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OPEN-FIELD HOST SPECIFICITY TEST OF *GRATIANA BOLIVIANA* (COLEOPTERA: CHRYSOMELIDAE), A BIOLOGICAL CONTROL AGENT OF TROPICAL SODA APPLE (SOLANACEAE) IN THE UNITED STATES

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ABSTRACT

An open-field experiment was conducted to assess the suitability of the South American leaf feeding beetle *Gratiana boliviana* Spaeth for biological control of *Solanum viarum* Dunal in the USA. An open-field test with eggplant, *Solanum melongena* L., was conducted on the campus of the University of Buenos Aires, Argentina, and a *S. viarum* control plot was established 40 km from the campus. One hundred adult beetles were released in each plot at the beginning of the experiment during the vegetative stage of the plants, and forty additional beetles were released in the *S. melongena* plot at the flowering stage. All the plants in each plot were checked twice a week and the number of adults, immatures, and eggs recorded. Results showed almost a complete rejection of eggplant by *G. boliviana*. No noticeable feeding damage was ever recorded on eggplant. The experiment was ended when the eggplants started to senesce or were severely damaged by whiteflies and spider mites. The results of this open-field experiment corroborate previous quarantine/laboratory host-specificity tests indicating that a host range expansion of *G. boliviana* to include eggplant is highly unlikely. *Gratiana boliviana* was approved for field release in May 2003 in the USA. To date, no nontarget effects have been observed either on eggplant or native species of *Solanum*.

Key Words: eggplant, open-field experiment, risk assessment, Solanaceae, *Solanum*, weed biological control

RESUMEN

Una prueba de campo fué conducida para evaluar la especificidad del escarabajo suramericano defoliador *Gratiana boliviana* Spaeth para control biológico de *Solanum viarum* Dunal en los Estados Unidos. La prueba con berenjena se realizó en el campo experimental de la Universidad de Buenos Aires, Argentina, y una parcela control con *S. viarum* fué establecida a 40 km. Cien escarabajos adultos fueron liberados en cada parcela al inicio del experimento durante la fase vegetativa, y cuarenta escarabajos adicionales fueron liberados en la parcela de berenjena durante la floración. Todas las plantas en cada parcela fueron inspeccionadas dos veces a la semana y el número de adultos, larvas, y posturas fueron registrados. Resultados indicaron un casi completo rechazo de la berenjena por *G. boliviana*. Ningún daño visible de defoliación en la berenjena fué detectado. Las pruebas concluyeron cuando las plantas de berenjena alcazaron su madurez o fueron severamente dañadas por mosca blanca y ácaros. Resultados corroboran previas pruebas de especificidad en laboratorio/cuarentena que indican que la berenjena no es un hospedero de *G. boliviana* y que la posibilidad de llegar a ser una plaga de este cultivo es muy remota. *Gratiana boliviana* fue aprobado para ser liberado en el campo en mayo del 2003. Ningún daño ha sido observado hasta la fecha a plantas no blanco.

Translation provided by the authors.

Solanum viarum Dunal (Solanaceae), known in the USA by the common name tropical soda apple, is an invasive shrub native to northeast Argentina, south Brazil, Paraguay, and Uruguay. It was first detected in the USA in 1988 and probably was accidentally introduced into Florida at the beginning of the 1980s with cattle importation from Brazil. Its dramatic spread has led to a current infestation of approximately 0.5 million hectares in Florida, Georgia, Alabama, Mississippi, Louisiana, Texas, South Carolina, North Carolina, Tennessee, and Puerto Rico (Mullahey & Colvin 1993; Mullahey et al. 1993; Bryson & Byrd 1996; Medal & Cuda 1999). This plant invades improved pastures, hammocks, ditch banks, and road sides, reducing livestock carrying capacity. Dense stands of this prickly shrub do not allow cattle to access shaded sites which results

in heat stress. Current control methods for this invasive plant in southeastern USA pastures are mostly based on herbicides and mechanical (mowing) practices (Mislevy et al. 1996; Mullahey et al. 1996; Sturgis & Colvin 1996; Akanda et al. 1997). These methods provide only temporary control of dense infestations of *S. viarum* at an estimated cost of US $185 per hectare (Mullahey et al.1996). Pasture production and stocking rates decline if this weed is left uncontrolled. *Solanum viarum* also causes ecological damage as it reduces biological diversity in natural areas, and interferes with restoration efforts following phosphate-mining operations (Albin 1994).

In addition *S. viarum* is known to harbor at least six viruses that affect cultivated solanaceous crops such as tomato, tobacco, and pepper (McGovern et al. 1994a,1994b; McGovern et al. 1996).This weed is used as an alternative host by some major insect pests such as the silverleaf whitefly *Bemisia argentifolii* Bellows and Perring, the tobacco budworm *Heliothis virescens* (Fabr.), the Colorado potato beetle *Stilodes decemlineata* (Say), the tobacco hornworm *Manduca sexta* (L.), the tomato hornworm *Manduca quinquemaculata* (Haworth), the green peach aphid *Myzus persicae* (Sulzer), the southern green stinkbug *Nezara viridula* (L.), and the suckfly *Tupiocoris notatus* (Distant) (Habeck et al. 1996; Medal et al. 1999a; Sudbrink et al. 1999).

Although *S. viarum* is able to spread vegetatively from the root system, the primary method of dispersal is by seed dissemination (Bryson et al. 1995; Medal et al. 1999b), which occurs mainly by livestock and wildlife that feed on the fruits and scarify the seeds (Akanda et al. 1996; Brown et al. 1996). A single plant of *S. viarum* can produces up to 150 fruits per year, with each fruit containing on average 400 seeds. The estimated seed production is 60,000 seeds/plant/season with a viability of more than 75% (Mullahey and Colvin 1993; Pereira et al. 1997).

The first exploratory survey for biological control agents of *S. viarum* in South America was conducted by University of Florida and Brazilian researchers in Jun 1994 (Medal et al.1996). In this initial 2-week survey, 16 species of insects were found feeding on *S. viarum* in southern Brazil and southeastern Paraguay. Two of these insects, *Metriona elatior* Klug and *Gratiana boliviana* Spaeth (Chrysomelidae), were selected as potential biological control agents for *S. viarum* based on field observations of the extensive defoliation caused by the immature and adult stages of these beetles (Medal et al. 1996; Medal et al. 2002; Medal et al. 2003). Host-specificity studies with *G. boliviana* were initiated at the Florida Biological Control Laboratory-quarantine and at the USDA-ARS South American Biological Control Laboratory in Hurlingham, Argentina in 1998. The biology of this insect was studied in Argentina (late D. G., unpublished data). The beetle laid eggs individually on leaves and petioles of *S. viarum* and *Solanum palinacanthum* Dunal, and not in masses as those of other *Gratiana* species (Sieber 1975; Hill & Hulley 1995). The adult stage of *G. boliviana* was redescribed by Buzzi (1995).

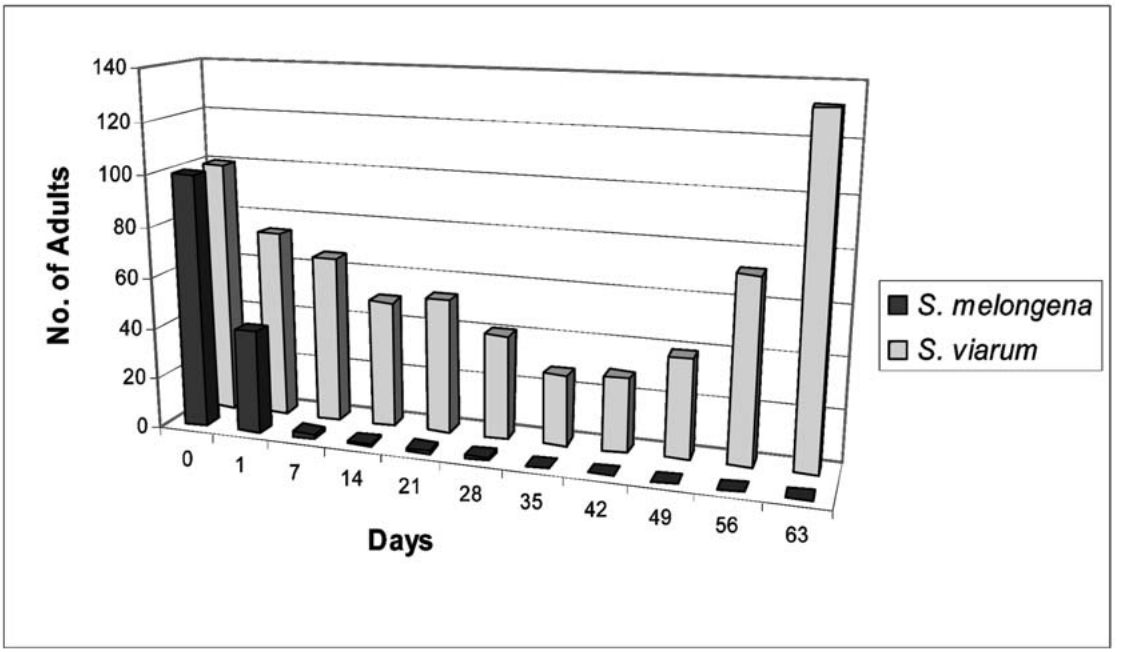
In this article we report the results of open-field tests in Argentina that were conducted to assess the risk of *G. boliviana* to eggplant if this beetle were released as a biological control agent of *S. viarum* in the USA.

Materials and Methods

An open-field risk assessment was conducted in Argentina during Jan and Feb 2000. A no-choice open-field experiment with organically grown eggplant, *S. melongena* L. cv 'Black Beauty', was set up in the experimental farm located on the campus of the University of Buenos Aires, and a *S. viarum* control plot at the USDA South American Biological Control Laboratory located in Hurlingham, Buenos Aires province. The plot sites were located 40 km from each other. This distance between the field plots was considered sufficient to ensure that the control plot with only *S. viarum* did not interfere with the results in the eggplant field, particularly as the suburbs of Buenos Aires located between the fields, acted as a physical barrier. Each plot measured 7 by 4 m, with 54 plants evenly distributed in 9 rows (6 plants/row). The location of both plots was outside the area of distribution of the beetle, the target weed, and other known hosts. Initially, 25 teneral and 75 mature adults (50% males, 50% females) were released in each plot when plants were in the vegetative growth stage. A few weeks later, a second release of 40 adults was made when plants were flowering and producing fruits. To avoid an induced preference due to previous experience with *S. viarum*, the mature adults were reared on *S. palinacanthum*, a natural host that does not occur in the USA. All plants in each plot were thoroughly examined visually by 2 technicians twice a week and the number of adults, immatures, and eggs recorded. Because *G. boliviana* deposits each egg individually inside an egg case, and discriminating between hatched and unhatched eggs is difficult, the number of eggs was recorded as total cumulative number of egg cases including some that had already hatched. However, hatched egg cases become delicate and some disappear 1 to 3 weeks after eclosion. The percentage of defoliation was visually estimated during the first 3 weeks. After that period, other phytophages colonizing the plots made it difficult to discriminate defoliation caused by them from the defoliation caused by *G. boliviana*. The plants of both plots were allowed to develop for a complete growing season without pesticide applications.

RESULTS

The results in the open field test showed almost complete rejection of eggplant by *G. boliviana* (Figs. 1-3). Twenty four h after 100 adults were released, 41 insects were recorded on eggplant. These adults were hidden in partially unfolded tender leaves, and most of them left the plot during the first week. Only 2 adults were found after 7 d. During the second week, 50



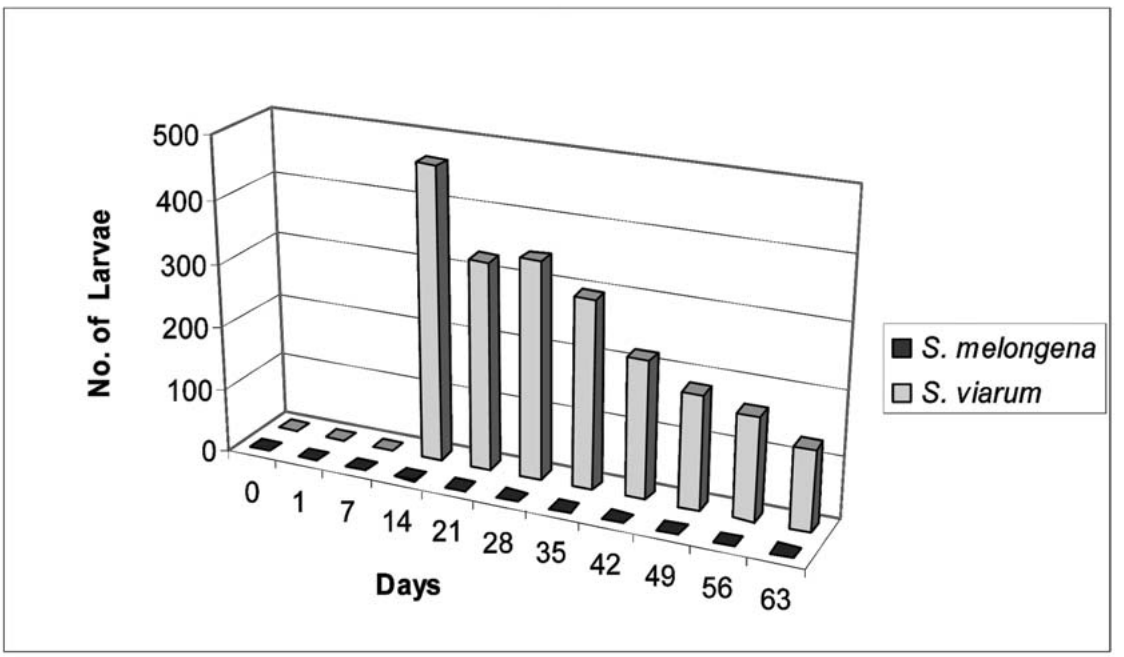


Fig. 1. Number of *Gratiana boliviana* adults found in 2 independent experimental plots of *S. viarum* and *S. melongena*.

Fig. 2. Number of *Gratiana boliviana* larvae found in 2 independent experimental plots of *S. viarum* and *S. melongena*.

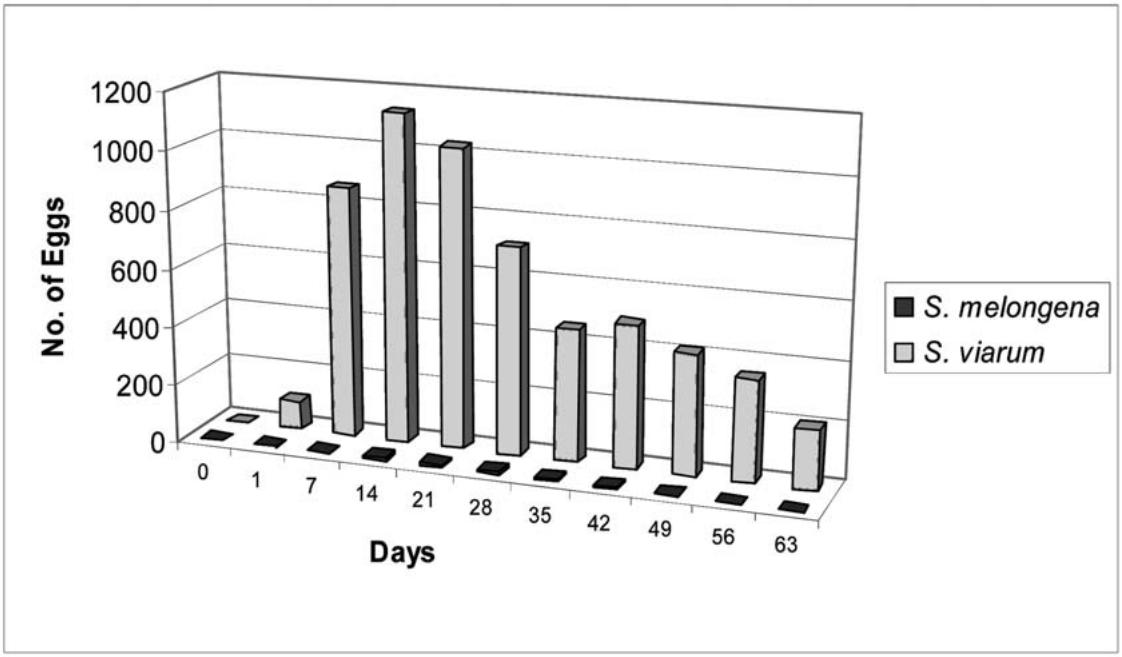


Fig. 3. Number of *Gratiana boliviana* eggs found in 2 independent experimental plots of *S. viarumi and S. melongena*.

adults (Fig. 1), 463 larvae (Fig. 2), and 1130 eggs (Fig. 3) were recorded on *S. viarum* compared to 1 adult, 1 larva, and 10 eggs on eggplant. The larva on eggplant died shortly after emergence and no other larvae were recorded in the following weeks (egg predation by mirid *Tupiocoris* sp. was observed in both plots). From the fourth to the seventh weeks, no adults or larvae of *G. boliviana* were found on eggplant. Therefore, 40 new adults were added to the eggplant plot (so that all the phenological stages of the plant were exposed), but they left in the following 7 d without ovipositing. No noticeable feeding damage was recorded on eggplant. Despite the egg and larval predation that was observed on the *S. viarum* plot during the experiment, a second generation of adults began to emerge during the sixth week, and during the last week (9th) 132 adults and 51 pupae were found. The experiment was concluded when the eggplants started to senesce or were severely damaged by whiteflies and spider mites.

Discussion

The primary advantage of open field tests is that they allow the candidate biocontrol agents to exercise free choice without constraints imposed by cages. Therefore, all behavioral decisions that lead to the acceptance of a host are tested. However, in our no-choice field experiment where the insects were randomly released between plants inside the plots, the ability of *G. boliviana* to locate eggplant at a considerable distance remain un-tested. Despite the favorable conditions of the plot set-up and relative short distance of the release for *G. boliviana* to locate and feed on eggplant, results showed clearly that eggplant is not an acceptable host and beetles fly away from the plants a few days after being released without feeding and development on the eggplants.

Eggplant is originally from Southeast Asia, and it was introduced by the Portuguese in Brazil where it has been cultivated in the native range of *G. boliviana* for more than a century, but the insect has never been recorded on it. In addition, it was not found during the 34 field surveys conducted in Argentina, Uruguay, Paraguay, and southern Brazil, where the surveys focused on unsprayed or neglected eggplant fields. On the contrary, we did find the insects on *S. viarum* plants growing intermixed or near eggplant (Medal et al. 2004). The open-field test results are supported by the fact that every test that has been conducted either in laboratory/or screen cages has shown a complete rejection of eggplant (choice feeding and oviposition test, late D. G. and J. M. unpublished data), or a strong decline of the fitness of *G. boliviana* when reared on eggplant (no choice larval development, and fecundity and longevity tests) (late D. G. and J. M., unpublished data).

Indiscriminate feeding on non-target hosts by biological control candidates for *Solanum* weeds under confined quarantine-laboratory conditions has been reported by South African researchers

(Neser et al. 1988; Hill & Hulley 1995, 1996; Olckers et al. 1995; Olckers 1996). For example, *Leptinotarsa texana* Schaeffer, *Leptinotarsa defecta* (Stal), and *Gratiana spadicea* (Klug) were introduced into South Africa for biological control of *Solanum elaeagnifolium* Cav., and *Solanum sisymbriifolium* Lam. It is noteworthy that the 3 species, under similar laboratory testing procedures, displayed a higher degree of acceptance of eggplant than *G. boliviana* (Olckers et al. 1995; Hill & Hulley 1995). The *Leptinotarsa* species were imported from the United States in 1992. Both species are established and are exerting some control of *S. elaeagnifolium* (Hoffmann et al.1998). *Gratiana spadicea* was imported from South America in 1994, and it is established (Olckers et al. 1999). None of the above mentioned beetles have been found on eggplant in South Africa; however, the time elapsed since they were released is relatively short. More importantly, the three beetle species have coexisted for more than a century with eggplant in their native range and have never been recorded on this crop.

These data suggest that a host range expansion of *G. boliviana* to include eggplant is highly unlikely. Furthermore, eggplant does not belong to the section Acanthophora, to which both the real and physiological host range of the insect seem to be restricted (Medal et al. 2002; Gandolfo et al. 1999). *Gratiana boliviana* was approved for field release in May 2003 in the United States (Medal et al. 2003), and more than 75,000 beetles have been released in Florida, Georgia, Alabama, and South Carolina. This beetle established in most of the locations where it was released, and is causing extensive defoliation on *S. viarum* plants. No non-target effects have been observed either on eggplant or native species of the genus *Solanum* (J. M., unpublished).

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