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ESTABLISHMENT AND INITIAL IMPACT OF THE LEAF-BEETLE *GRATIANA BOLIVIANA* (CHRYSOMELIDAE), FIRST BIOCONTROL AGENT RELEASED AGAINST TROPICAL SODA APPLE IN FLORIDA

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ABSTRACT

Gratiana boliviana Spaeth (Coleoptera: Chrysomelidae), the first biocontrol agent introduced against tropical soda apple, *Solanum viarum* Dunal (Solanaceae), was released in Florida in the summer 2003. Post-release evaluations have focused on insect population changes and the impact of insect feeding damage on the plant defoliation and fruit production of the target weed. *Gratiana boliviana* quickly established in the release sites, and dispersed causing extensive defoliations to tropical soda apple plants and significant reduction of fruit production. Plant defoliation during the first growing season was directly associated with the increase in number of beetles observed on the tropical soda apple plants. Beetles showed a dispersal ability from the release sites of 1.6 km per year. Two years after the beetle release, most of the tropical soda apple plants at the 3-ha release site in Polk County and at the release site in Okeechobee County, Florida have been replaced by other plant species, resulting in improved pastures. No negative non-target effects have been observed, even in plants closely related, such as the non-natives *Solanum capsicoides* Allioni and *Solanum torvum* Swartz that were growing in the proximity or mixed with tropical soda apple in the monitored sites.

Key Words: *Gratiana boliviana*, *Solanum viarum*, tropical soda apple, Solanaceae, field release, weed biocontrol, monitoring, Florida

RESUMEN

La liberación en el campo en Florida del primer agente de control biológico *Gratiana boliviana* Spaeth (Coleoptera: Chrysomelidae) para la maleza invasora tropical soda apple, *Solanum viarum* Dunal (Solanaceae) fue iniciada en el verano del 2003. Las evaluaciones pos-liberación se han concentrado en los cambios de la población del insecto y el impacto del daño por la alimentación del insecto en la defoliación y producción de frutos de la maleza objetivo. *Gratiana boliviana* se estableció en los sitios liberados y se está dispersando causando defoliaciones extensivas a tropical soda apple y reduciendo significativamente la producción de frutos. La defoliación de las plantas durante el primer ciclo de crecimiento estuvo directamente asociada con el aumento en el número de escarabajos observados en las plantas de tropical soda apple. Los escarabajos mostraron una habilidad de dispersión de 1.6 km/año desde los puntos de liberación. Después de dos años de la liberación del escarabajo, la mayor parte de las plantas de tropical soda apple en el sitio de tres hectáreas en que fueron liberados en el condado Polk en Florida, han sido reemplazadas por otras especies de plantas incluyendo gramas y plantas de hojas anchas. Ningún efecto dañino a plantas no objetivos se ha observado ni en plantas cercanas tales como las no nativas *Solanum capsicoides* Allioni, y *Solanum torvum* Swartz, las cuales estuvieron creciendo en las proximidades o mezcladas con tropical soda apple en los sitios monitorados.

Translation provided by the authors.

Tropical soda apple, *Solanum viarum* Dunal (Solanaceae), which is native to southern Brazil, northeast Argentina, south Paraguay, and Uruguay was first reported in south Florida, USA in 1988 (Coile 1993; Mullahey & Colvin 1993). Since then it has spread throughout Florida and into other states (Alabama, Georgia, Mississippi, South Carolina, and Texas) where it has infested over 400,000 ha of pastures and natural areas (Mullahey et al. 1993; Bryson & Byrd 1996; Medal et al. 1999; Medal & Cuda 1999; Medal et al. 2010b). Current management practices for

tropical soda apple in the United States of America are mostly based on herbicide applications or mowing or combination of these methods (Mislevy et al. 1996, 1997; Sturgis & Colvin 1996; Akanda et al. 1997). These control methods provide temporary weed control at an estimated cost of US \$75 ha⁻¹ to control dense infestations of tropical soda apple (Mullahey et al. 1996). Florida cattle ranchers spend an estimated US \$6.5 to \$16.0 million every year in herbicides and mechanical control methods of tropical soda apple (Thomas 2007). In addition, cost and accessibility

may limit control by herbicides or mowing. In this situation, the use of biological control may become an economically and ecologically viable management alternative for this invasive plant.

Explorations for biocontrol agents of *S. viarum* in its area of origin were initiated in summer 1994 by University of Florida researchers in collaboration with Universidade Estadual Paulista scientists, Jaboticabal campus, Brazil. Sixteen species of insects, including the leaf-beetle *Gratiana boliviana* Spaeth (Coleoptera: Chrysomelidae), were found associated with tropical soda apple in Brazil and Paraguay (Medal et al. 1996). A high level of specificity and significant defoliation of tropical soda apple were demonstrated in host-specificity tests conducted with *G. boliviana* at the Florida Biological Control Laboratory quarantine facilities in Gainesville, at the USDA-ARS (Agriculture Research Service) South American Biological Control Laboratory in Hurlingham, Argentina, and at the USDA-ARS quarantine facility in Stoneville, Mississippi, as well as in extensive field surveys and open-field tests conducted in South America (Gandolfo et al. 1999, 2007; Medal et al. 2002, 2003, 2004). Host-specificity tests including 126 plant species in 35 families were conducted with *G. boliviana* from 1998 to 2001 (Medal et al. 2002, 2003). After 4 years of intensive plant feeding and oviposition tests, *G. boliviana* was the first biological control agent approved for field release by the USDA-APHIS-PPQ (Plant Protection Quarantine) in the southeastern United States for management of tropical soda apple.

Initial releases of this agent in Florida began on 14 May 2003 in Polk County, and up to now more than 200,000 larvae and adult *G. boliviana* have been released in 39 Florida counties, and 33,550 beetles in 2 counties in Georgia, 3 counties in Alabama, and 1 county in Texas (Medal et al. 2008; Overholt et al. 2010). In this article we report the establishment, post-release monitoring, and initial impacts of *G. boliviana* on tropical soda apple plants in 2 release sites in Florida.

MATERIAL AND METHODS

Post-release Evaluation in Polk County, Florida

The first release of the control agent *G. boliviana* was made in north-central Florida (N 28°09'62", W 82°06'05"), in screened cages that enclosed 25 to 47 medium to large *S. viarum* plants 48 to 91 cm in height. The beetles released in this study were the F1 adult progeny of those collected in Paraguay and colonized at the Florida Biological Control Laboratory Quarantine, Florida Department of Agriculture and Consumer Services, Gainesville, Florida. The beetles were sexed with a microscope before release. This first release (initially in screened cages) of *G. bolivi-*

ana was made on 14 May 2003 in a cattle ranch in Polk County, Florida. Six cages (2 × 4 × 3 m) had been set up 1 week before at the release site to prevent the dispersal of not yet mated beetles and, thus, to allow them to build up their numbers. The caged tropical soda apple plants were not sprayed with a chemical pesticide before they were enclosed in cages to determine the effectiveness of the *G. boliviana* beetles. The cages were set up as follows: (1) 40 *G. boliviana* adults were introduced per cage into each of 3 cages (50% females, 50% males), and (2) no *G. boliviana* life forms were introduced into the 3 other cages (control). Therefore, there were several predators (spiders, ants, wasps) inside the cages.

The covers of the cages were removed 13 weeks later (21 Aug 2003) to allow the natural dispersal of the 1,042 adult beetles that were recorded inside the 3 rearing cages. Defoliation of the tropical soda apple plants in all cages was visually estimated, and the tropical soda apple plant heights and number of beetles inside the cages were recorded. After removal of the cage covers, post-release monitoring of *G. boliviana* was conducted from Aug 2003 to Dec 2005 at this release site in Polk County, Florida. To assess the effect of the *G. boliviana* beetles on the target weed tropical soda apple, 20 infested medium to large plants within 100 m of the initial release site were marked with orange tape and permanent water-proof black markers. Plants 4-5 m distant from each other were randomly selected along a transect east of the release site. As a control (plants with no beetles), 10 tropical soda apple plants each 40 to 100 cm tall were randomly selected at approximately 300 m east of the release site and used for comparison during the first 8 months of the evaluation period. This control site was found to be infested by adult *G. boliviana* at the end of May 2004, and the plants could no longer serve as a control. No control plants (free of beetles) were used during the second yr of the evaluation period because the beetles had dispersed throughout an area with a radius of at least 1,600 m by the end of the first year after their initial release in the field. Evaluations of the feeding effects of the beetles on the marked tropical soda apple plants, i.e., % defoliation, plant height, plant canopy diameter, number of fruits per plant, and changes in the density of the beetle population were made every 8 to 12 weeks during the plants' growing season.

The plant cover was also visually estimated in 3 permanently marked quadrats (4 × 3 m) and the plant species composition and number of insects on the plants were recorded twice a year. The percentage defoliation in the quadrats was visually estimated by 2 field technicians. Dispersal of the beetles from the initial release site was evaluated by randomly inspecting tropical soda apple plants within 1,600 m both to the east and to the west of the initial release site. For the cage-beetle exclu-

sion period the plant height was compared with analysis of variance (PROC GLM, SAS INSTITUTE 2001). For the post-release evaluations (after removal of the cover of each cage), the heights and canopy diameters of 20 marked tropical soda apple plants and of 10 control plants were analyzed by the same statistical procedure (PROC GLM) at a significance level of $P \leq 0.05$; and means were separated by the LSD comparison test. A correlation analysis was conducted to determine the relationship between the percentage tropical soda apple plant defoliation and the number of *G. boliviana* on the 20 marked plants.

Field Observations in Okeechobee County, Florida

To evaluate the impact of the *G. boliviana* beetles on a dense stand of tropical soda apple plants (>1,000 plants on approximately 0.5 ha), 500 adult beetles, separated in 5 groups of 100 insects each, were randomly released over the foliage of the tropical soda apple plants on 11 Aug 2004 in Okeechobee County (N 27°41'15", W 81°01'71"). At a second site (N 27°40'75", W 81°02'60") with a dense 1 ha stand of tropical soda apple, 300 and 500 *G. boliviana* beetles were released on 2 Jun 2005 and 7 Jul 2005, respectively. Defoliation was visually estimated 2 months post-release (26 Oct 2004) at the first release site and 1 month post-release (7 Jul 2005) at the second release site. The number of fruits on each of 20 tropical soda apple plants randomly selected within 100 m of the release site in all directions were recorded when the beetles were released and 1 year later.

RESULTS

Post-release Evaluations in Polk County, Florida

When the cage covers were removed 3 months after *G. boliviana* had been released, the tropical soda apple plants inside the 3 cages with *G. boliviana* were completely defoliated (100%) by the 1,042 adult beetles recorded; in contrast only light defoliation ($10 \pm 5\%$) had occurred in the control cages with no *G. boliviana* beetles. Herbivores of tropical soda apple responsible for this light defoliation in the control cages included few larvae and adults of the Colorado potato beetle, *Leptinotarsus decemlineata* Say (Chrysomelidae), and few Lepidoptera larvae (leaf-rollers, leaf-tiers) (Noctuidae). The tropical soda apple plant heights in cages with and without *G. boliviana* were not significantly different (mean \pm SD: 68 ± 12 with beetles vs. 72 ± 14 without beetles; $P \leq 0.05$). The cage covers were removed in Aug 2003 to allow the *G. boliviana* to disperse in the area. The open-field post-release evaluations at this location can be observed in Figs. 1 and 2. The estimated (visual) defoliation increased on average from 46% (Dec 2003) to 94% (Dec 2004), and it

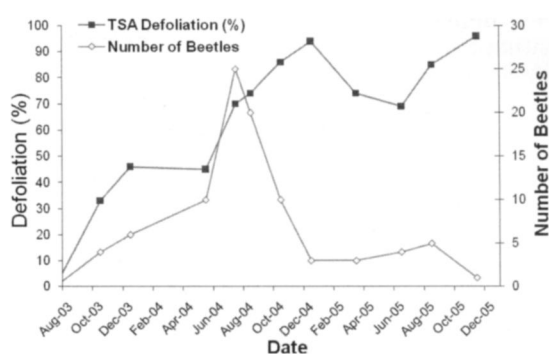


Fig. 1. Mean defoliation (%) of 20 tropical soda apple (TSA) plants and number of beetles per plant at the release site in Polk County, Florida, USA following the release of *Gratiana boliviana* in Aug 2003.

was directly associated ($r = 0.75$) with the increase in number of adult and larval beetles recorded on the tropical soda apple plants during the same period except from Aug to Dec 2004 when the number of beetles per plant decreased (Fig. 1).

In 2005, tropical soda apple plant defoliation remained averaged 69 to 96%. At least half of the 20 marked plants were not able to regrow after complete defoliation by the beetles in the previous yr and also due to competition by other plant species growing nearby. There was a negative correlation ($r = -0.55$) between the level of tropical soda apple plant defoliation and number of *G. boliviana* beetles per plant during 2005 because the beetles had dispersed due to lack of food. Beetles showed a dispersal ability of approximately 1.6 km year^{-1}

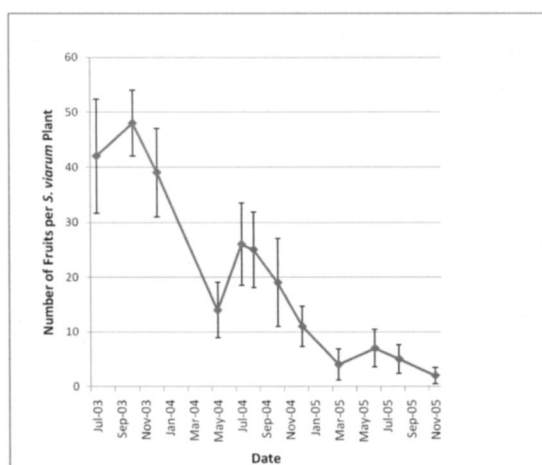


Fig. 2. Mean number of fruits per plant on 20 tropical soda apple plants at the release site in Polk County, Florida, USA following the release of *Gratiana boliviana* in Aug 2003.

during the evaluation period, which is in agreement with annual dispersal distances reported by Medal et al. (2010a) in post-release evaluations conducted during 2006-07 with the same insect in Sumter County, Central Florida. However shorter dispersal distances of the same biocontrol agent were reported by Overholt et al. (2009) in St. Lucie County, Florida. The heights and canopy diameters of the 20 marked tropical soda apple plants within 100 m of the initial release site and of the 10 control plants recorded during 2003-04 can be observed in Table 1. The heights of plants defoliated by *G. boliviana* were significantly lower ($P \leq 0.05$) than control plants with no beetles in Dec 2003 and May 2004, but no significant differences were obtained in the corresponding plant canopy diameters during the same period (Table 1). The number of tropical soda apple fruits produced per beetle-defoliated plant significantly decreased, with none or very few small fruits produced compared with the large number of fruits observed during the summer of 2003 at the time the beetles were released (Fig. 2). Most of the tropical soda apple plants in the 3-ha release site have been replaced by other plant species including the introduced widely used bahiagrass forage (*Paspalum notatum* Flüggé), the natives: *Rubus* sp., dayflower (*Commelina diffusa* Burman), roadside flat-sedge (*Cyperus sphacelatus* Rottb.) and oak (*Quercus* sp.); and the non-natives: Caesar weed (*Urena lobata* L.), air-potato (*Dioscorea bulbifera* L.), and other grasses or broadleaf plants (Figs. 3 and 4). In the 3 quadrats (4×3 m), most of the tropical soda apple plants have been replaced by grasses and broadleaf plants about a yr after the release of *G. boliviana*, and no tropical soda apple plants were recorded in the 3 quadrants 2 years after the release of the beetles at the release site in Polk County (Table 2). Other herbivores, which sometimes were found in relatively low numbers on the tropical soda apple plants, were the Colorado potato beetle, the tomato hornworm, *Manduca quin-*

quemaculata Haworth, and the mired, *Tupiocoris notatus* Distant. These native oligophages seem to have limited impact on the tropical soda apple plants (Sudbrink et al. 2000). The whole release area (3 ha) at the end of Nov 2005 showed an estimated ground cover of tropical soda apple of 5-10%, which is significantly lower than the tropical soda apple population ground cover (80-90%) present in the summer of 2003 before the beetles had been released in the area (Figs. 3 and 4).

Field Observations in Okeechobee County, Florida

The tropical soda apple plants at the release site in Okeechobee County, Florida, were completely defoliated (100%) 2 months after the release of 500 *G. boliviana* adults at the beginning of Aug 2004. This defoliation combined with the impact of 2 months of flooded conditions, produced by rain in the aftermath of Hurricane Jeanne, and resulted in the complete replacement of the tropical soda apple plants by improved grasses such as bahiagrass in this release site.

The visually estimated tropical soda apple defoliation at the second release site, located 3 km away, ranged from 60 to 90% 1 month after the release of the beetles. Fruit production of the tropical soda apple plants was significantly decreased from 35 ± 8 fruits per plant (mean \pm SD) when the beetles were released to 12 ± 7 fruits per plant a year later at this release site.

DISCUSSION

The post-release evaluations and field observations conducted during 2 years in Polk and Okeechobee counties, respectively, indicated that the leaf-feeding beetle *G. boliviana* established and caused significant defoliation and reduction of fruit production of the tropical soda apple plants in both release sites. Despite the great

TABLE 1. HEIGHTS (CM) OF *SOLANUM VIARUM* PLANTS AND DIAMETERS (CM) OF THEIR CANOPIES IN POLK COUNTY, FLORIDA AT VARIOUS DATES FOLLOWING THE RELEASE OF *GRATIANA BOLIVIANA* IN AUG 2003.

Date	Height with Beetles	Height with No Beetles	Diameter with Beetles	Diameter with No Beetles
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
Oct 30/03	90 \pm 15 a	95 \pm 20 a	35 \pm 15 a	30 \pm 20 a
Dec 2/03	85 \pm 12 b	110 \pm 10 a	80 \pm 24 a	90 \pm 16 a
May 05/04	105 \pm 10 b	135 \pm 13 a	85 \pm 14 a	90 \pm 9 a
Jul 06/04	95 \pm 25		88 \pm 11	
Aug 19/04	80 \pm 18		75 \pm 20	
Oct 05/04	40 \pm 20		55 \pm 23	
Dec 14/04	45 \pm 22		40 \pm 17	

Means of heights and diameters within a row followed by the same letter are not significantly different (LSD, $P \leq 0.05$).

The heights of plants and diameters of their canopies with no beetles were only recorded from Oct 2003 to May 2004. After this period beetles were infesting the control plants to which neither beetles nor pesticide had been applied.



Fig. 3. Tropical soda apple stand before *Gratiana boliviana* beetles were released. Tropical soda apple covered 100% of the surface. Polk County, Florida, USA, May 2003.

abundance of potential predators at the release sites such as the green lynx spider, *Peucetia viridans* (Hentz), observed preying on adult beetles, and the red imported fire ants, *Solenopsis invicta* Buren, observed preying on small and large larvae, this biocontrol agent was capable not only of surviving but of rapid population increase and dispersal to target plants elsewhere. The most likely explanation for the relatively low numbers (average <5 per plant) of beetles recorded on the 20 marked tropical soda apple plants during 2005 was due to the beetles' dispersal from the initial release site to tropical soda apple plants as far as 1,600 m away observed in Sep 2005. Dispersal of the beetles can be attributed to the lack of tropical soda apple foliage caused by the beetle feeding on the target plant since they were released in the previous growing season. Dispersal ability of *G. boliviana* adults is similar to that of other chrysomelids monitored in weed biocontrol programs (Grevstad & Herzig 1997; DeLoach et al. 2007), or even greater (1.6-16.0 km/year) as indicated by dispersal assessment conducted throughout Florida since 2003 (Medal et al. 2008; Overholt et al. 2009, 2010).

The tropical soda apple plants defoliated by *G. boliviana* have been replaced by other plant spe-

cies and no negative non-target effects have been observed in seven years of post-release monitoring even by closely related plants such as the non-native spiny shrubs, red soda apple (*Solanum capsicoides* Allioni), and turkeyberry (*Solanum torvum* Swartz), that were growing in the proximity or mixed with tropical soda apple in the monitored sites (Medal et al. 2008). Results of this study indicated that the presence of *G. boliviana* was associated with smaller tropical soda apple plants and fewer fruits per plant. A similar decrease in fruit production was found by Medal et al. (2010a) in Sumter County, Florida during 2006-07. Further this result is in agreement with a 2008 statewide survey conducted to determine the geographic distribution and abundance of *G. boliviana* and to obtain information on the impact of *G. boliviana* on the tropical soda apple plant performance (Overholt et al. 2009). This survey indicated that plants at sites with beetles had 66% fewer fruits than plants at other locations. This clearly indicates that *G. boliviana* is having a negative impact on tropical soda apple growth and reproduction (Overholt et al. 2009, 2010). This is the first case of a biocontrol project undertaken in North America for managing a *Solanum* invasive. A similar project was initiated earlier in



Fig. 4. Site of former Tropical soda apple stand in Polk County, Florida, USA in April 2008. In Aug 2003 *Gratiana boliviana* beetles had been released at this site. During the intervening yr tropical soda apple coverage of the surface was reduced to 0%.

South Africa for managing the non-native invasive plant *Solanum sisymbriifolium* Lam. A South-American leaf-feeder beetle, *Gratiana spadicosa* (Klug) (Coleoptera: Chrysomelidae), released in South Africa in 1994, became established; however, its effect on the target weed has not been as successful as *G. boliviana* on tropical soda apple in Florida due to different biotic (predation), abiotic (climate), and management (burning regimes) factors at the release sites in South Africa (Olckers 1996; Byrne et al. 2002).

Gratiana boliviana is now well established and widespread in central and south Florida. A recent field survey of randomly selected Florida locations revealed that *G. boliviana* was present at 70% (of a total of 340 release sites) of the locations between latitude 26° and 29° (Overholt et al. 2009). However, it seems that new agents are required for northern Florida because no beetles or beetle damage to *S. viarum* north of 29° latitude were found in the survey during the fall of 2008 (Overholt et al. 2009). Management of tropical

soda apple infestations at the central and south Florida release sites is now mostly based on the biological control agent with fewer applications of herbicides and mowing.

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TABLE 2. PLANT SPECIES AND PERCENT OF GROUND COVERED BY EACH SPECIES AT THE SITE IN POLK COUNTY, FLORIDA WHERE *GRATIANA BOLIVIANA* WERE RELEASED IN AUG 2003.

Plant species	Aug-03 Mean ^a	Nov-03 Mean	May-04 Mean	Oct-04 Mean	Jul-05 Mean	Nov-05 Mean
<i>Solanum viarum</i>	95 (5.0)	70 (9.6)	10 (7.0)	5 (3.0)	0 (0.0)	0 (0.0)
<i>Paspalum notatum</i>	3 (3.0)	20 (3.0)	65 (10.0)	60 (17.0)	55 (10.0)	40 (13.0)
<i>Urena lobata</i>	2 (2.0)	2 (0.3)	5 (3.0)	10 (3.0)	25 (8.0)	55 (11.0)
<i>Eupatorium capillifolium</i>	0 (0.0)	8 (2.0)	15 (3.0)	20 (10.0)	10 (7.0)	0 (0.0)
<i>Parthenocissus quinquefolia</i>	0 (0.0)	0 (0.0)	3 (2.0)	5 (2.0)	0 (0.0)	0 (0.0)
<i>Bidens alba</i>	0 (0.0)	0 (0.0)	2 (0.3)	0 (0.0)	0 (0.0)	2 (1.0)
<i>Dioscorea bulbifera</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (3.0)	2 (2.0)
<i>Commelina diffusa</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)
<i>Quercus</i> sp.	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0)	1 (1.0)
<i>Trifolium pratense</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.3)	0 (0.0)

^aMean (%) of the visually estimated plant species composition in 3 quadrants. Numbers in parentheses represent the standard deviation.

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