American nightshade	0	0
Solanum carolinense	Horse nettle	0	0
Solanum citrullifolium	Watermelon nightshade	0	0
Solanum dimidiatum	Western horsenettle	0	0
Solanum diphillum	Two-leaf nightshade	0	0
Solanum donianum	Mullein nightshade	0	0
Solanum elaeagnifolium	Silverleaf nightshade	1	0
Solanum jamaicense	Jamaican nightshade	0	0
Solanum jasminoides	White potato vine	0	0
Solanum melongena	Eggplant		
cv Black Beauty		0	0
cv Market		0	0
cv Asian Long Purple		0	0
Solanum nigrescens	Black nightshade	0	0
Solanum pumilum	Rock-outcrop	0	0
Solanum ptycanthum	Wonder berry	0	0
Solanum retroflexum	Sunberry	0	0
Solanum scabrum	Garden huckleberry	0	0
Solanum tampicense	Wetland nightshade	3	3-6
Solanum torvum	Turkeyberry	1	0
Solanum tuberosum	Potato	0	0
Solanum viarum	Tropical soda apple	0	0
CONVOLVULACEAE			
Ipomoea batata (L.) Lam.	Sweet-potato	0	0

Each test included 3-4 replications with 10 adults (5 females, 5 males) per replication.
*0 = No feeding, 1 = Probing (<5% of leaf/flower bud area), 2 = Light feeding (5-20%), 3 = Moderate feeding (21-40%), 4 = Heavy feeding (41-60%), 5 = Intense feeding (>60% of the area).

No-Choice Adult Feeding Tests

The no-choice host specificity tests with *A. elutus* adults exposed to individual potted plants (30 species in 3 families) in cages at the Gainesville-quarantine facility (Table 2) indicated that the insects fed and laid eggs (range: 3-6, average: 5 eggs per female) only on wetland-nightshade. Feeding on wetland-nightshade was moderate (21-40% of the area offered) compared to a probing or exploratory feeding (<5%) observed on *S. elaeagnifolium* and on *S. torvum*. No eggs were laid on any of the 29 non-target plant species tested including the 3 eggplant cultivars (Black Beauty, Market, Asian Long Purple).

The high specificity showed by this weevil in the host-range tests feeding and development only on the target weed in the Micrantha section, 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 associated with release and establishment of *A. elutus* may be expected due to recolonization by native plants that have been displaced by the rapidly growing and highly competitive wetland-nightshade plants.

The host-specificity tests at the Gainesville quarantine indicated that *A. elutus* is safe to release. Occasional temporary feeding might occur on some close related *Solanum* species. These species are the non-native weeds *S. torvum* (in the Federal Noxious Weed list, introduced from Central/South America (Kissman & Groth 1995), *S. capsicoides*, *S. sisymbriifolium*, and *S. jamaicense*. Noticeable damage to economic/native Solanaceae plants is unlikely to occur based on our host tests. The lack of records as a crop pest in the native range of the weevils support our findings on the specificity and safety of *A. elutus* as a biocontrol agent of wetland-nightshade in Florida.

One of the strongest arguments in favor of releasing *A. elutus* against wetland-nightshade in Florida was the inability of this weevil to feed, oviposit, or develop on eggplant, tomato, potato, bell-pepper, chile, native Solanaceae plants, and other economic crops in the no-choice and choice experiments. These findings suggest that a host range expansion of *A. elutus* to include any of the tested non-target plants is highly unlikely. The quarantine feeding-oviposition tests exposing *A. elutus* to 87 plant species in 17 families indicated that this weevil is nearly a monophagous herbivore which feeds on a few *Solanum* species, including *S. torvum* which is an exotic weed of increasing concern in Florida due to its potential to invade wildlife areas and displace native vegetation. The primary goal of this biological control program is to bring down the wetland-nightshade population density below a threshold that does not cause economic or ecological damage. We do not consider eradication of an invasive plant that has already been established to be a realistic goal. However, if local eradication were observed (e.g., as a consequence of herbicide/intense mowing applications), and occasional feeding on a non-target plant occurred, the evidence accumulated indicates that the possibility of *A. elutus* becoming a problem on a non-target plant is remote.

Based on the specificity of *A. elutus* feeding and developing only on the target weed in the Micrantha section, we consider this weevil safe for field release against wetland-nightshade. Therefore, a petition to release the Mexican-Central-American flower-bud weevil *A. elutus* for the control of wetland- nightshade in Florida was submitted to the USDA-APHIS-PPQ Technical Advisory Group (TAG) members on Dec 2008.

Acknowledgments

We thank Howard Frank (University of Florida), and Julieta Brambila (United States Department of Agriculture, Animal and Plant Health Inspection Service) for reviewing the manuscript. We thank Wayne Clark (Auburn University) for identification of *Anthonomus elutus*. This research was funded by Florida Fish and Wildlife.

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