Releases, Distribution and Abundance of Gratiana boliviana (Coleoptera: Chrysomelidae), a Biological Control Agent of Tropical Soda Apple (Solanum viarum, Solanaceae) in Florida

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RELEASES, DISTRIBUTION AND ABUNDANCE OF *GRATIANA BOLIVIANA* (COLEOPTERA: CHRYSOMELIDAE), A BIOLOGICAL CONTROL AGENT OF TROPICAL SODA APPLE (*SOLANUM VIARUM*, SOLANACEAE) IN FLORIDA

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ABSTRACT

From 2003 to 2008, 176,643 *Gratiana boliviana* Spaeth (Coleoptera: Chrysomelidae) were released in Florida as part of a biological control program targeting tropical soda apple (TSA) *Solanum viarum* Dunal (Solanaceae). The spatial distribution of releases was clustered with more beetles released in south/central Florida than further north. A survey conducted in the fall of 2008 found *G. bolviana* present at >70% of randomly selected locations between 26° and 29° latitude, but no beetles were found at sites further north. The presence of beetles and beetle damage were associated with smaller TSA plants and fewer fruits per plant. The absence of beetles in northern Florida may be due to the fewer number released in that area, but also could be influenced by land cover and climate.

Key Words: Biological control, tropical soda apple, *Gratiana boliviana*, establishment, spread, invasive species

RESUMEN

Del 2003 hasta el 2008, se liberaron 176,643 individuos de *Gratiana boliviana* Spaeth (Coleoptera: Chrysomelidae) en la Florida como parte de un programa de control biológico del "tropical soda apple" (TSA) (*Solanum viarum* Dunal (Solanaceae)). La distribución espacial de las liberaciones fue agrupada con más escarabajos liberados en el sur/central de la Florida que en áreas más al norte. Un sondeo realizado en otoño de 2008 encontró *G. bolviana* presente en un >70% de los lugares seleccionados al azar entre la latitud de 26° y 29°, pero ningún escarabajo fue encontrado en los sitios mas al norte. La presencia de los escarabajos y el daño causado por ellos fueron asociados con plantas de TSA mas pequeñas y menos frutos por planta. La ausencia de los escarabajos en el norte de la Florida puede ser debido al número menor de individuos liberados en esta área, pero también puede haber sido influenciada por la cubertura de la tierra y el clima.

Tropical soda apple (TSA), *Solanum viarum* Dunal (Solanaceae), is a prickly, perennial weed from South America that was first reported in Florida in 1988 (Mullahey et al. 1993). The plant quickly spread throughout Florida and into several other states including Georgia, South Carolina, North Carolina, Alabama, Georgia, Mississippi, Tennessee and Texas (EDDMapS 2008, The Plants Database 2008). TSA invades rangelands, improved pastures, and natural areas with an estimated 1 million acres infested in Florida (Mullahey 1996). Although cattle do not consume TSA leaf tissue, they readily consume fruits, and in doing so vector seeds to new areas in their digestive tracts (Brown et al. 1996). Cattle ranchers in Florida spend an estimated $6.5 to $16 million annually to control TSA (Thomas 2007).

Exploration for classical biological control agents of TSA was initiated in 1994 in South America. One of the agents discovered in Argentina and Paraguay was *Gratiana bolviana* Spaeth (Coleoptera: Chrysomelidae) (Medal et al. 1996). *Gratiana boliviana* is a highly specialized herbivore (Gandolfo et al. 2007; Medal et al. 2002); larvae and adults feed on foliage in the upper third of the canopy of TSA plants, resulting in a distinctive shotgun-hole feeding pattern on leaves. Eggs are laid individually on the upper or lower surfaces of leaves, and eclose after about 5 d at 25°C. Larvae complete 5 instars in 15-18 d before pupating on the underside of leaves (Diaz et al. 2008). The beetles enter an adult reproductive diapause during winter months from about Nov to Apr in central Florida. During this period, beetles are found in leaf litter under TSA plants (Overholt, unpublished data). *Gratiana boliviana* was first released in Florida in 2003 in Polk Co. and has since been released at many other locations in the state.

During the summer of 2008, beetles were found at a number of locations several kilometers from where they had been released in previous years, and thus efforts were being expended travelling to locations to make new releases, only to find that beetles were already present. The primary objectives of the present study were to document the numbers and locations of beetles released in Florida, and to estimate the abundance and spatial distribution of *G. boliviana* in Florida. This information will be used to select future release sites. Additionally, plant size, cover and number of fruits per plant were measured at survey sites to examine possible impacts of *G. boliviana* on plant performance. Finally, we investigated differences in land cover and climate along a south-north gradient which may influence the presence and abundance of beetles at various latitudes in Florida.

Materials and Methods

Insect Rearing

Gratiana boliviana was reared by 3 institutions at 5 locations in Florida: University of Florida (UF) at Gainesville, Fort Pierce and Immokalee; Florida Department of Agriculture and Consumer Services (FDACS) at Fort Pierce; and USDA/APHIS in Miami. Beetles were reared on whole live plants (30-60 cm in height) either in a greenhouse, screenhouse or large outdoor screen cages (2 m × 2 m × 2.5 m). Immature and adult beetles were transferred to new plants every 2-4 weeks when plants began to exhibit signs of excessive feeding (approximately 50% of leaf tissue consumed). Non-diapausing colonies were maintained over the winter at 2 locations (FDACS/Fort Pierce and UF/Gainesville) by rearing insects under a 14h:10h (L:D) photoperiod. Each spring, field collected beetles were added to these colonies, and to re-establish seasonal colonies at other rearing facilities.

Releases

Property owners and land managers were informed of the availability of *G. bolviana* as a TSA biological control agent through Cooperative Extension Service agents, extension publications (Medal et al. 2006, 2007), an article in a ranching trade journal (Overholt et al. 2008), and by word of mouth from neighbors. A database of releases was maintained throughout the project and included the date of release, geographic coordinates of release sites, number of beetles released, TSA density, and property owners' names. At the time of release, property owners and land managers were asked not to apply herbicides or mow release areas to increase the likelihood of beetle establishment. In total, 176,643 *G. boliviana* were released in Florida between May 2003 and Oct 2008. Because of a few missing records, geo-coordinates are available only for the release of 163,185 beetles on 374 occasions. Beetles were released at 340 different sites, some sites receiving repeated releases (2-4). The vast majority of releases were of adult beetles, although a few releases were made by placing plants infested with a mixture of life stages directly in the field. The number of beetles released on each occasion ranged from 25 to 2700 and averaged 439 ± 20 beetles (mean ± SE) per release.

Statewide survey

From Sep 4 to Oct 30, 2008, an extensive survey was conducted to estimate the distribution and abundance of *G. boliviana* throughout Florida. The survey was made in the fall because the abundance of *G. boliviana* was typically highest at this time of year (Overholt, unpublished data). Because tropical soda apple is mainly a weed of pastures and rangelands, the survey was primarily conducted in counties that had more than 10,000 head of cattle (USDA/NASS 2008). Three counties (Miami-Dade, Seminole, and Union) with less than 10,000 cattle were included in the survey. Random points (10) were generated for each of the 40 counties included in the survey with a random geospatial coordinate generator included in the GIS software program AWHERE® Professional (AWHERE, Inc., Golden CO). Personnel conducting the survey selected 3-5 random sites per county based on land cover (pasture), presence of TSA, and accessibility. In a few counties, only 1 or 2 sites were sampled due to difficulty in finding locations with TSA, and in 2 counties (Bradford and Gilchrist), no TSA was found. In total, 113 sites in 38 were surveyed (Fig. 1). At each site, 10 plants were sampled by walking a straight line through the center of the infested area and selecting a plant every 4-5 steps. The following parameters were measured or estimated for each selected plant:

- Height from the ground to the highest foliage.
- Diameter of the crown at the widest point.
- Number of fruit.
- Plant cover (visual estimate; scale of 1-5 with 1 = 0-19% of surface area under the plant canopy covered, 2 = 20-39% cover, 3 = 40-59% cover, 4 = 60-79% cover, and 5 = >80% cover.

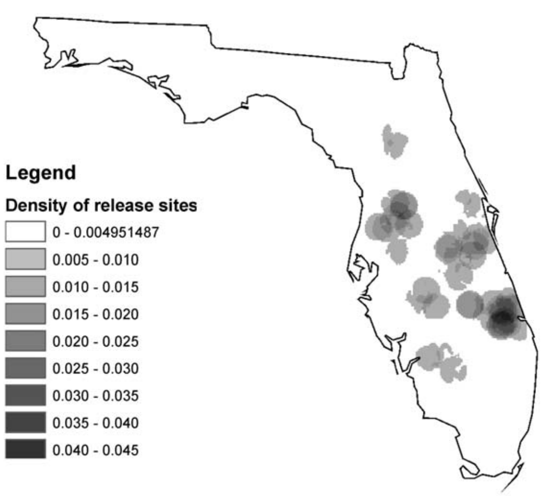

Fig. 1. The spatial distribution of *Gratiana boliviana* releases in Florida from 2003-2008. Density estimates are interpolated values in units of releases/m^2.

- Damage due to *G. boliviana* (visual estimate; scale of 0-5 with 0 = no damage, 1 = 1-19% of leaf tissue consumed, 2 = 20-39% consumed, 3 = 40-59% consumed, 4 = 60-79% consumed, and 5 = >80% consumed.
- Number of *G. boliviana* larvae, pupae, and adults.

Data analyses

All spatial analyses were conducted with ArcMap 9.2 (ESRI, Redlands CA). Nearest neighbor analysis was conducted with the 'Spatial Statistical Tools' to examine the spatial distribution of release points. This tool calculates a *Z* statistic to test the hypothesis that points are randomly distributed in space. The average distance between nearest release sites was calculated with the 'Calculate Distance from Neighbors Utility'. The average distance between survey sites and the nearest release site was calculated by the 'Near' tool. The density of release points was analyzed with the 'Point Density' tool in 'Spatial Analyst Tools' with a neighborhood setting of 20 km.

The effect of latitude and sampling date on the number of *G. boliviana*/plant was examined with simple linear regression. To determine whether there was a latitudinal bias in the temporal pattern of sampling, latitude was regressed on sampling date. An interpolated surface of *G. boliviana* intensity across the surveyed areas in Florida was generated by the Inverse Distance Weighted (IDW) method in the Geostatistical Analyst, with 6 nearest neighbors and a power setting of 2. IDW is a simple deterministic interpolator which estimates values at un-sampled locations as weighted averages of observed values within the designated neighborhood, with weights decreasing as distance increases. The influence of the intensity of releases (number of beetles released in an area) on the number of beetles found at survey sites was examined by creating raster datasets (20 km × 20 km) from the IDW interpolated surface of *G. boliviana* intensity and a similarly interpolated surface of numbers released. Grid cell values of beetle intensity and release density were extracted from the 2 raster datasets and a multiple regression was performed to examine the relationship between *G. boliviana* intensity and 2 independent variables; density of releases and latitude.

To visualize the possible influence of cold temperatures on the distribution of *G. boliviana*, the IDW method with 15 neighbors and a power setting of two was used to generate a surface of the first occurrence of freezing temperature (≤0°C) in the winters between 2003, when beetles were first released, and 2008. Weather data from 120 climate stations in Florida were extracted from the Applied Climate Information System (CLIMOD 2008).

Pasture cover in 6 latitudinal zones of Florida (25°-26°, 26°-27°, 27°-28°, 28°-29°, 29°-30°, and 30°-31°) was examined by constructing boundary polygons for each zone. Vegetation cover for Florida was acquired from the Habitat and Landcover raster dataset (30 m × 30 m resolution), which classified ground cover from remote sensing data into 43 categories (FFWCC 2004). A GIS layer including 2 of the categories, unimproved pasture and improved pasture, was extracted from the dataset and converted from raster to polygon. The intersection of each zone and pasture cover was used to calculate the total amount of pasture in each zone, and the mean, minimum and maximum patch size per zone. Mean patch size was compared between zones with analysis of variance and means separated with LSD by the PROC GLM procedure of SAS (SAS Institute 2001).

Multiple linear regressions with backward elimination of non-significant ($P > 0.05$) independent variables were performed to examine the relationships between plant performance variables (plant height, diameter, cover, and number of fruit) and the independent variables of *G. bolviana* damage score, site latitude, and sampling date (model 1), and the number of *G. boliviana*/plant, latitude, and sampling date (model 2). Latitude and sampling date were included in the models to estimate the effects of *G. boliviana* on plant performance independent of these variables. All regressions were performed with PROC REG procedure of SAS (SAS Institute 2001).

RESULTS

The average distance between nearest release sites was 4.4 ± 0.7 km, with a minimum of 2.3 km and a maximum of 217 km. The distribution of release sites was clustered ($Z = -26.1, P < 0.01$) with several patches of high intensity as indicated in Fig. 1. More beetles were released between 27° and 29° than in areas further north or south (Fig. 2). The number of beetles released per year increased with time and peaked at 86,205 in 2008 (Fig. 3).

Gratiana boliviana was found at 48% (54/113) of the surveyed sites, with intensity averaging 3.2 ± 0.6 beetles/plant (range 0-38.2 beetles/plant). Damage due to *G. boliviana* was found at 2 sites where no beetles were found. Latitude and sampling date affected the presence of beetles with significantly fewer beetles as latitude increased ($F_{1,111} = 10.6, P=0.0015$) and as the date of sampling increased ($F_{1,111} = 6.23, P = 0.014$). The regression between latitude and sampling date was significant ($F_{1,111} = 4.09, P = 0.046$), with sampling occurring slightly later in the season as latitude increased (0.16 d later with each increase of 1 degree latitude). The average date of sampling at latitudes below 29° was Sep 27 (± 1.7 d), and at latitudes greater than 29° was Oct 1 (± 2.7 d). The average distance between sites where beetles were present and the nearest release site was 6.3 ± 0.9 (SE) km, and the furthest that beetles were found from a release site was 32.4 km. On average, there were 488 ± 62.6 d between the date of release at the nearest point and the date of the survey at sites where beetles were found. Assuming that beetles arrived at the survey sites from the nearest release site, the average distance beetles traveled per year since their release was about 4.7 km. The beetles found at 32.4 km from the nearest release site had moved about 8.1 km/year since their release.

Beetles were present at 77% (10/13) of sites between 26° and 27° latitude, 79% (30/38) of sites between 27° and 28° and 54% (14/26) of sites between 28° and 29°. No beetles were found at 32 sites sur-

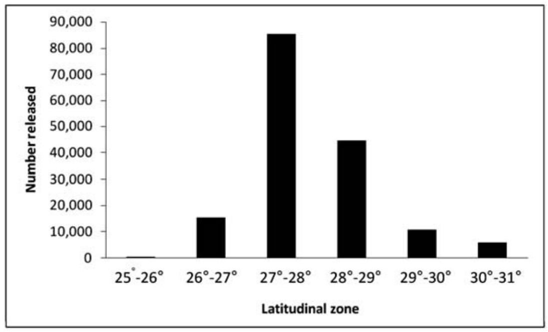

Fig. 2. Number of *Gratiana boliviana* released in Florida by latitudinal zone.

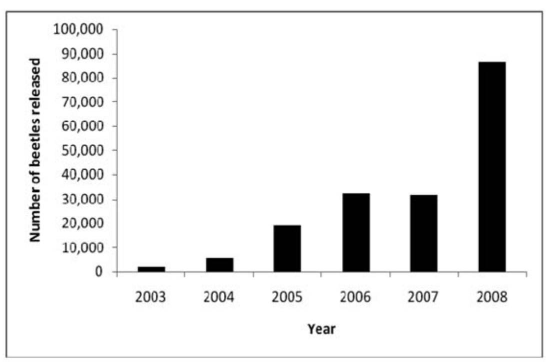

Fig. 3. Number of *Gratiana boliviana* released per year in Florida.

veyed north of 29° latitude, at 2 sites sampled in extreme south Florida or in a small area of the west coast near Sarasota. The northernmost occurrence of *G. boliviana* was at 28.77° in Seminole Co. near the town of Sanford. The interpolated intensity surface indicates highest densities along a band running from approximately Naples on the west coast to Fort Pierce on the east coast, and northward on the east coast in Volusia county (Fig. 4). It should be noted that the spatial interpolation was based on data from 38 counties, but projected into counties not sampled. However, the beetle was not predicted to occur in any counties that were not sampled, and thus, the interpolation is likely a conservative estimation of the actual distribution. The earliest occurrence of freezing temperatures in Florida during the period of study (2003-2008) is shown in Fig. 5.

The number of beetles released in 20 km × 20 km cells, and the latitude of the cells influenced beetle intensity in those cells with the number of beetles increasing as the number released increased (partial regression coefficient = 0.69, t = 11.5, P < 0.0001) and decreasing as latitude increased (partial regression coefficient = -0.0004, t = -13.8, P < 0.0001).

The amount of pasture cover in different zones ranged from 2,205 ha between 25° and 26° latitude to 500,674 ha between 27° and 28° latitude. The mean patch size ranged from 2.91 ha between 28° and 29° to 9.34 ha between 27° and 28°. Patch size was smaller in the 28°-29° zone than all other zones except 25°-26°, where there was very little pasture ($F_{5,238218}$ = 28.5, P < 0.0001, LSD) (Table 2).

Sampling date was not significant (P > 0.05) and therefore removed from multiple regression models of plant parameters against *G. boliviana* damage estimates and beetle numbers, but latitude was significant and retained. Plant height, diameter, and cover were all negatively affected by the number of *G. boliviana*/plant, but the number of fruit was not different. As the *G. boliviana* damage score increased, plant height, diameter, cover, and the number of fruit were reduced (Table 1).

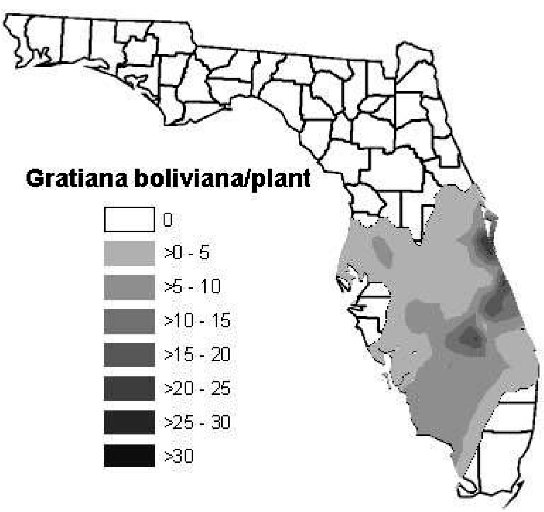

Fig. 4. Interpolated values of *Gratiana boliviana* intensity based on a state-wide survey conducted in the fall, 2008.

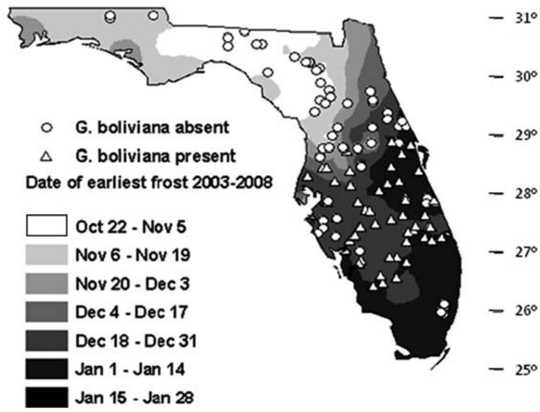

Fig. 5. Date of earliest frost in Florida during the period 2003-2008 and locations where *Gratiana boliviana* was found to be present or absent during a state-wide survey conducted in fall, 2008.

Discussion

The survey demonstrated that *G. boliviana* is firmly established in south/central Florida, and has dispersed from the original release sites. TSA plants were smaller and produced fewer fruit as the number of beetles and damage increased, clearly suggesting that beetles are having a negative impact on TSA plants. The absence of beetles at the southernmost survey sites in Miami-Dade and Broward counties, and in the west coast area near Sarasota, is not surprising as no beetles were released in these areas prior to the time of the survey. However, the absence of beetles and beetle damage in northern peninsular Florida was unexpected, particularly considering evidence that beetles are established at some locations in northern Florida (Medal et al. 2007), and in southern Georgia and Alabama (Roda, unpublished data). Moreover, cold tolerance studies suggested that *G. boliviana* could establish as far north as 32°-33° (central Georgia) (Diaz et al. 2008). Thus, it appears that direct effects of cold temperatures may not explain the absence of beetles at surveyed locations in north Florida. At least 4 non-mutually exclusive hypotheses could explain the absence of *G. boliviana* at sites surveyed in north-central Florida. First, fewer beetles were released in this area compared to southern locations, and therefore it may be simply a matter of time until population densities increase and beetles spread in northern areas. The signifi-

TABLE 1. PARTIAL CORRELATION COEFFICIENTS OF THE NUMBER OF *GRATIANA BOLIVIANA* AND LATITUDE (MODEL 1) AND *G. BOLIVIANA* DAMAGE SCORE AND LATITUDE (MODEL 2) AGAINST PLANT HEIGHT, DIAMETER, COVER, AND NUMBER OF FRUIT.

	Dependent variable							
	TSA Height		TSA Diameter		TSA Cover		TSA Fruit	
Regression models	Partial correlation coefficient	*P* value	Partial correlation coefficient	*P* value	Partial correlation coefficient	*P* value	Partial correlation coefficient	*P* value
Model 1								
G. boliviana /plant	-0.63	0.01	-1.28	0.01	-0.03	0.03	-0.37	0.13
Latitude	5.80	<0.0001	12.01	<0.0001	-0.18	0.005	4.18	0.001
Model 2								
G. boliviana damage score	-4.78	0.0007	-9.88	0.0005	-0.29	<0.0001	-3.42	0.01
Latitude	4.52	0.0008	8.87	0.0009	-0.28	<0.0001	2.85	0.02

cant relationship between the number of beetles released in 20 km × 20 km grid cells and the number of beetles found at randomly selected sites in those cells provides support for this hypothesis.

A second possible explanation is that the survey was conducted slightly later in the year at more northern latitudes. *Gratiana boliviana* enters an adult reproductive diapause in the fall, during which time they are found in leaf litter under TSA plants. Sampling at northern latitudes may have occurred after beetles had entered diapause, such that they were not found actively feeding on TSA leaves. However, differences in sampling dates between latitudes were small, and therefore we do not believe that the presence of beetles was greatly affected. Moreover, damage characteristic of *G. bolviana* feeding was not found at any sites north of 29° latitude, strongly suggesting that beetles were absent from northern survey sites for at least several weeks prior to the survey.

Landscape features may provide an explanation for the lack of beetles at northern Florida latitudes. TSA is primarily an invader of pastures/rangelands, and the spatial distribution of habitats suitable for TSA may vary with latitude. Comparison of patch size of pasture between latitudinal zones does not provide support for this hypothesis, as patch size was lower in the 28°-29° latitudinal zone, where beetles were found, compared to zones further north where they were absent. However, the presence of pasture is not equivalent to the presence of TSA. Unfortunately, there is no information available on the spatial distribution of TSA in Florida at a resolution that would allow an examination of patch size, and connectivity between patches. Moreover, the spatial distribution of TSA is likely dynamic. The plant can rapidly colonize new areas through transport in the digestive tracks of livestock and wild mammals (Mullahey et al. 1998), and may disappear from areas due to active management by mowing and herbicide application, or from natural events such as flooding (Mullahey et al. 1998).

The most likely explanation for absence of *G. boliviana* at northern survey sites may be asynchrony in seasonal phenologies of TSA and *G. boliviana*. Freezing temperatures do not usually kill TSA plants, but all above-ground parts die back (Mullahey et al. 1998); the only food source for the beetles. *Gratiana boliviana* enters diapause in the fall as day length decreases, although the critical day length for diapause induction is not yet established. If a freeze arrives prior to beetles entering diapause, immatures would certainly be killed as they do not migrate from the plant on which eggs that they hatched from were laid, and food availability for adults would be greatly diminished. The earliest occurrence of freezing temperatures during the period of the study (2003-2008) coincides with the time of year when bee-

TABLE 2. PASTURE COVER IN DIFFERENT LATITUDINAL ZONES OF FLORIDA.

Latitudinal zone	Number of patches	Mean patch size ± SE (ha)*	Minimum patch size (ha)	Maximum patch size (ha)	Total pasture (ha)
25°-26°	505	4.37 ± 1.1ab	0.06	454	2,205
26°-27°	22,806	5.66 ± 0.5a	0.001	8377	128,997
27°-28°	30,306	9.34 ± 0.7a	0.0006	22,782	500,674
28°-29°	86,231	2.91 ± 0.2b	0.0007	14,657	303,820
29°-30°	34,435	4.64 ± 0.3a	0.0001	5780	178,663
30°-31°	18,501	7.34 ± 0.3a	0.01	2129	135,869

*Means followed by the same lower case letter are not statistically different (LSD, $P < 0.05$).

tles enter diapause, and may be a factor that influences the distribution of *G. boliviana*. Similarly, the increase of TSA in the spring, through regrowth from root tissue and seed germination occurs later in more northern areas of Florida than in the south, where plants may even continue growing throughout the winter in some years (Overholt, unpublished data). If *G. boliviana* diapause terminates before TSA increases in abundance in the spring, food would be scarce and population increase of beetles may be affected. A lack of synchrony between diapause and climate has been shown to negatively affect the distribution of *Diorhabda elongata deserticola* (Brullé) (Coleoptera: Chrysomelidae), a biological control agent released in the western US for control of *Tamarix* spp. The insect established north of 38° latitude, but not further south due to day length not being long enough to maintain a non-diapausing population for more than 1 generation (Bean et al. 2007; Lewis et al. 2007). Bean et al. (2007) also speculated that there was a lack of synchrony between univoltine populations south of 38° and their host plants that negatively affected survival.

The fall 2008 survey provides information on the distribution and abundance of *G. boliviana* in Florida that will be useful for selecting locations for future releases. With the exception of the area near Sarasota where no beetles were found, no further releases appear to be warranted in central/south Florida below 29° latitude, where beetles were found at > 70% of release sites. However, releases should be made in the spring/summer of 2009 at locations north of 29°.

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