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Source: Florida Entomologist, 97(1) : 179-190

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.097.0124

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SUCCESSFUL BIOLOGICAL CONTROL OF TROPICAL SODA APPLE (SOLANALES: SOLANACEAE) IN FLORIDA: A REVIEW OF KEY PROGRAM COMPONENTS

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Abstract

Tropical soda apple (*Solanum viarum* Dunal) (Solanaceae) is a small shrub native to South America that is invasive in pastures and conservation areas across Florida. Dense patches of tropical soda apple not only reduce cattle stocking rates and limit their movement, but also serve as reservoirs for pests of solanaceous crops. A classical biological control program was initiated in 1994 with exploration for natural enemies of tropical soda apple in Argentina, Brazil, and Paraguay. Host specificity tests conducted under laboratory and field conditions demonstrated that the leaf feeding beetle *Gratiana boliviana* Dunal (Coleoptera: Chrysomelidae) was a specialist herbivore that completes development only on the target weed. After obtaining appropriate permits, field releases of *G. boliviana* were initiated in Florida in May of 2003. Larvae and adults of *G. boliviana* feed on tropical soda apple leaves and may completely defoliate their host plants, resulting in reduced growth and fruit production. Mass rearing facilities for the beetle were established in northern, central and southern Florida, and adults were either hand-carried or transported to release sites by overnight courier. From 2003 to 2011, a total of 250,723 beetles were released and they became established throughout Florida, however, their impact is more noticeable in regions below latitude 29 °N. Reductions of tropical soda apple densities caused by damage by the beetle were visible 2-3 yr after initial release, or in some cases, within a few months. Various methods of technology transfer were used to inform the public, land owners, funding agencies and scientists about the biological control program, including articles in trade magazines, extension publications, websites, videos, field days and scientific publications. The project was successful because of the coordinated efforts of personnel from federal, state and county agencies.

Key Words: Chrysomelidae, exotic weed, *Gratiana boliviana,* insect herbivore, pastures, *Solanum viarum*

Resumen

Tropical soda apple (*Solanum viarum* Dunal) (Solanaceae) es un arbusto pequeño nativo de Sur América el cual es invasivo en pastizales y áreas de conservación en Florida. Las aglomeraciones de tropical soda apple no solo limitan la densidad y movimiento de ganado, sino además sirven como reservorio de plagas de cultivos solanáceos. Un programa de control biológico clásico fue iniciado en 1994 con exploraciones de enemigos naturales de tropical soda apple en Argentina, Brasil, y Paraguay. Ensayos de especificidad realizados bajo condiciones de laboratorio y campo demostraron que *Gratiana boliviana* Dunal (Coleoptera: Chrysomelidae) fue un herbívoro especialista el cual completó su desarrollo solo en la maleza objetivo. Luego de obtener los permisos respectivos, liberaciones de campo de *G. boliviana* fueron

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iniciadas en Florida en Mayo del 2003. Larvas y adultos comen hojas de tropical soda apple y pueden defoliar completamente las plantas, resultando en un crecimiento reducido y baja producción de frutas. Laboratorios de crianza masiva de *G. boliviana* fueron establecidos en el norte, centro y sur de Florida, y los adultos fueron llevados al sitio de liberación por entrega directa o por correo rápido. Entre los años 2003 y 2011, un total de 250,723 adultos fueron liberados y se establecieron a través de Florida, no obstante, su impacto es más notorio en regiones por debajo de la latitud 29 °N. Reducciones en la densidad de tropical soda apple debido al daño de *G. boliviana* fueron visibles luego de 2 a 3 años de su liberación, o en algunos casos, luego de unos pocos meses. Varios métodos de transferencia de tecnología fueron usados para informar al público, dueños de propiedades, agencias estatales y científicos acerca del programa de control biológico, incluyendo artículos en revistas, publicaciones de extensión, páginas de internet, videos, días de campo y publicaciones científicas. El éxito del proyecto se puede atribuir al esfuerzo coordinado de personal de agencias federales, estatales y de condados.

Palabras Clave: Chrysomelidae, maleza exótica, *Gratiana boliviana,* insecto herbívoro, pastizales, *Solanum viarum*

Evaluation of classical biological control programs of invasive weeds provides critical information to stakeholders (e.g., property owners, researchers, and funding and regulatory agencies) about successes and setbacks that would facilitate the reformulation of ongoing programs and the planning of future programs. Important knowledge is generated during the different stages of a biological control program. For example, identification of insect herbivores and establishment of a collaboration network with scientists in the native range is essential for success of foreign exploration (Goolsby et al. 2003). One of the critical steps in classical biological control is defining the agent's host specificity in the laboratory before releases are undertaken, followed by validation of these results in the field (Blossey 1995; Pratt et al. 2009). Extension efforts are critical during field releases to assist with agent deployment and measurement of spread in the weed's introduced range (Wiedenmann et al. 2007). In addition, impact studies of the biocontrol agents are conducted at different spatial and temporal scales in the field (Morin et al. 2009). Finally, the benefits of the program should be quantified through economic analyses and changes in the perspectives and behaviors of land managers (Coombs et al. 1996). The goal of this review is to provide a comprehensive account of the major steps taken during the program on classical biological control of tropical soda apple in Florida using the leaf feeding beetle *Gratiana boliviana* Dunal.

Tropical Soda Apple

Tropical soda apple (TSA), *Solanum viarum* Dunal, is a perennial South American shrub in the Acanthophora section, subgenus *Leptostemonum* ('spiny solanums') in the family Solanaceae (Nee 1991). The native range of TSA includes southern Brazil, northern Argentina and Paraguay (Wunderlin et al. 1993), and from there it has spread to India, Africa, China, Vietnam, Australia, Central America, Mexico and the West Indies (Nee 1991; Wunderlin et al. 1993; Diaz et al. 2008; Ensbey et al. 2011; GBIF 2012). In its native range, TSA is found in scattered patches but is not considered to be a major weed (Bianco et al. 1997). In Florida, TSA was first collected in Glades County in 1988 (Mullahey et al. 1993), and although it may have arrived as early as 1981 (Coile 1993), the pathway of introduction into Florida is unknown.

TSA spread rapidly in the state through the movement of contaminated hay, manure, sod and grass seed, and in the guts of livestock and wild animals that feed on TSA fruits (Wunderlin et al. 1993; Brown et al. 1996; Mullahey 1996). In 1992, the area infested in Florida was estimated to be 61,000 ha (Mullahey et al. 1993) but by 1994, TSA had spread to 200,000 ha and was found in all of Florida's 67 counties (Mullahey & Cornel 1994). The area infested expanded to 400,000 ha in Florida by 1996, and by then TSA had reached Alabama, Georgia, and Mississippi (Mullahey 1996). In addition to those states, TSA has been reported in South Carolina, Tennessee, Louisiana, Texas, Arkansas and Oklahoma (EDDMAPS 2012). A population reported in Pennsylvania in the summer of 1996 (Lingenfelter & Curran 1998) apparently did not become established (D. D. Lingenfelter, personal communication). A study of the physiological limitations of TSA suggested that it may be able to establish as far north as areas in Virginia, Illinois, Kansas and Colorado (Patterson et al. 1997), although a recent climate modeling exercise (Mukherjee et al. 2012) suggested that TSA may have already colonized the majority of suitable areas in North America. TSA invades a variety of habitats including ditch banks, citrus groves, sugarcane fields, and natural areas, but is primarily an economic problem in pastures (Mullahey et al. 1993; Salaudeen et al. 2012a). The foliage is not palatable to cattle, which results in a loss of grazing land and the consequent lowering of stocking rates (Mullahey et al. 1993). TSA has also been reported to

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increase the incidence of heat stress in livestock due to heavy infestations in wooded areas, which restricts access of cattle to shade (Mullahey et al. 1998). An economic assessment of TSA in Florida conducted in 2006 found that this invasive species cost Florida ranchers nearly $15 million/yr, with the greatest losses (~ $12 million) in central Florida (Salaudeen et al. 2012a). In addition to the impacts to cattle producers, TSA is a reservoir of several plant pathogens and serves as a host for a number of insect pests of solanaceous crops (McGovern et al. 1994, 1996; Adkins et al. 2007; Diaz et al. 2012a;). TSA also invades natural areas where it may out-compete native species (Langeland & Burks 1998).

Because of the rapid spread of TSA and the threat it posed to Florida's cattle producers, the Florida Department of Agricultural and Consumer Services (FDACS) established a TSA Task Force in 1993. Task Force membership included representatives of the USDA/APHIS, the University of Florida, cattle producers and other land managers. The stated purpose of the group was to "carefully review all aspects of the TSA problem and develop a recommended strategy to pursue in bringing this weed under control" (FDACS 1993). A year later, the membership was expanded to include representatives from the Florida Department of Environmental Protection, the Florida Fish and Game Commission, chemical company representatives and other interested parties. A regional task force with a similar mandate was established in 2003 to share information among southeastern states. Both state and regional task forces performed an instrumental role in the coordination of research and the acquisition of research funding. In addition, the task forces contributed to the dissemination of reliable information about TSA to stakeholders and the general public. Their most useful role was to provide a forum for the discussion of voluntary measures in order to limit the spread of TSA within Florida and into neighboring states.

Management of TSA initially relied on mowing and the application of chemical herbicides (Mullahey et al. 1994), though these tactics were refined over time (Mullahey 1996; Ferrell et al. 2006; Sellers & Ferrell 2008; Sellers et al. 2010). Current recommendations include herbicide applications, which vary according to the level of infestation. Dense infestations are treated with aminopyralid (Milestone®) or aminopyralid plus 2.4D (GrazonNext®) herbicides, with or without mowing, or with triclopyr (Remedy®) and periodic mowing. Sparse infestations can be spot treated with the same herbicides (for detailed recommendations, see Sellers et al. 2010).

Research on biological control of TSA was initiated soon after its arrival in Florida. Exploration for natural enemies began in South America in 1994 (Medal et al. 1996), and resulted in the release of a leaf feeding beetle, *Gratiana boliviana* Spaeth (Coleoptera: Chrysomelidae), in 2003 (discussed in detail below). Additionally, a bioherbicide was developed from a locally collected virus (*Tobacco mild green mosaic virus* [TMGMV]), which received the trade name Solvinix™. The virus is highly effective in killing TSA plants because of a hypersensitive response of the plant to infection (Charudattan & Hiebert 2007). An Experimental Use Permit for Solvinix™ was approved in 2007, but it has not received full EPA registration (Charudattan 2010).

Foreign Exploration of Natural Enemies and Host Specificity Testing

Foreign exploration for insect herbivores and plant diseases of TSA was initiated in 1994 with surveys conducted in Argentina, Brazil, Paraguay and Uruguay by the University of Florida in collaboration with the Universidade Estadual Paulista, Jaboticabal Campus, Brazil. During a 2-wk survey, 16 species of insects were found feeding on TSA and at least 2 were considered promising as biological control agents: *Gratiana boliviana* (Fig. 1), and *Metriona elatior* Klug (Coleoptera: Chrysomelidae) (Medal et al. 1996). In 1997, a classical biological control project against TSA was formally initiated with funding from USDA/APHIS. The goal of this project was to identify host specific natural enemies that feed on TSA in its native range for possible introduction into Florida as biological control agents. Additional foreign exploration was conducted from 1997 to 2010 in Brazil, Argentina, Paraguay, and Uruguay by the University of Florida and South American collaborators. These surveys resulted in the identification of 3 additional candidate natural enemies; a flower-bud feeding weevil, *Anthonomus tenebrosus* Boheman (Coleoptera: Curculionidae), collected from TSA in Rio Grande do Sul, Brazil, and 2 leaf-feeding beetles; *Platyphora* sp., and *Gratiana graminea* Klug (Coleoptera: Chrysomelidae) (Medal et al. 2010; Olckers et al. 2002).

Host specificity tests included plant species related to TSA as well as economically and ecologically important species, and this process followed the centrifugal phylogenetic method (Wapshere 1989). Because eggplant (*Solanum melongena* L.) belongs to the same subgenus (*Leptostenomum*) as TSA, and is important to Florida agriculture, several varieties of eggplant were included. The first insect evaluated was *M. elatior*. Non-target effects were observed on eggplant with adult feeding (Freitas et al. 2008) and complete development on several eggplant varieties (Medal et al. 1999a). However, host range testing under laboratory conditions has resulted in false positives because of the inhibitory cage environment, associative learning and host habituation of agents to non-hosts (Marohasy 1998; Heard 2000). Thus,

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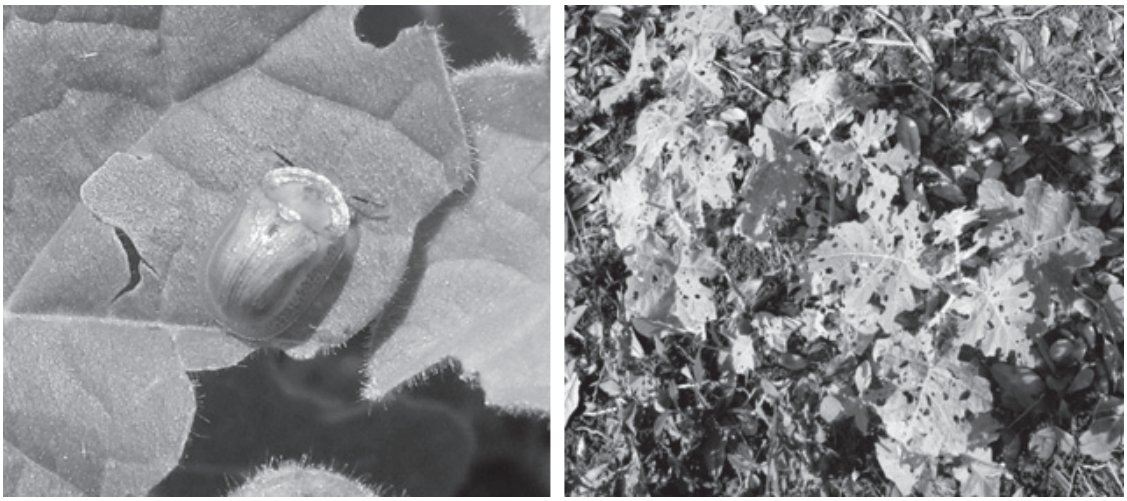


Fig. 1. *Gratiana boliviana* adult and shot-hole type of damage on the foliage of TSA.

open field experiments in the native range are recommended in order determine the "ecological host range" of these agents under more natural conditions (Briese et al. 2002). Field studies conducted in Brazil and Argentina indicated that this beetle was highly specific to TSA (Medal et al. 1999a, 1999b), and only minor feeding and oviposition were observed on eggplants (Bredow et al. 2007). Similarly, host specificity tests with *A. tenebrosus* and *G. graminea* revealed that TSA was the preferred host but minor damage was observed on eggplant and other non-target plants (Medal et al. 2010, 2011; Diaz et al. 2013). Despite only minor damage to eggplant, *M. elatior*, *A. tenebrosus* and *G. graminea* were rejected as potential biological control agents of TSA.

Host specificity tests with *G. boliviana* were conducted from 1998 to 2001 at the Florida Biological Control Laboratory quarantine facility in Gainesville, at the USDA-ARS quarantine facility in Stoneville, Mississippi and at the USDA-ARS South American Biological Control Laboratory in Hurlingham, Argentina (Medal et al. 2002, 2003). Exposure of a 126 plant species in 35 families to *G. boliviana* revealed a high level of host specificity to TSA. Under no-choice conditions, females laid eggs only on TSA and immatures transferred to non-target species experienced 100% mortality (Medal et al. 2002). Moreover, open field tests and extensive field surveys in South America revealed that *G. boliviana* was only found on TSA (Gandolfo et al. 1999, 2007; Medal et al. 2002, 2003, 2004) and *S. palinacanthum* Dunal (F. McKay personal observation). After 4 years of intensive examination, *G. boliviana* was approved for field release by the USDA/APHIS/PPQ in Apr 2002 and by the US-Fish and Wildlife Service in Oct 2002. *Gratiana boliviana* was released for the first time in Florida on 14 May 2003 in a cattle pasture in Polk County.

In Florida, TSA occasionally grows in close proximity to 2 closely related species, Jamaican nightshade (*Solanum jamaicense* Mill.) which belongs to the same subgenus (*Leptostenomum*) as TSA and red soda apple (*Solanum capsicoides* All.), which belongs to the same section (Acanthophora) as TSA. A field study showed that *G. boliviana* did not utilize Jamaican nightshade for feeding or oviposition (Overholt et al. 2008), and this beetle has never been observed on red soda apple (R. Diaz personal observations). This confirms that *G. boliviana* is a specialist herbivore of TSA, which was in agreement with the host range found prior to its release.

Biology and Ecology of *Gratiana Boliviana*

Adults of *G. boliviana* lay eggs individually on the leaves and stems of TSA. Larvae feed on the foliage and can be recognized by a distinctive accumulation of frass and shed integuments on the dorsal side of their abdomens. Pupation occurs on the underside of the leaves usually near leaf veins. *Gratiana boliviana* completed development from egg to adult in 30 days at 25 °C (Diaz et al. 2008). Adult females can live for several months laying ≈253 eggs during their lifetime (Manrique et al. 2011). Larvae and adults are folivores and produce a shot-hole type pattern of feeding damage on the leaf (Fig. 1). Leaf quality greatly affects the performance of *G. boliviana*. For example, when beetles were exposed to TSA plants infected with a virus, the developmental time was approximately 10% slower, adults consumed only about 50% as much leaf tissue and had decreased fecundity compared to beetles fed on uninfected plants (Overholt et al. 2009). Additionally, *G. boliviana* had shorter immature development time, and higher immature survival and adult fecun-

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dity when feeding on shade-acclimated plants compared to plants grown in full sunlight (Diaz et al. 2011). Despite experiencing better leaf quality and lower mortality in the shaded areas, *G. boliviana* densities in central Florida were higher in open habitats (Diaz et al. 2011).

To understand the influence of beetle density and time of the day on the dispersal of *G. boliviana*, field experiments were conducted in Miami. Beetle adults emigrated from plants when densities were higher than 40 beetles per plant; additionally, beetle dispersal occurred most frequently at noon (Chong et al. 2009). Seasonal dynamics of *G. boliviana* populations in Florida reflect a strong correlation with climate and host availability (Overholt et al. 2010). Populations increase rapidly from Apr to Oct when TSA is available and temperatures are warm. By late Nov, cold fronts kill the foliage of TSA in northern and central Florida, which time also coincides with the migration of *G. boliviana* adults to overwintering sites in leaf litter beneath plants. Laboratory experiments showed that adults enter diapause during short photoperiods. Diapause is characterized by a lack of reproduction, change in elytra coloration from green to yellow-brown, and migration to overwintering sites (Diaz et al. 2011a). Thus, during the winter in central and northern Florida, adult beetles remain in reproductive diapause in leaf litter. In contrast, in southern Florida adults can be observed feeding on TSA leaves during winter months because the foliage is available throughout the yr, however, beetle reproduction is non-existent during the winter. By mid-Mar, adults emerge from overwintering sites and start laying eggs in all areas of Florida.

Successful establishment of biological control agents following field release is greatly affected by abiotic and biotic factors found in the areas of introduction. For example, similar climatic conditions in the native and introduced ranges increase the chances of survival and persistence of an agent in the new environment (Sutherst & Maywald 1985; Byrne et al. 2003; Senaratne et al. 2006). In addition to climate, biological control agents may also encounter predation and parasitism that may limit their success (e.g. Goeden & Louda 1976; Briese 1986; Ghosheh 2005). In the case of *G. boliviana*, successful establishment and impact have been reported in southern and central Florida, while beetle densities remain low in northern Florida (Overholt et al. 2009, 2010; Diaz et al. 2012c). To examine the mortality factors that influence beetle populations in central and northern Florida, life-tables were constructed from laboratory and field data (Manrique et al. 2011). Intrinsic mortality (laboratory data) and biotic factors (field predation) together accounted for 75% of the mortality of immature stages, while abiotic factors were less important (Manrique et al. 2011). Survival to adulthood in the field was similar (ca. 15%) between central and northern Florida, which suggests that predation rates are comparable in both regions. Therefore, other factors may affect beetle performance in northern Florida including colder winters, mismatch in the seasonal phenologies of the beetle and its host-plant, and changes in host-plant quality. According to Diaz et al. (2012a), a high diversity of predators was found associated with TSA in Florida, including 19 species of spiders and 30 species of predatory insects. In addition, several parasitoids were reared from *G. boliviana* pupae collected in Florida including *Conura side* (Walker) (Chalcidae), *Brasema* sp. (Eupelmidae), and *Aprostocetus* nr. *cassidis* (Eulophidae) (Diaz et al. 2012a). The most abundant predator group in central Florida was a complex of 3 mirid species (*Engytatus modesta* Distant, *Tupiocoris notatus* Distant, and *Macrolophus* sp.) (Manrique et al. 2011). Even though significant mortality (81-95%) of immature stages of *G. boliviana* was found in the field, positive growth rates ($r_m = 0.3$) during the summer and early fall allow the beetle population to increase and provide suppression of TSA in central Florida (Manrique et al. 2011).

Rearing and Field Release of *Gratiana Boliviana*

Mass rearing of *G. boliviana* was conducted by the University of Florida, the Florida Department of Agriculture and Consumer Services and the United States Department of Agriculture at locations in northern, central, and southern Florida (Overholt et al. 2009). Beetles were reared on potted TSA plants in screen-houses mostly during the warmer months of the year. Non-diapausing colonies of *G. boliviana* were maintained over the winter months in laboratories in northern and central Florida by extending day length and maintaining warm temperatures in order to insure their availability for release early in the spring. Beetles for release were hand collected, placed in ventilated plastic containers, and hand-carried to ranchers or shipped overnight inside coolers. Upon arrival at release sites, groups of 10 to 20 adult beetles were manually transferred to individual TSA plants. To document beetle releases, an online database was maintained throughout the entire program (May 2003-Nov 2011). After each release, the following information was entered into the database: name of person making the release and their affiliation, type of property (ranch, conservation area or park, residential, other), date of release, geographic coordinates of release site, number of beetles released, and type of habitat of the release site (open pasture, hammock, pine flatlands, other) (Overholt et al. 2009). Beetles were also released in Texas, Georgia and Alabama, although not in the numbers released

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in Florida. Successful overwintering was demonstrated in Georgia and Alabama, but permanent establishment in those states and Texas has not been confirmed (A. Roda personal observation).

A total of 250,723 *G. boliviana* beetles were released in Florida between May 2003 and November 2011 (Diaz et al. 2012b) (Fig. 2). By the end of the program, releases had been made throughout most of the state in 42 of Florida's 67 counties, with the majority released in central Florida. Fewer counties in northern Florida received beetles compared to those in central and southern Florida. The total number of beetles released per county ranged from 250 in Suwannee to more than 16,000 in Alachua, Martin, Okeechobee, St. Lucie and Sumter counties (Fig. 3). Releases were concentrated in counties with large numbers of cattle, because TSA is primarily a problem in cattle pastures.

Field releases of *G. boliviana* were a coordinated effort that included property owners (ranchers and other land managers), federal employees (USDA/NRCS, USDA/APHIS and USDA/ARS) and state employees (FDACS/Division of Plant Industry, FDACS/Division of Forestry, Water Management Districts, Cooperative Extension Service, University of Florida and Florida Fish and Wildlife Conservation Commission). The majority of properties (80%) receiving beetles were private lands including ranches, dairy farms and equestrian centers (Fig. 4). Parks and conservation areas, including county lands, water management district lands and state parks accounted for 15% of properties where beetles were released (Fig. 4). With the assistance of the Florida Cattlemen's Association, which has 4,222 members throughout the state, a large proportion of ranchers benefitted from releases. The private/public partnership was instrumental in identifying release sites, delivering extension materials and organizing field days.

Establishment and Impact of *Gratiana boliviana*

In the fall of 2008, an extensive survey was conducted to estimate the establishment, distribution, and abundance of *G. boliviana* in Florida (Overholt et al. 2009). A total of 113 randomly selected sites with TSA were surveyed in 38 counties. *Gratiana boliviana* was found to be established at 48% of the surveyed sites, with an average density of 3.2 beetles/plant. Beetles were present at 77% of sites between N 26° and 27° latitude, 79% of sites between 27° and 28° and 54% of sites between 28° and 29°. No beetles were found at 32 sites surveyed north of 29°. The northernmost occurrence of *G. boliviana* was at 28.8° in Seminole Co., near the town of Sanford. Assuming that beetles arrived at survey sites from the nearest release site, the average distance beetles traveled per yr since their release was about 4.7 km. The survey demonstrated that *G. boliviana* was firmly established in southern/central Florida, and had spread from release sites. Later surveys conducted in 2009 and 2010 revealed that *G. boliviana* overwintered in several counties above latitude N 29°, however, beetle densities were low (0 to 0.1 beetles/plant) (Diaz et al. 2012c) (Fig. 5). A possible explanation for the low densities of *G. boliviana* at northern survey sites is asynchrony in seasonal phenologies of TSA and *G. boliviana*. Freezing temperatures in northern Florida do not usually kill TSA plants, but all vegetation aboveground dies back (Mullahey et al. 1998). *Gratiana boliviana* enters diapause in the fall as day length decreases. If a freeze arrives prior to the entry of beetles into diapause, they may starve to death because of lack of food. Similarly, increased abundance of TSA in the spring, through regrowth from root tissue and seed germination, occurs later in more northern areas of Florida than in the southern areas, where plants continue growing throughout the winter in most years. If *G. bo-*

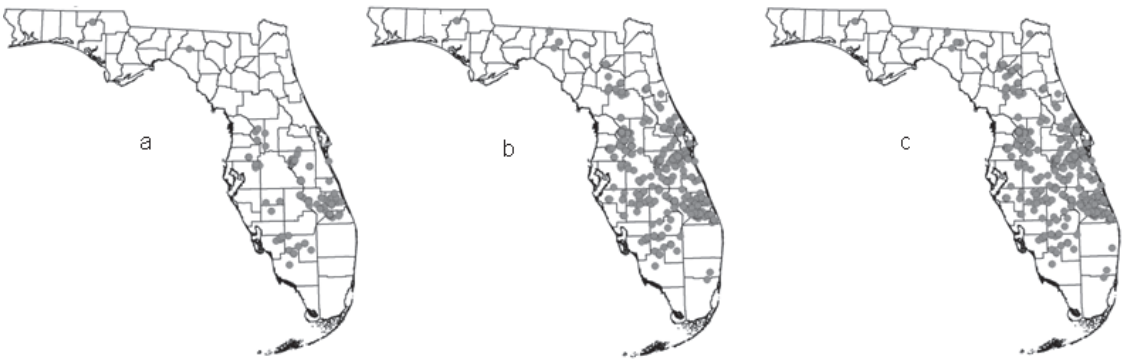


Fig. 2. Cumulative release sites of *Gratiana boliviana* in Florida from 2003 to 2006 (a), 2003 to 2009 (b), and 2003 to 2011(c).

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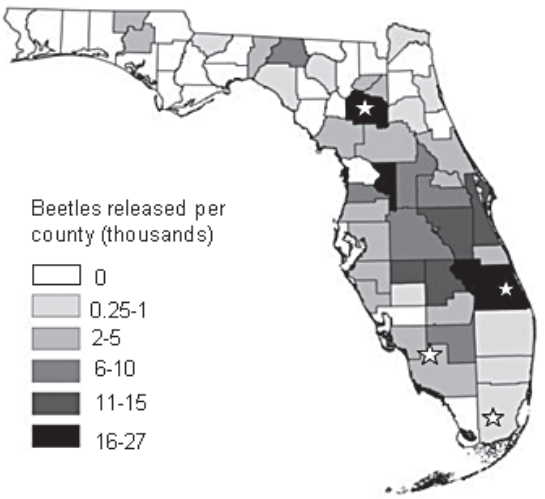


Fig. 3. Total *Gratiana boliviana* adults released from 2003 to 2011 in Florida counties. Stars indicate the locations of *G. boliviana* rearing facilities.

liviana diapause terminates before TSA increases in abundance in the spring, food would be scarce and beetle populations will be negatively affected.

Several studies have been conducted to measure the effect of *G. boliviana* on performance and population dynamics of TSA, and all strongly showed that the beetle has significant negative impacts on TSA densities in Florida. The first study was conducted in the summer of 2005, when 500 beetles were released in a densely infested patch of TSA located in Sumter Co. (Medal et al. 2010). Post-release monitoring showed that defoliation increased from 20% in Mar 2006 to 70% in Oct of the same yr, and from 10% in Apr 2007 to 90% in Aug., 4 months later (Medal et al. 2010). This increase in defoliation was associated with an 88% decrease in fruit production for the yr. A second study examined the performance of TSA at 113 locations in 38 Florida counties and found that plant height, canopy diam, cover and number of fruit on TSA declined as feeding damage by *G. boliviana* increased (Overholt et al. 2009). A 2-yr exclusion study was conducted on a ranch in Saint Lucie County, Florida to compare the performance of insecticide protected plants (no herbivory) to unprotected plants (Overholt et al 2010). In both years of the study, TSA plants treated with insecticide were taller, had greater canopy cover, and higher fruit production compared to unprotected plants. Overall, plant survival was higher in plots protected with insecticide than in unprotected plots (Fig. 6).

A long-term field study was conducted to monitor the population dynamics of *G. boliviana* and TSA every 3 months for a 40-month period at 4 locations on a ranch in St. Lucie County, Florida (Fig. 7) (Overholt et al. 2010). A preliminary survey found no evidence of *G. boliviana*, and 4 wooded areas along a northwest to southeast axis were selected as study sites. Adults of *G. boliviana* were released at Beetle site 1 (800 beetles) and in Beetle site 2 (350 beetles) in Jun 2006, and the number of beetles released was proportional to the area covered by woods in each site. Two other areas were selected as controls (Control sites 1 and 2), and these were separated by at least 1.6 km from the nearest release site. However, the 2 control sites were quickly compromised as beetles were found at both sites during the second sampling in Oct 2006, indicating that the beetles had moved at least 1.64 km in 4 months. At Release site 1, which had a much higher density of TSA at the beginning of the study than the other 3 sites,

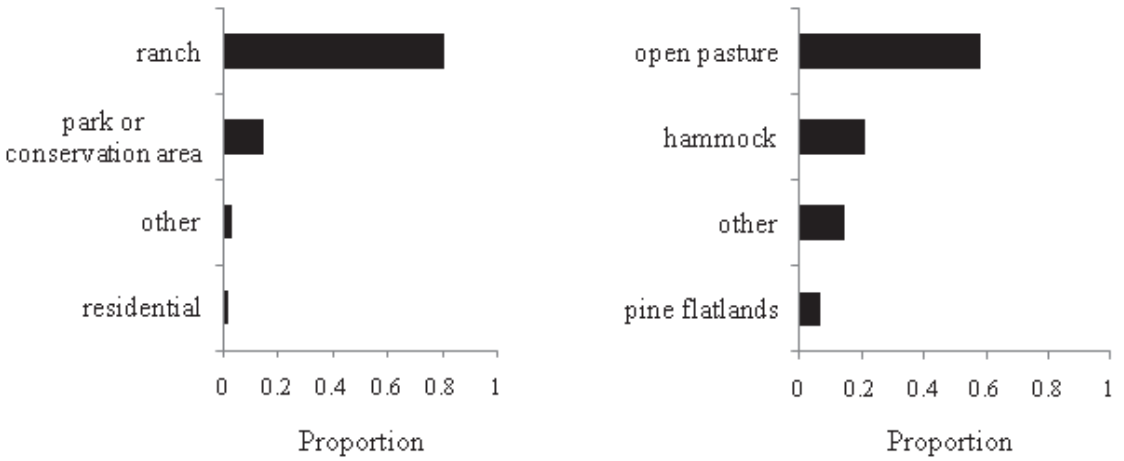


Fig. 4. Type of property (a) and habitat (b) where *Gratiana boliviana* was released in Florida from 2003 to 2011.

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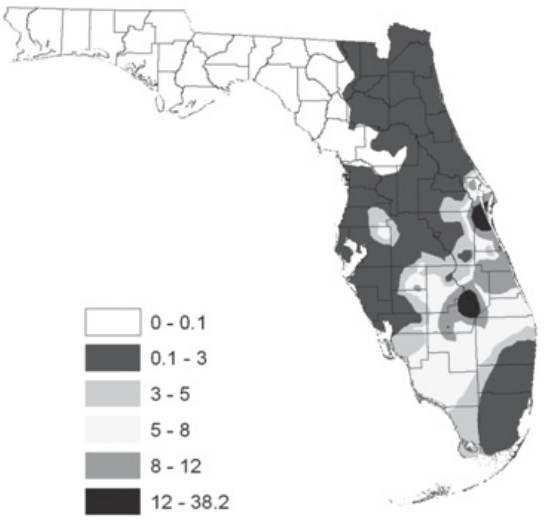


Fig. 5. Densities of *Gratiana boliviana* per plant reported in field surveys conducted in 2008, 2009 and 2010.

the TSA population declined approximately 90% (Fig. 7). At the other 3 sites, the TSA density was initially low, and remained more or less stable during the next 3 yr. This study showed that *G. boliviana* was able to reduce TSA densities to an average of 1 plant/4 m^2 after 2 yr (Overholt et al. 2010).

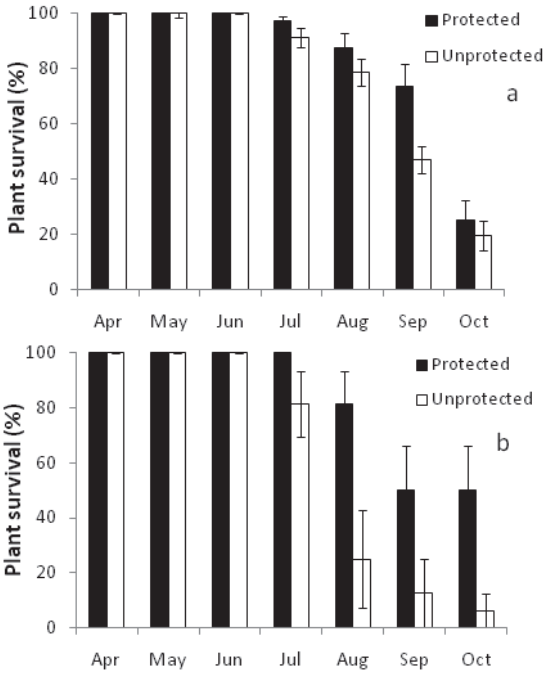


Fig. 6. Survival of unprotected and insecticide protected TSA plants in 2008 (a) and 2009 (b).

Outreach and Technology Transfer

A collaborative effort among federal, state and county agencies facilitated the successful implementation of the biological control program against TSA in Florida. In order to better inform land managers about biological control and promote the use of *G. boliviana* as a management tool against TSA, several types of materials and methods were used to communicate with stakeholders. Demonstrations during field days allowed direct communication between scientists and ranchers, websites served as repositories of pictures, factsheets and videos, and non-technical publications (newspaper and trade magazine articles, brochures), permitted a wide-range distribution of information by mail. A 'how-to' manual with information about beetle biology and recognition, release methods, expected impact and the integration of biological control with other management practices (Diaz et al. 2010) was delivered free of charge to thousands of ranchers across Florida. Additionally, short videos (2-3 min) were produced to deliver information to a broader audience about identification of the plant, recognition of beetle damage and a rancher's perspective on beetle impact.

Economic Impact of the Biological Control Program

Field observations of the reduction of TSA densities after the release of *G. boliviana* motivated questions about land-owners' perceptions of the program. In 2010, a questionnaire designed to determine ranchers' views on TSA, and knowledge about its biological control was mailed to 4,222 members of the Florida Cattlemen's Association. By comparing the surveys from 2006 (Salaudeen et al. 2012a) and 2010, we evaluated changes in perspective by land owners. Responses revealed that the biological control program had an overall positive impact on the ranchers' management of TSA. Ranchers located in central and southern Florida were more aware of the presence of *G. boliviana* on their properties than ranchers in northern Florida, and they reported a reduction on TSA densities. Preliminary analysis suggests that the biological control program reduced the management costs of TSA by 50% statewide, leading to a savings of $3.25 to $8 million annually, or assuming the savings are permanent, $108 to $266 million in total savings (Salaudeen et al. 2012b).

Take Home Messages

1. Soon after the arrival of TSA in Florida, there was wide recognition of its negative effects to

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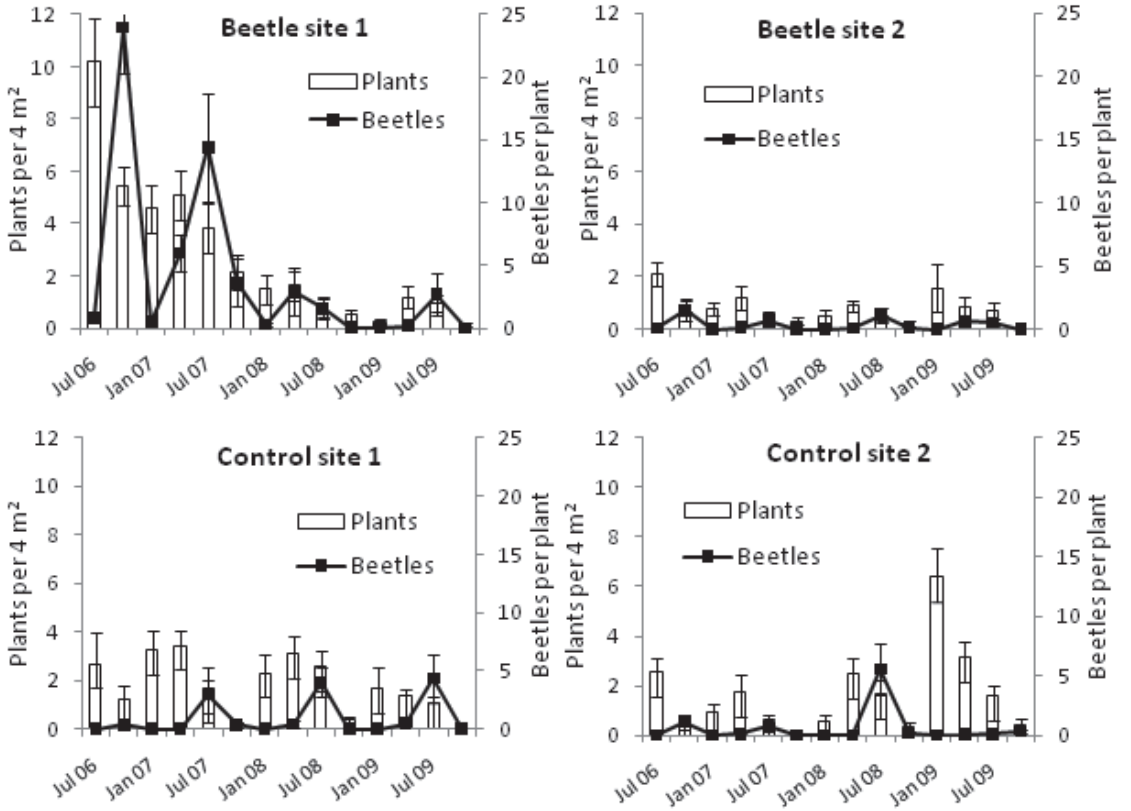


Fig. 7. Density of TSA and *Gratiana boliviana* during a 40 month period in four hammocks in western St. Lucie Co., Florida.

pasture lands and natural areas. The consensus among ranchers and other stakeholders that action was urgently required to identify effective management options was instrumental in convincing funding agencies to provide financial support to initiate a classical biological control program.

2. Close collaboration with scientists in South America was critical during the early stages of the program for facilitation of foreign exploration for natural enemies and subsequent open-field host range testing in the native range.
3. Studies on the biology of *G. boliviana* provided baseline information required for host range testing, development of mass rearing techniques, and selection of release sites in Florida.
4. Monitoring densities of TSA before and after the release of the beetle provided critical information that was used to deliver realistic expectations to ranchers and land managers about the likely impact of the beetle.
5. Statewide surveys conducted in the later stages of the program gave a clear picture of the spatial distribution of the beetle and its impacts; additionally, they provided baseline information required for periodic reformulation of release efforts.
6. Reaching the stakeholders of the program was facilitated by the use of several technology transfer methods such as trade magazines, videos, field days and scientific publications.
7. Twice per yr meetings of the program leaders helped to coordinate release efforts, identify new infestations and evaluate the impact of the beetle across Florida.

ACKNOWLEDGMENTS

The biological control program of TSA was funded in part by the United States Department of Agriculture, Animal Plant Health Inspection Service and the Florida Department of Agriculture and Consumer Services, Division of Plant Industry. We would like to thank the

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hundreds of ranchers and land managers in Florida for allowing access to areas infested with TSA. The release of beetles and technology transfer of the program were greatly facilitated by agents of the Cooperative Extension Service. We thank all the high school, undergraduate and graduate students and foreign interns for their assistance with experiments.

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