EFFECTS OF BEETLE DENSITY AND TIME OF DAY ON THE DISPERSAL OF *GRATIANA BOLIVIANA* (COLEOPTERA: CHRYSOMELIDAE)

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Abstract

The leaf beetle, *Gratiana boliviana* Spaeth, is a biological control agent of tropical soda apple (*Solanum viarum* Dunal), a noxious weed invading the rangeland and agricultural fields of Florida and other southeastern states. In caged experiments, we examined the influence of beetle density and the time of day on the dispersal of the leaf beetle. Increasing beetle density from 2 to 100 beetles per plant did not increase *G. boliviana* dispersal, as long as the host plants were not severely defoliated. An increase in beetle density from 10 to 100 beetles per plant significantly reduced the per capita fecundity from 0.3 to 0.02 eggs over a 24-h period. Thus, a mass rearing method for *G. boliviana* should consider the potential detrimental effect of increasing density on the fecundity of the leaf beetle. When the dispersal activity of the leaf beetle was observed between 8:00 and 18:00 h, the proportion of beetles dispersing from a given plant increased throughout the morning hours and peaked at noon. A monitoring or sampling program of *G. boliviana* should be conducted in the early morning or late afternoon when the beetles' tendency to disperse is the lowest in order to more accurately sample for the population density in a certain area. Beetle density and the time of day interacted to influence the proportion of *G. boliviana* dispersing from a given plant. The proportions of beetles that dispersed were not different among the different time periods at densities of less than 40 beetles per plant. At or above 40 beetles per plant, the proportion of beetles that dispersed peaked at noon. This study suggests that a myriad of factors, including density, feeding damage, photoperiod and host plant quality, interact to determine the dispersal pattern of *G. boliviana*.

Key Words: biological control, emigration, mass rearing, noxious weed, tropical soda apple

Resumen

El crisomélido, *Gratiana boliviana* Spaeth, es un agente de control biológico de *Solanum viarum* Dunal, una maleza nociva que invade las pasturas y campos agrícolas de la Florida y otros estados del sureste de los EEUU. En experimentos en jaulas, se examino la influencia de la densidad del escarabajo y la hora del día en que el escarabajo crisomélido se dispersa. Al aumentar la densidad del crisomélido de 2 a 100 escarabajos por planta no aumento la dispersión de *G. boliviana*, esto cuando las plantas hospederas no fueron severamente defoliadas. Un aumento en la densidad del escarabajo de 10 a 100 escarabajos por planta redujo significativamente la fecundidad por hembra de 0.3 a 0.02 huevos durante un periodo de 24 horas. Por lo tanto, un método de cría masiva de *G. boliviana* debe considerar el efecto potencialmente prejudicial del aumento de la densidad sobre la fecundidad del crisomélido. Cuando la actividad de dispersión del crisomélido en una planta dada fue observada entre los 8:00 y 18:00 horas, la proporción de los escarabajos que se dispersan aumento durante las horas de la mañana con su máximo al medio día. Se debe realizar un programa de monitoreo y muestreo de *G. boliviana* temprano en la mañana o al final de la tarde cuando la tendencia de los escarabajos para dispersarse es la mas baja para obtener una muestra mas precisa de la densidad de la población en una área especifica. La densidad del escarabajo y la hora del día se relacionan para influenciar la proporción de *G. boliviana* que se dispersa de una planta dada. La proporción de los escarabajos que se dispersaron no fue diferente entre los periodos de tiempo en las densidades de menos de 40 escarabajos por planta. A un nivel de 40 escarabajos por planta o mas, la proporción de escarabajos que se dispersaron fue la mas alta al medio día. Este estudio sugiere que hay un gran número de factores, incluyendo la densidad, el daño por alimentación, el fotoperíodo y la cualidad de la planta hospedera, que se interactúan en la determinación del patrón de dispersión de *G. boliviana*.

Tropical soda apple (TSA), *Solanum viarum* Dunal, is a noxious weed introduced into Florida from South America in the 1980s (Medal et al. 2002). Because of its large reproductive capability (40,000-50,000 seeds per plant reported by Mullahey et al. 1993 and Pereira et al. 1997) and easy dissemination by cattle and wildlife that ingested the fruits, TSA spread quickly throughout Florida (Medal et al. 2002). TSA has also established in Alabama, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee (SE-EPPC 2008). Infestation by TSA not only reduces suitable grazing land and cattle carrying capacity (Mullahey et al. 1993), they also serve as host plants or reservoirs for at least six viruses that affect vegetable production (McGovern et al. 1994). Management options against TSA include combinations of chemical (herbicide applications), mechanical (mowing), and biological (herbivores) practices.

A biological control program against TSA was initiated in 1994 (Medal et al. 1996). The leaf beetle, *Gratiana boliviana* Spaeth (Coleoptera: Chrysomelidae), collected from Argentina and Paraguay, was approved for release in 2003. The leaf beetles have been released in 29 counties in Florida, as well as several sites in Alabama, Georgia, and Texas (UF-BCRCL 2008). *Gratiana boliviana* has established in almost all release sites (Medal et al. 2008). A recent study by Diaz et al. (2008) suggested that *G. boliviana* may be able to establish near 32-33° north latitude. The nymphs and adults of *G. boliviana* feed on the foliage of TSA, causing moderate to high level of defoliation, gradual weakening of the TSA plants, and decrease in fruit production (Medal et al. 2008). Laboratory and field studies have confirmed that *G. boliviana* attacks only TSA, even when the closely related, non-target red soda apple (*Solanum capsicoides* All.) and turkey berry (*Solanum torvum* Sw.) were available in close proximity of the TSA plants (Medal et al. 2002).

The dispersal ability of *G. boliviana* is not known. Field observations at release sites in central and southwestern Florida indicated that the leaf beetle is capable of dispersing an average of 1.6-16 km (1-10 miles) per year (Medal et al. 2008; W. Overholt, unpublished data). The cues responsible for initiating local dispersal of *G. boliviana* are even less well understood. Factors affecting dispersal behavior of insects include environmental factors, life history strategy, crowding, physiological status of the insects, and habitat and resources conditions (Schowalter 2000). In this study, we evaluated the dispersal patterns of *G. boliviana* in relation to the beetle density and the time of day. Results of this study will help researchers in designing more effective rearing and monitoring methods for the leaf beetle.

Materials and Methods

Study Plants

Eight-week old TSA plants were grown from seeds in plastic pots (15 cm in diameter) at the USDA-ARS Subtropical Horticulture Research Station (SHRS), Miami, FL. The plants were fertilized with 10g of slow release Osmocote™ (The Scotts Company, Marysville, OH). In order to minimize the difference in structural characteristics, the TSA plants were selected and grouped by height, width, and the numbers of leaves and branches. The selected plants were on average (± SEM) 36.29 ± 0.5 cm high, 25.58 ± 0.6 cm wide, and had 2.54 ± 0.1 branches and 12.6 ± 0.4 leaves. The TSA plants were carefully examined and any pre-existing eggs, larvae, and adult *G. boliviana* were removed. The plants were then placed individually in fine-mesh cages (60cm × 60cm × 60cm) a short time before the release of beetles.

All experiments were conducted in outdoor settings in Jun and Jul 2006 under ambient environmental conditions. During the experimental period, the average daily temperature fluctuated between 23 and 28°C, daily relative humidity between 72 and 93%, daily precipitation between 0 and 15 cm, and daily solar radiation between 204 and 288 W/m^2.

Effect of Beetle Density on the Dispersal and Fecundity of *G. boliviana*

Gratiana boliviana were placed on the TSA plants at the densities of 2, 10, 20, 40, 60, 80, and 100 beetles per plant. Each beetle density treatment was replicated eight times. Equal numbers of male and female leaf beetles (i.e., 50-50 sex ratio) were collected for each density treatment. Mature female and male leaf beetles can be easily distinguished as early as 1 week after adult eclosion by the appearance of reproductive organs, which are often shown through the underside of the body. Two orange testes (one of each side of abdomen) can be observed in a male while a pair of white ovaries is in the middle of a female's abdomen. The adult beetles (about 2 weeks old) were collected from a colony maintained in a rearing facility at USDA-ARS-SHRS. The beetles were released at one of the seven densities into each cage at noon and were allowed to feed, mate and reproduce on the TSA plants for 24 h. At noon the next day, the beetles that had dispersed from the plant to the screen of the cage were collected, counted, and sexed. The proportion of dispersal was calculated by dividing the number of beetles dispersed from the plants with the initial number of beetles released onto each TSA plant. The plants were carefully examined for eggs deposited by the leaf beetles over the 24-h period. The per capita fecundity was calculated by dividing the total number of eggs with the number of female leaf beetle per cage.

Effect of the Time of Day on the Dispersal of *G. bolivana*

Sixty leaf beetles were placed on a TSA plant in a cage. This particular density was chosen because the preceding 'beetle density' experiment indicated that the percent dispersal was the highest at this density. The sizes of plants and cages were the same as those used in the 'beetle density' experiment. A total of 15 cages (each represented a replicate) were used in this experiment. Male and female adult beetles (about 2 weeks old) were collected from the colony and released in each cage at equal numbers. The beetles were allowed to feed on the plants overnight before the commencement of the experiment. The next day, the beetles were observed and the numbers of beetles that dispersed from the plants to the screen of the cage were recorded hourly over a 10-h period (from 8:00 to 18:00). All 15 cages were observed in the same day. The dispersed beetles were not removed from the cages; thus, allowing them to return to the host plants. The proportion of dispersing beetles was calculated for each hour by dividing the number of beetles dispersed from the plant by the initial number of beetles on the plant.

Effect of Beetle Density on the Time of Dispersal

The interactive effect of beetle density and time of day on the dispersal of *G. boliviana* was studied by placing young adult leaf beetles onto a caged TSA plant at one of the seven densities (2, 10, 20, 40, 60, 80, and 100 beetles per plant). The sizes of plants and cages were the same as in the two previous experiments. Equal numbers of female and male leaf beetles were collected from the colony and released into the cages. The beetles were allowed to feed overnight and the dispersal of the leaf beetles from the plant was observed the next day from 8:00 to 18:00 h. The proportion of dispersal was calculated as previously described. Each density treatment was replicated 4 times.

Statistical Analysis

The respective effects of beetle density and time of day on the arcsine-transformed proportions of dispersal and the fecundity of *G. boliviana* were analyzed with one-way analysis of variance (ANOVA) at a significance threshold of 0.05 (SAS Institute 1999). The proportion of dispersal from the third experiment (density × time) were analyzed with a repeated measures analysis with a univariate ANOVA with a split-plot model (SAS Institute 1999), with the beetle density as the main plot factor and the time of day as the split-plot factor. The means were separated with Tukey's test when significant difference was detected among the treatment levels.

RESULTS

Effect of Beetle Density on Dispersal Patterns and Fecundity

The percentages of *G. boliviana* dispersed from TSA plants were not different among the seven densities ($F = 0.52$, $df = 6, 49$, $P = 0.7900$) (Fig. 1). About 6% of the leaf beetles dispersed from the TSA plants when there were only 2 beetles per plant. The percentage of beetles dispersing was on average 14.5% when the beetle density was between 10 and 100 beetles per plant. Male and female leaf beetles had a similar tendency to disperse from a given plant ($F = 1.29$, $df = 1, 98$, $P = 0.2583$).

An increase in the beetle density from 10 to 100 beetles per plant significantly decreased the average number of eggs produced by each female from 0.3 to 0.02, respectively ($F = 5.90$, $df = 6, 49$, $P = 0.0001$) (Fig. 2). No eggs were produced when there were only 2 beetles per plant.

Effect of the Time of Day on Dispersal

The dispersal of *G. boliviana* was significantly influenced by the time of day (8:00 and 18:00 h) ($F = 13.87$, $df = 10, 154$, $P < 0.0001$) (Fig. 3). Some

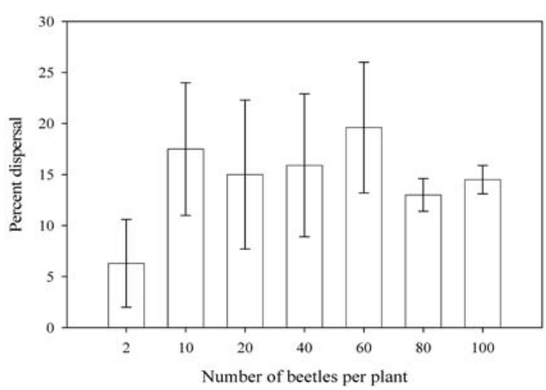

Fig. 1. Mean percent (± SEM) dispersal of 7 densities (2-100 beetles/plant) of *Gratiana boliviana* from tropical soda apple plants to the mesh side of cages after a 24-h acclimation period.

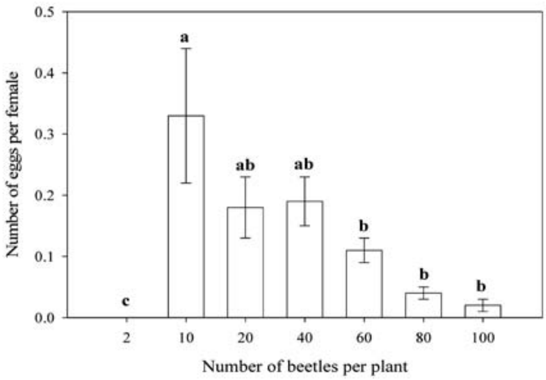

Fig. 2. Mean (± SEM) per capita fecundity of female *Gratiana boliviana* over a 24-h period at 7 densities (2-100 beetles/plant). Bars with the same letters are not significantly different.

beetles began dispersing before 8:00 h as indicated by a low number of beetles on the cages at 8:00 h (Fig. 3). The proportion of beetles dispersing from the TSA plants continued to increase during the morning hours (starting at 8:00 h) and peaked at noon. By 16:00 h, few beetles left the host plants. The proportion of dispersing beetles was lower than that observed in the density experiment. In this experiment, only 6% of a total of 60 beetles left their host plant at noon (Fig. 3). In the density experiment, percent dispersal of 60 beetles at noon was about 20% (Fig. 1).

Interactions Between the Time of Day and Beetle Density

The dispersal of *G. boliviana* was significantly influenced by the beetle density ($F = 11.44$, $df = 6, 21$, $P < 0.0001$), the time of day ($F = 17.22$, $df = 10, 210$, $P < 0.0001$), and the interaction between beetle density and time of day ($F = 5.72$, $df = 60, 210$, $P < 0.0001$) (Fig. 4). No leaf beetles dispersed from the host plant when the density was 2 beetles per plant. The proportions of beetles dispersing were not different among the different time periods when there were 10 or 20 beetles per plant. When the beetle density was increased to 40 or more beetles, more leaf beetles dispersed from the TSA plants at noon than at any other time. The peak percentage of beetles that dispersed increased from 9% with 40 beetles per plant, to slightly more than 10% with 60 and 80 beetles per plant, and to approximately 14% with 100 beetles per plant.

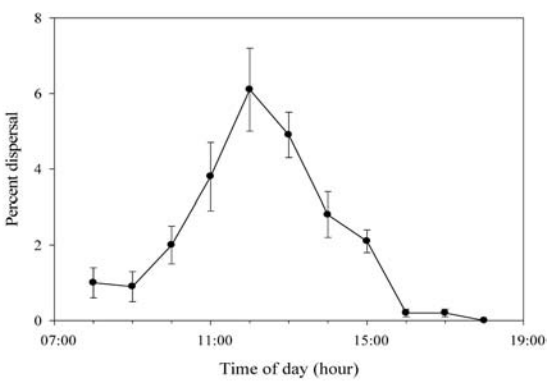

Fig. 3. Mean percent (± SEM) dispersal of 60 *Gratiana boliviana* between 8:00 and 18:00 h from tropical soda apple plants to the mesh side of cages.

Discussion

The success of a biological control program depends in part on the abilities of the biological control agent to establish a viable population, increase the population, and disperse from the original release point. Crowding at the release site is one of the many factors that can promote the dispersal of the biological control agent. In this study, *G. boliviana* showed a tendency to remain on a host plant as long as the host plant was not severely defoliated (Chong, personal observation). An average of 15% of the leaf beetles dispersed from the host plants after being caged for 24 h, regardless of the numbers of beetles released onto the same TSA plant. The tendency of *G. boliviana* to remain at the original release point, combined with the reduction in fecundity at high beetle densities, presents a challenge for TSA biological control practitioners. If a large number of beetles are released at a single location, the beetles may eventually disperse from the site after the quantity and quality of the host plants deteriorate. However, the high beetle density will reduce the per capita fecundity of the beetles and lead to a slower growth of the population. Alternatively, a large number of beetles could be released over a wider area, with each TSA plant receiving only a few beetles. The beetles would likely remain at the same location over a longer period of time but their fecundities will not be negatively impacted.

The choice for an appropriate release method will depends on several factors, such as the number of available beetles and the distribution and accessibility of TSA plants. Currently, *G. boliviana* is mostly released in an inoculation on several plants within an infested site. The ultimate result of the inoculative release method is the reduction of tropical soda apple density through the establishment, growth and dispersal of *G. boliviana* population over time. This release method has the potential of managing the density of scattered tropical soda apple plants within a large area using only a small number of beetles in the initial release. Based on the results of this study, the optimal number of *G. boliviana* to be released on each plant should be 20 to 40 beetles, at which

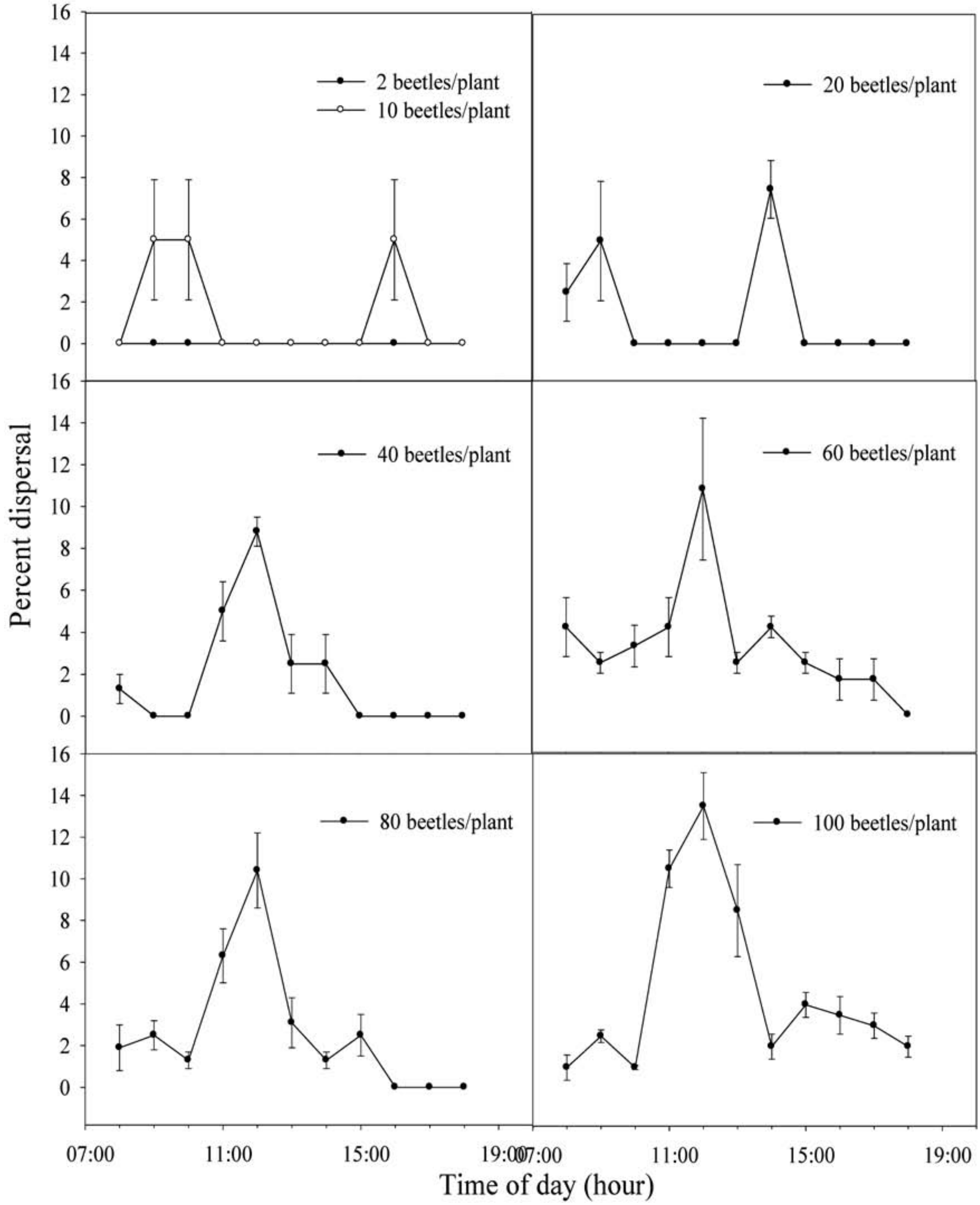

Fig. 4. Mean percent (± SEM) dispersal of 7 densities of *Gratiana boliviana* (2-100 beetles/plant) between 8:00 and 18:00 h.

density the leaf beetles are encouraged to disperse (15-16%/d) while the remaining beetles could still achieve a relatively high fecundity (0.18-0.19 eggs/female/d).

A high beetle density did not encourage the dispersal of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), (Sandeson et al. 2002, 2004) and the flea beetle

Aphthona lacertosa (Rosenhauer) (Coleoptera: Chrysomelidae) (Van Hezewijk & Bourchier 2005). Alternatively, an increase in population density increases the larval dispersal in Lepidoptera (Rhainds et al. 2002). A higher herbivore density is correlated to a higher level of damage to plant tissues; thus the dispersal of an herbivore from the food plant may not be directly affected by density (Sandeson et al. 2002). Instead, dispersal is influenced by the level of deterioration of food plant quality. The ability to disperse in response to crowding and deterioration in host quality is a significant adaptation for an herbivore that has the potential to deplete their local food source (Southwood 1974; Denno et al. 1991). In this study, *G. boliviana* were moved onto the uninfested TSA plants and might not have caused enough damage to encourage their dispersal within a 24-h period. If the leaf beetles were allowed to feed for more than 24 h, we might have seen a difference in the dispersal rates among the beetle densities and the damage levels.

The dispersal behavior of the beetles also has the advantages of achieving uniform distribution, reducing competition for food, and increasing reproductive potential. By dispersing to a new habitat or host plant of a higher quality, *G. boliviana* will be able to improve its development and reproduction. *Gratiana boliviana* decreased its fecundity by 17 times when the conspecifics density was increased from 10 to 100 beetles per plant. The negative effect of an increased density on the fecundity and fitness of herbivores is well-documented in other insects (e.g., Robins & Reid 1997; Achiano & Gilliomee 2004; Wardhaugh & Didham 2005; Hari et al. 2008), and is a major concern in the mass rearing biological control agents in laboratory and insectary (Grundy et al. 2000). Oddly, *G. boliviana* did not produce any egg when only 2 beetles were present on each tropical soda apple plant. The underlying reason for the observed cessation of oviposition by *G. boliviana* at the lowest density is unknown. In some insect species, particularly some scolytid species, low-density aggregation could confer reproductive benefits to individual female insects by overcoming host plant defenses (Raffa & Berryman 1983) or improving host plant quality through the introduction of symbiotic microbes (Whitney 1982). In this case, we do not know if *G. boliviana* requires the presence of conspecifics to initiate reproduction on the tropical soda apples.

A mass rearing method of biological control agent in the laboratory represents a balance among myriads of interrelated factors (Singh & Moore 1985; Thompson 1999). Temperature, humidity, photoperiod, host and natural enemy biology, nutrition, and the availability of alternative hosts influence the potential of chrysomelids to be mass reared in laboratory or nursery for experimental or field releases (Tauber et al. 1996; Blossey & Hunt 1999; Schachter-Broide et al. 2003; Herrera et al. 2005; Ojima et al. 2005; Bredow et al. 2007; Gandolfo et al. 2007; Simelane 2007; Overholt et al. 2008). A successful mass rearing program for *G. boliviana* should be able to produce a large number of adult beetles for field releases and retain sufficient mature beetles to maintain a growth in the culture. For *G. boliviana*, the optimal density of beetles to be maintained on each plant in a colony should be 20 to 40 beetles per plant. Colony density higher than 40 beetles per plant will reduce the fecundity of individual female and the growth and productivity of the colony. Other factors that should be considered, but not addressed in this study, when using a density lower or higher than 20-40 beetles per plant include a significant increase in costs (space and labor) and waste of rearing materials.

Gratiana boliviana was most actively dispersing from the TSA plants at noon than at any other time. The diurnal rhythm in the dispersal behavior of *G. boliviana* is perhaps related to the ambient temperature. The effects of temperature on the flight pattern of many insects are well-known (e.g., Blackmer et al. 2006; Achiano & Gilliomee 2008; Zhang et al. 2008). The relationship between temperature and dispersal efficiency is often dome-shaped with the highest rate and distance of dispersal occurring at a median temperature (Zhang et al. 2008). Although the ambient temperature at the study site reached its peak (at 39°C at one point during the experiment) between 1 to 2 PM, *G. boliviana* might have preferred to disperse at a slightly cooler temperature and early time.

The tendency of *G. boliviana* to become active in flight in the later hours of morning also has significant implication for post-release monitoring of beetle population in the field. In order to account for the majority of the leaf beetles in a particular locale or site and provide a more accurate estimate of the resident population, sampling of the beetle population should coincide with the lower level of dispersal activity of the leaf beetles in the early morning or late afternoon.

The design of this study was not without shortcomings. The densities of beetles tested in this study were higher than realistically observed in the field. The low densities of 2 to 20 beetles per plant were representative of those observed in the field on the similarly sized tropical soda apple plants (Chong and Roda, personal observations). Densities of 40 to 100 beetles per plant were higher than those observed in the field, even for plants receiving the initial releases. However, the high beetle density was characteristic of an insectary situation (Chong, personal observation) and applicable to the discussions of mass rearing procedure. The second shortcoming was the relatively small size of the arena, which could discourage normal dispersal and reproductive behavior (van Alphen & Jervis 1996). In this study, the movement of *G. boliviana* was limited to within the cages and any dispersal could only happen from the plant to the cage and vise versa. In the field, however, the movement of *G. boliviana* is undoubtedly more complex, with the movement of beetles leaving and arriving on the same plant and among the different host plants occurring simultaneously. When summing up all the movements, the dispersal rate from the infested plant may be less than what was observed in this study. A study conducted in the field or large cages using commonly observed densities will provide more realistic prediction of the dispersal behavior of *G. boliviana*.

In summary, there was a significant interaction between density and the time of day in influencing the dispersal rate of *G. boliviana*. Similar to the second dispersal experiment, the most active dispersal of *G. boliviana* was detected at noon. However, the leaf beetles did not emigrate as readily from the host plants when the beetle density was lower than 40 beetles per plant. As the beetle density increased from 40 to 100 beetles per plant, the peak dispersal rate also increased. This study shows that the dispersal of *G. boliviana* was not dependent on only one factor but on the interactions between the beetle density and the time of day. To better understand the dispersal and population distribution of *G. boliviana* in the field, the interactive effects of density, feeding damage, photoperiod, age or physiological status of the beetles, and host plant nutritional quality or phenology should be studied in greater details.

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