OPEN FIELD HOST SPECIFICITY TESTS IN BRAZIL FOR RISK ASSESSMENT OF METRIONA ELATIOR (COLEOPTERA: CHRYSOMELIDAE), A POTENTIAL BIOLOGICAL CONTROL AGENT OF SOLANUM VIARUM (SOLANACEAE) IN FLORIDA

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OPEN FIELD HOST SPECIFICITY TESTS IN BRAZIL FOR RISK ASSESSMENT OF *METRIONA ELATIOR* (COLEOPTERA: CHRYSOMELIDAE), A POTENTIAL BIOLOGICAL CONTROL AGENT OF *SOLANUM VIARUM* (SOLANACEAE) IN FLORIDA

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ABSTRACT

Open field experiments were conducted in Brazil to assess the suitability of the South American leaf feeding beetle *Metriona elatior* Klug for biological control of tropical soda apple, *Solanum viarum* Dunal in the USA. A multiple choice open field test with eggplant (*Solanum melongena* L.), bell-pepper (*Capsicum annuum* L.), potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill.), giló (*Solanum gilo* Raddi), falsa-jurubeba (*Solanum fastigiatum* Willd.), and the target weed *Solanum viarum*, was conducted at the Universidade Federal do Paraná Experimental Station in Curitiba. A second multiple choice-test was conducted 2 years later at the same location excluding the target weed and exposing *M. elatior* adults to *S. melongena*, *S. tuberosum*, *L. scullentum*, and *C. annuum*. An *S. viarum* control plot was established 60 km from the choice-test field. In total, 276 teneral adult beetles were released in the first multiple choice-test. In the second test, 176 beetles were released in the choice-test plot that excluded *S. viarum*, and 172 adult beetles were released in the *S. viarum* control plot at different developmental stages of the tested plants. All the plants in each plot were visually checked once a week and the number of adults, immatures, and eggs recorded. Results in the first multiple choice-test showed a complete rejection of the crop plants by *M. elatior*. Minor feeding (<3%) was observed on eggplant in the second experiment in which *S. viarum* was excluded from the crop plots, but all *M. elatior* larvae died in less than a week. The tests were ended when the plants started to senesce. The results of these open field tests corroborate previous quarantine/laboratory host specificity tests indicating that host range expansion of *M. elatior* to include the solanaceous crops tested is highly unlikely. A petition to release *M. elatior* in the USA was submitted to the TAG committee in September 2006.

Key Words: Brazil, biological control, tropical soda apple, *Solanum viarum*, *Metriona elatior*, open field experiment, risk assessment

RESUMEN

Pruebas de campo fueron realizadas en Brasil para evaluar la especificidad del escarabajo suramericano defoliador *Metriona elatior* Klug para control biológico de tropical soda apple, *Solanum viarum* Dunal en los Estados Unidos. La prueba de 'escogencia' con berenjena (*Solanum melongena* L.), pimiento dulce (*Capsicum annuum* L.), papa (*Solanum tuberosum* L.), tomate (*Lycopersicon esculentum* Mill.), giló (*Solanum gilo* Raddi), falsa-jurubeba (*Solanum fastigiatum* Willd.), y la maleza blanco *Solanum viarum*, fué realizada en el campo experimental de la Universidade Federal do Paraná en Curitiba. Una segunda prueba de 'escogencia sin la maleza blanco' fué realizada 2 años después en el mismo campo liberando adultos de *M. elatior* en parcelas con *S. melongena*, *S. tuberosum*, *L. scullentum*, y *C. annuum*. Una parcela control fué establecida a 60 km. Doscientos setenta y seis escarabajos adultos fueron liberados en la primera prueba. Durante la segunda prueba se liberaron ciento setenta y seis escarabajos adultos en las parcelas de escogencia que excluían *S. viarum*, y ciento setenta y dos en la parcela control. Todas las plantas en cada parcela fueron inspeccionadas una vez por semana y el número de adultos, larvas, y posturas registrados. Resultados en la primera prueba indicaron un completo rechazo de los cultivos Solanaceous por *M. elatior*. Una ligera defoliación (<3%) en berengena por *M. elatior* fué observada durante la segunda prueba pero las larvas murieron en menos de una semana sin completar su desarrollo. Las pruebas concluyeron cuando las plantas alcanzaron su madurez. Resultados corroboran previas pruebas de especificidad en laboratorio/cuarentena que indican que *M. elatior* es altamente específico desarrollandose solamente en *S. viarum*. La solicitud para ser liberado en el campo fué enviada al comité 'TAG' en septiembre del 2006.

Translation provided by the authors.

Open field host specificity tests can be important complementary techniques for risk assessment of potential weed biological control agents under more natural conditions than enclosed cage tests in quarantine. These kinds of tests provide a more accurate determination of the ecological host range of a potential agent that would have been rejected on the basis of confined quarantine testing alone. Contrary to their restricted movement by screened cages, the tested agent in the open field arena is allowed to express behavioral decisions such as to move or fly away from plants that are not natural hosts. Open field tests are useful for clarifying contradictory results (or false positives) obtained with potential biological control agents in quarantine tests feeding on non-target plants that have never been reported as hosts in the native range of the tested agent (Briese 1999; Blossey 1995; Clement & Cristofaro 1995). However, the use of open field tests has been limited because these have to be conducted in the country of origin of the potential biological control agent. Furthermore, quarantine restrictions prohibit the introduction of non-native plants in the country where the tests will be conducted. Open-field tests have proven valuable in some cases for resolving host-specificity concerns which led to the release of important biological control agents such as the flowerbud weevil, *Anthonomus santacruzi* Hustache, against *Solanum mauritianum* Scopoli in South Africa (Olckers 2004), and the leaf feeding beetle *Gratiana boliviana* Spaeth against *Solanum viarum* Dunal in the southeastern United States (Gandolfo et al. 2007; Medal et al. 2003).

The insect evaluated in this study was *Metriona elatior* Klug (Coleoptera: Chrysomelidae: Cassidinae), a potential biological control agent of the invasive weed *S. viarum*, a prickly perennial shrub native to northeastern Argentina, southern Brazil, Paraguay, and Uruguay. *Solanum viarum* has invaded 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 2000). This weed causes problems in improved pastures by reducing livestock carrying capacity and invades hammocks, ditch banks, and road sides. Current control methods for this invasive plant in the southeastern United States 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*. The first biological control agent released against *S. viarum* in 2003 was *G. boliviana* (Medal et al. 2003; Medal et al. 2004). In this study, we report the results of open field tests in Brazil that were conducted to assess the risk of *M. elatior* to economic crops if this beetle were released as a biological control agent of *S. viarum* in the USA. The Florida quarantine host specificity tests were reported by Medal et al. (1999).

Materials and Methods

Multiple Choice Feeding and Oviposition Field Test

The open field multiple choice test was conducted at the Universidade Federal do Paraná-Agriculture Experimental Farm 'Canguiri' located 60 km northeast of Curitiba in Paraná state. *Metriona elatior* immatures and adults were collected in Paraná and Rio Grande do Sul states, Brazil, on 5 occasions in Oct and Nov 2003. These field collected insects were placed in screened cages ($0.6 \times 0.6 \times 0.9$ m) at the Neotropical Biological Control Laboratory in Curitiba with *S. viarum* plants growing in 1-2-gallon pots to establish a colony of the beetles for the open field multiple choice test. In total, 276 *M. elatior* teneral adults were released in a field plot (20×40 m^2) with 7 plant species (tropical soda apple *S. viarum* Dunal, eggplant *Solanum melongena* L., bell-pepper *Capsicum annuum* L., potato *Solanum tuberosum* L., tomato *Lycopersicon esculentum* Mill., giló *Solanum gilo* Raddi, and the native Brazilian falsa-jurubeba *Solanum fastigiatum* Willd.). Six of each plant species (42 plants/plot) were randomly assigned in each of the experimental plots following a Randomized Complete Block Design with 4 replications. In total, 168 plants were transplanted in September 2003, and the insects were released during the second week of Dec 2003. All plants were thoroughly examined visually once a week (from Dec 10, 2003 to Feb 3, 2004) and feeding, and number of egg masses, larvae, pupae, and adults recorded. The percentage defoliation was estimated visually by 2 field observers. The insect data recorded were analyzed by the nonparametric procedure Kruskal-Wallis One-Way Analysis of Variance by Ranks (Daniel 1990; Silva & Azevedo 2002).

Multiple choice Minus the Target Feeding and Oviposition Field Test

An open field multiple choice test exposing *M. elatior* adults to eggplant, tomato, potato, and bell pepper (*S. viarum* was excluded from the plots) was conducted at the same location from Jan to Mar 2006. Control plots with only *S. viarum* plants were set-up at the Neotropical Biological Control Laboratory in Curitiba located at approximately 60 km from the cultivated crop plots. *Metriona elatior* adults were collected in Paraná and Rio Grande do Sul states, Brazil, on two different occasions in Oct and Dec 2005. These field collected insects were placed in screened cages ($0.6 \times 0.6 \times 0.9$ m) at the Neotropical Biological Control Laboratory in Curitiba with *S. viarum*

plants growing in 1-2 gallon pots to establish a colony for providing beetles for the open field tests. Greenhouse reared *M. elatior* teneral adults (total: 348 adults) were released at the beginning, and subsequently every 2 weeks during the field tests for a total of 176 adults released in crops to be tested and 172 adults in *S. viarum* plots. The beetles were randomly released in groups of 10-20 on the ground at approximately 1m from the plants but not on any specific test plant to allow the beetles to exhibit normal host location behaviors. Evaluations by visual estimation of feeding and number of insects were made weekly by thoroughly checking each of the plants tested. The insect data recorded were analyzed by the nonparametric procedure Kruskal-Wallis One-Way Analysis of Variance by Ranks (Silva & Azevedo 2006).

RESULTS

Multiple Choice Plus Control Plant: Feeding and Oviposition Field Test

In the open field planted with *S. viarum* (control), *S. melongena*, *C. annuum*, *S. tuberosum*, *L. esculentum*, *S. gilo*, and *S. fastigiatum*, the *M. elatior* adults fed intensively and laid eggs only on *S. viarum*. *Metriona elatior* egg masses (total: 349; mean ± SD = 44 ± 29), larvae (1,001; 125 ± 85), pupae (147; 18 ± 18), and adults (279; 35 ± 12) recorded on *S. viarum* plants are shown in Table 1. The Kruskal-Wallis test indicated that the *M. elatior* egg masses, larvae, pupae, and adults recorded on *S. viarum* plants were highly significantly different ($P < 0.01$) from those obtained on the other plants tested. This choice test corroborated previous findings in cages and open field tests conducted by D. Ohashi and the late D. Gandolfo in Argentina, and by R. Pitelli and A. Santana in São Pablo state, Brazil (unpublished data), indicating that *M. elatior* is highly specific in feeding and develops only on *S. viarum*. The beetle does not represent a threat to eggplant, tomato, potato, bell-pepper, giló, and the native Brazilian falsa-jurubeba.

Multiple Choice Minus Control Plant: Feeding and Oviposition Field Test

In the open field multiple choice test exposing *M. elatior* adults (176) to *S. melongena* (Black Beauty cultivar), *L. esculentum*, *S. tuberosum*, and *C. annuum* (*S. viarum* excluded from the commercial crop plots, and planted in a separate experiment at 60 km distance), there was no feeding or oviposition by *M. elatior* on *L. esculentum*, *S. tuberosum*, and *C. annuum*. The number of egg masses (total: 74; mean ± SD = 11 ± 5) laid by *M. elatior* adults on *S. viarum* plants were significantly higher ($P < 0.01$) than the number of egg masses (8; 1 ± 2) laid on *S. melongena* when *S. viarum* plants were not present (Table 2). A significant ($P < 0.01$) number of *M. elatior* larvae (498; 71 ± 47) were able to develop and reach the pupal stage only on *S. viarum* (Table 2). The 29 first instars that were recorded on *S. melongena* died in less than 7 d. Feeding by *M. elatior* on *S. viarum* plants was 40 to 90%, contrary to the minor feeding or probing (<3% of the leaf area) by the *M. elatior* on *S. melongena*. Results indicated that *M. elatior* was able to feed, lay eggs, and develop only on *S. viarum*, although minor incidental feeding may occur on *S. melongena* when *S. viarum* is not present.

DISCUSSION

The primary advantage of open field tests is that they allow the candidate biological control agents to exercise free choice without constraints imposed by cages. Therefore, all behavioral decisions that lead to the acceptance of a host are

TABLE 1. NUMBER OF *METRIONA ELATIOR* RECORDED ON SEVEN PLANT[1] SPECIES IN AN OPEN FIELD MULTIPLE CHOICE TEST IN BRAZIL, DEC 2003-FEB 2004.

	S. viarum			
Date	Egg Masses	Larvae	Pupae	Adults
10-Dec-03	27	173	0	31
19-Dec-03	50	137	6	27
26-Dec03	63	161	18	39
5-Jan-04	92	220	64	45
12-Jan-04	66	217	22	31
16-Jan-04	30	73	18	24
26-Jan-04	16	17	16	59
3-Feb-04	5	3	3	23
Total	349	1001	147	279

[1]No *Metriona elatior* beetles were recorded on tomato, bell-pepper, potato, eggplant, jurubeba, and gilo.

TABLE 2. NUMBER OF *METRIONA ELATIOR* RECORDED ON 5 PLANT[1] SPECIES IN AN OPEN FIELD MULTIPLE CHOICE TEST IN BRAZIL, JAN-MAR 2006.

	S. viarum			Eggplant		
Date	Egg Masses	Larvae	Pupae	Egg Masses	Larvae	Pupae
9-Jan-06	9	22	3	0	0	0
24-Jan-06	18	92	18	6	24	0
4-Feb-06	11	113	37	0	0	0
13-Feb-06	16	147	85	2	5	0
24-Feb-06	8	57	137	0	0	0
1-Mar-06	10	38	97	0	0	0
20-Mar-06	2	29	48	0	0	0
Total	74	498	423	8	29	0

[1]No *Metriona elatior* beetles were recorded on tomato, bell-pepper, and potato.

tested. However, in our multiple choice field experiment where the insects were randomly released between plants inside the plots, the ability of *M. elatior* to locate the economic crops at a considerable distance remains untested. Despite the favorable conditions of the plot set-up and relative short distance of the release for *M. elatior* to locate and feed on the crops, results showed clearly that eggplant, potato, tomato, bell-pepper, giló, and the native Brazilian *Solanum fastigiatum* are not acceptable hosts and beetles flew away from the plants a few days after being released without feeding and development on the native plant and economic crops.

Eggplant is originally from Southeast Asia, and it was introduced by the Portuguese into Brazil where it has been cultivated in the native range of *M. elatior* for more than a century, but this beetle had never been recorded in the Brazilian literature 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 with screen cages has shown a complete rejection of eggplant, potato, bell-pepper, and tomato (choice feeding and oviposition test, late D. Gandolfo and J. Medal 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* (Stål), and *Gratiana spadicea* (Klug) were introduced into South Africa for biological control of *Solanum elaeagnifolium* Cav., and *Solanum symbriifolium* Lam. It is noteworthy that the three species, under similar laboratory testing procedures, displayed a higher degree of acceptance of eggplant than *M. elatior* (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 also 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 3 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 *M. elatior* to include eggplant, potato, tomato, or bell-pepper 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).

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