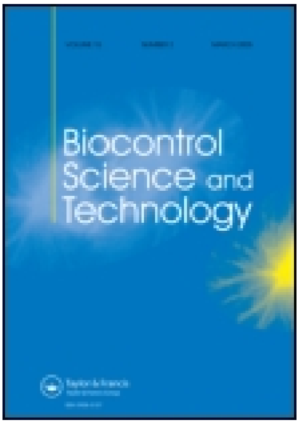

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The leafmining Leurocephala schinusae (Lepidoptera: Gracillariidae): not suitable for the biological control of Schinus terebinthifolius (Sapindales: Anacardiaceae) in continental USA

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RESEARCH ARTICLE

The leafmining *Leurocephala schinusae* (Lepidoptera: Gracillariidae): not suitable for the biological control of *Schinus terebinthifolius* (Sapindales: Anacardiaceae) in continental USA

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The host range of *Leurocephala schinusae* Davis & Mc Kay (Lepidoptera: Gracillariidae) was studied to assess its suitability as a biological control agent of *Schinus terebinthifolius* Raddi (Anacardiaceae), a serious environmental weed in the USA and elsewhere in the world. The host range was determined in the laboratory with adult no-choice oviposition (Argentina and USA) and larval development tests (USA). Seventeen plant species in ten genera were selected based on taxonomic relatedness to *S. terebinthifolius*, economic importance, and availability. Additional information was obtained by sampling foliage of *S. terebinthifolius* and six other South American native Anacardiaceae species in north-eastern Argentina. In the laboratory, except for *Lithrea molleoides* and *Spondias mombin*, all of the tested species were accepted for oviposition with a marked preference for *Rhus aromatica*. Incipient mines successfully developed into complete mines, pupae and adults on *R. aromatica, Rhus copallinum*, *Schinus molle, Schinus lentiscifolius* and *S. terebinthifolius.* In the field, although *L. schinusae* showed a clear preference for *S. terebinthifolius*, the host range, as determined by samples of host use in the native range, included three other *Schinus* species (*S. lentiscifolius*, *Schinus longifolius, Schinus weinmannifolius*) and one *Astronium* species (*Astronium balansae*). In conclusion, *L. schinusae* will not be considered for the biological control of *S. terebinthifolius* in continental US. However, the utilisation of this species in other infested areas such as Hawaii and Australia should be further discussed.

Keywords: Brazilian pepper; biological weed control; host range; specificity

Introduction

Schinus terebinthifolius Raddi, Anacardiaceae, known as Brazilian peppertree, broad leaf pepper or Christmas berry, is a Neotropical perennial shrub native to Brazil, Uruguay, north-eastern Argentina and adjacent Paraguay (Barkley 1944, 1957; Muñoz 2000; Mc Kay unpublished data). This species has been introduced into many countries around the world as an ornamental and has successfully naturalised in sub-tropical areas (15–30°) of both the northern and southern hemispheres

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(Morton 1978; Ewel 1986; Panetta and McKee 1997). Currently, *S. terebinthifolius* is listed as a prohibited plant and a noxious weed in Florida, and is considered an invasive species in Florida, California, Texas and Hawaii (Randall 2000; HSASC 2001; FLEPPC 2005; USDA NRCS 2009). In its exotic range the plant decreases the biodiversity of infested natural areas by aggressively invading a variety of coastal and upland habitats (Mytinger and Williamson 1987; Gann, Bradley, and Woodmansee 2001). *S. terebinthifolius* constitutes a threat not only to natural areas but also to agriculture and cattle production in Florida and Hawaii (Morton 1978; Ewel 1986; Yoshioka and Markin 1991). This species produces allelopathic compounds that suppress the growth of other plant species (Gogue, Hurst, and Bancroft 1974; Morgan and Overholt 2005; Donnelly, Green, and Walters 2008) and is also suspected of causing allergic reactions and respiratory illness in sensitive humans from volatiles released by the leaves, flowers and fruits (Morton 1978). Biological control efforts against *S. terebinthifolius* began in Hawaii in the 1950s and resulted in the release of three insect species: a gall-forming caterpillar, *Crasimorpha infuscata* Hodges (Lepidoptera: Gelechiidae), a leaf-tying caterpillar, *Episimus unguiculus* Clarke (= *Episimus utilis* Zimmerman) (Lepidoptera: Tortricidae), and a seed-feeding beetle, *Lithraeus atronotatus* (Pic) (Coleoptera: Chrysomelidae: Bruchinae) (Davis and Krauss 1962; Krauss 1962, 1963; Hight, Cuda, and Medal 2002). Only the last two species established field populations in Hawaii, and they are exerting only negligible control of the weed population (Hight et al. 2002). Exploration in South America for potential natural enemies for the classical biological control of *S. terebinthifolius* in Florida conducted in the 1980s and 1990s revealed the presence of at least 200 species of natural enemies (Bennett, Crestana, Habeck, and Berti-Filho 1990; Bennett and Habeck 1991). Three insects were selected for further studies in Florida: the leaf-feeding sawfly *Heteroperreyia hubrichi* Malaise (Hymenoptera: Pergidae), the sap-sucking thrips *Pseudophilothrips ichini* Hood (Thysanoptera: Phlaeothripidae), and the defoliating caterpillar *E. unguiculus* (Medal et al. 1999; Hight et al. 2002; Martin et al. 2004; Cuda, Medal, Gillmore, Habeck, and Pedrosa-Macedo 2009). Due to sawfly toxicity (Oelrichs et al. 1999; Dittrich, Macedo, Cuda, and Biondo 2004), incorrect thrips identification during testing (Mound, Wheeler, and Williams 2010), or a lack of sufficient specificity (Wheeler unpublished data), none of these biological control candidates has been released in the continental US.

The continuous spread of invasive *S. terebinthifolius* and the environmental concerns regarding pesticide use motivated the search for additional natural enemies against this weed in Argentina and Brazil. These surveys revealed the presence of four previously unknown species of plant-mining gracillariids (Mc Kay et al. 2009; Davis, Mc Kay, Oleiro, Diniz Vitorino, and Wheeler 2011; Wheeler unpublished data). Among these, *Leurocephala schinusae* Davis & Mc Kay were selected for further investigations. Preliminary host-range studies conducted in Argentina and at the quarantine facility of the USDA-ARS-Invasive Plant Research Lab (IPRL) in Ft Lauderdale, FL, USA indicated a preference of *L. schinusae* for *Schinus* species, but also revealed that *L. schinusae* would oviposit and complete larval development on some native North American Anacardiaceae (*Rhus* species) (Mc Kay et al. 2009; Davis et al. 2011; Wheeler unpublished data). These preliminary results suggested an unacceptable host range for *L. schinusae.* However, considering (1) the severity of the problems caused by existing infestations of *S. terebinthifolius*, (2) the insufficient alternative methods of control, and (3) the expected further spread of the weed, we decided to complete the host-specificity studies to assess the real risk posed by *L. schinusae* on non-target species. In this paper, we present the results of field and laboratory studies for this leaf-mining moth.

Materials and methods

Cultures of *L. schinusae* were established at the USDA-ARS-South American Biological Control Laboratory (SABCL) in Hurlingham, Argentina and at the USDA-ARS-IPRL in Ft. Lauderdale, FL, USA from fresh leaf mines collected on *S. terebinthifolius* between 2006 and 2008 in Argentina and Brazil. *L. schinusae* was found at several localities in Corrientes and Misiones Provinces in north-eastern Argentina and from Salvador, Bahia, to Porto Alegre, Rio Grande do Sul, in Brazil (Mc Kay et al. 2009; Davis et al. 2011; Wheeler unpublished data) (Figure 1).

Host range of L. schinusae

The host range of *L. schinusae* was determined with adult no-choice oviposition (Argentina and USA), and larval development tests (USA). Unless otherwise described, experiments were carried out in controlled environmental chambers (25 ± 2°C: 60–80% RH; 16:8 L:D). Tests were performed on native South American, agricultural, and ornamental species at the SABCL and on native North American, Hawaiian, Caribbean and agricultural species at the IPRL. In addition, field host range of *L. schinusae* was obtained from sampling the foliage of native South American Anacardiaceae species coexisting with *S. terebinthifolius* populations in north-eastern Argentina.

Studies conducted in Argentina

No-choice oviposition test

Eight plant species in five genera of Anacardiaceae were selected based on taxonomic relatedness to *S. terebinthifolius*, economic importance, and availability, as follows: *S. terebinthifolius* haplotype B from Florida (commercially available in Argentina) (Williams, Overholt, Cuda, and Hughes 2005; Wheeler unpublished data), used as control, *Schinus fasciculatus* (Griseb.) ('molle'), *Schinus lentiscifolius* Marchand ('caroba'), *Schinus molle* L. ('Peruvian peppertree'), *Astronium balansae* Engl. ('urunday'), *Lithrea molleoides* (Vell.) Engl ('aroeira blanca'), and the agricultural species *Pistacia integerrima* J.L. Stewart ex Brandis, *Pistacia vera* L. ('pistachio nut'), and *Mangifera indica* L. ('mango') (all from subfamily Anacardioideae) (Pell, Mitchell, Miller, and Lobova 2011). Special attention was given to *Pistacia* species, important crops in the western USA.

In each replicate, 20 adults were confined inside three-litre plastic jars containing a bouquet of freshly excised leaves with their petioles inserted in 15 ml glass vials filled with water. Moistened tissue paper was placed in the bottom of each jar. A piece of cotton soaked in water-sucrose solution tied with a wire and hung from the

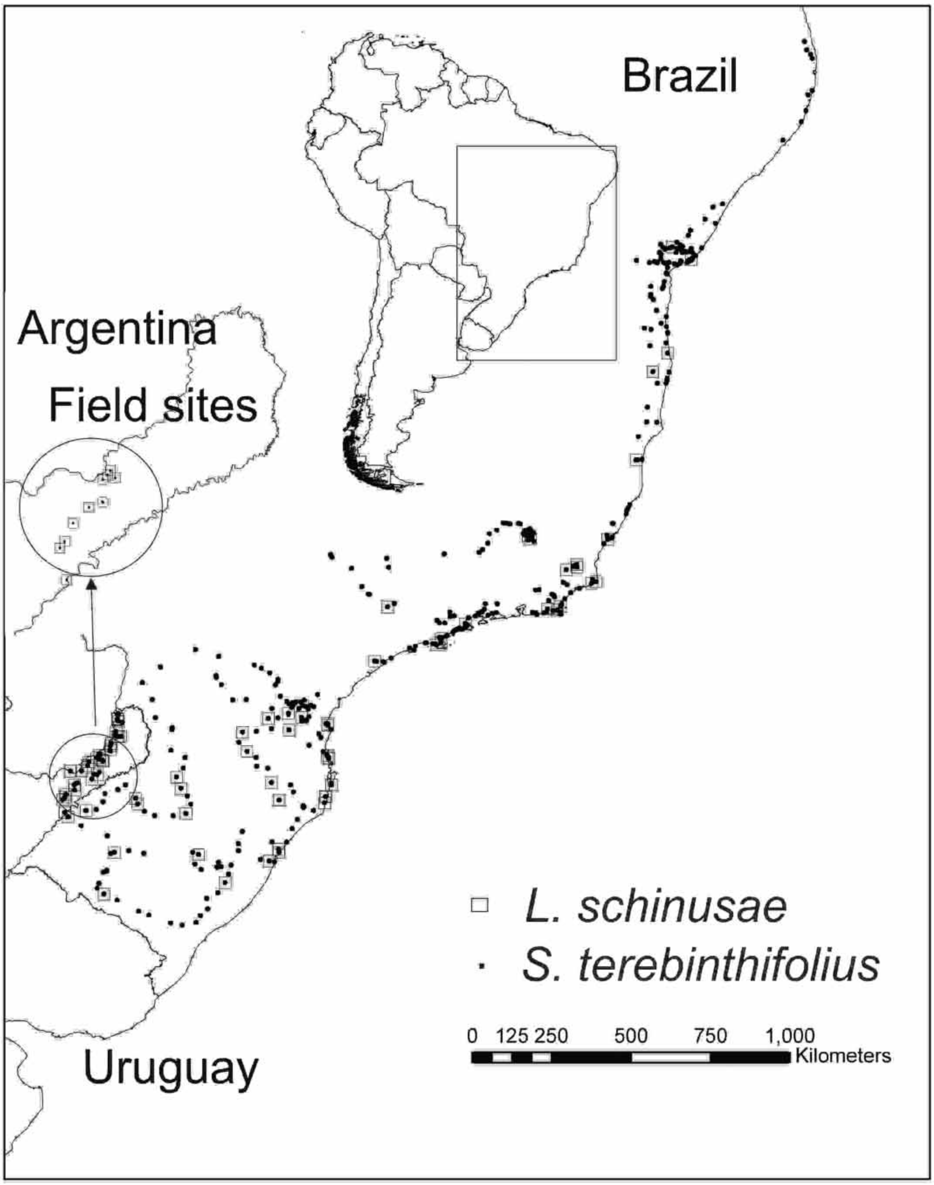

Figure 1. Distribution of the host *Schinus terebinthifolius* and its gracillariid leafminer, *Leurocephala schinusae,* in South America. Inset shows Argentinian field sites where field host range was assessed on South American native Anacardiaceae.

lid constituted the adults' food source. Adults used in tests were previously fed in empty jars for 24–48 h. The experiment ended when all adults were dead (about 15 d). Test-plant leaflets were checked under the stereomicroscope (10 $\times$) and the number of eggs recorded.

Multiple-choice oviposition and larval development test

In order to evaluate the host specificity of *L. schinusae*, but under less conservative conditions, a one-replicate multiple-choice adult oviposition and larval development test was conducted in an outdoor screen cage (2 m^3) set-up at SABCL's garden. Plant species included were: *M. indica, L. molleoides, S. lentiscifolius* and *S. terebinthifolius.* Three potted plants of each test species (1.5–2 m high) with similar foliage area were placed inside the cage. A total of 450 adults were released during 10 days and allowed to oviposit until death. When all adults were dead, we counted the number of eggs and incipient mines and the number of adults that emerged for each test plant.

Seasonal occurrence and plant-use under field conditions in Argentina

Foliage of *S. terebinthifolius* and six other South American native Anacardiaceae species were sampled at 10 sites along 150 km in north-eastern Argentina (Corrientes and Misiones provinces) (Figure 1) on four dates between June 2008 and May 2009. At each site, we randomly collected leaflets (each species filling a 10-liter container) from 2 to 7 plant species growing intermixed and 10 plants of each species, separated by 5–10 m. The number of leaflets collected for each species/site/sample date was estimated for *A. balansae* (3772 leaflets), *L. molleoides* (6088 leaflets), *S. lentiscifolius* (8860 leaflets), *Schinus longifolius* (Lindl.) Speg. (3176 leaflets), *S. molle* (11,984 leaflets), *S. terebinthifolius* (4376 leaflets), and *Schinus weinmannifolius* Engl. (6375 leaflets).

Samples were kept in plastic bags inside a cooler and brought to the laboratory within 1–2 d. Once in the lab, the occurrence of empty or active (with the larva present) blotch mines in leaflets was determined under a stereo microscope (10 $\times$). The blotch mines created by this species are distinct and can be distinguished from those made by other *S. terebinthifolius* herbivores (Davis et al. 2011).

Studies conducted in USA

No-choice oviposition and larval development test

Plants were grown in a research garden and fertilised with both liquid (Miracle-Gro for acid loving plants, 30N-10P-10K) and slow-release (Multicote 4, 14N-14P-16K) formulations according to label directions. No pesticide was applied within 3 months of the beginning of these experiments. Plant species included the North American Anacardiaceae species *Metopium toxiferum* (L.) Krug & Urb. ('Florida poisontree'), *Toxicodendron radicans* (L.) Kuntz ('eastern poison ivy'), *Rhus aromatica* Aiton ('fragrant sumac'), *R. glabra* L. ('smooth sumac'), *Rhus copallinum* L. ('winged sumac'), Hawaiian species *R. sandwicensis* A. Gray ('neneleau'), South American species *S. molle* ('Peruvian peppertree'), and the agricultural species *M. indica* L. ('mango') *Anacardium occidentale* L. (cashew), *Pistacia terebinthus* L. (all from subfamily Anacardioideae) and the Caribbean species *Spondias mombin* L ('yellow mombin') (subfamily Spondioideae) (Pell et al. 2011).

The insects were collected as larvae in July 2008 from the state of Rio Grande do Sul, Brazil (S29.59451; W50.00427; 6 m elevation) and introduced into quarantine at the IPRL. The insects were colonised in insect rearing sleeve cages (BugDorms 30 $\times$ 70 cm, white, Bioquip, Rancho Dominguez, CA) covering *S. terebinthifolius* branches and sealed around the branch with a foam stopper. The insects were reared in a greenhouse (28 ± 5°C) under ambient photoperiod. The rearing sleeves containing the insects were lightly misted daily to increase humidity. For each test, 20 freshly emerged adults (generally 1 d old) were released inside each sleeve cage and allowed to oviposit until death. After 50 d we removed the cages and counted the number of leaflets aborted, incomplete and complete mines, pupae and adults. The experiment included four replicates of each test plant species and 20 replicates of *S. terebinthifolius*.

Statistical analysis

Data from the Argentinean results were analysed with statistical package Statistica (version 7.0; StatSoft. Inc., 1984–2000). For the adult no-choice oviposition tests, the number of eggs was compared using a one-way ANOVA. Prior to analysis the data were (Log_{10} +1) transformed. Means were compared using Tukey's HSD for unbalanced designs (Zar 1996). To analyze the field host range, Chi-square analyses were conducted to examine the association between active and old mines collected on different species on different sampling dates. The North American no-choice results were analysed with SAS (1990) (version 9.1; SAS Institute, Cary, NC, USA). Results of no-choice oviposition (incipient mines), complete mines, pupal and adult emergence were analysed with one-way ANOVA and means were compared with a Tukey's HSD ($P < 0.05$). In addition, the number of leaflets aborted was determined and analysed similarly. To examine the relationships between the percentage of aborted leaflets versus incipient mines and the percentage of aborted leaflets versus adult emergence Pearson Correlation analyses were conducted.

Results

Seasonal occurrence and plant-use under field conditions in Argentina

At the four sampling dates, *L. schinusae* blotch mines were found on leaflets of *A. balansae, S. lentiscifolius, S. longifolius*, *S. weinmannifolius* and *S. terebinthifolius.* However, over the entire sampling period the majority of active (92.9%; $\chi^2 = 41.9$; df = 6; $P < 0.0001$) and old (94.7%; $\chi^2 = 900.3$; df = 6; $P < 0.0001$) mines was found on *S. terebinthifolius.* Even at their peak density, a very low percentage of the collected leaflets were mined by this species on the primary host, *S. terebinthifolius.* Only 11 (0.26%) of the estimated 4376 *S. terebinthifolius* leaflets collected in June 2008 contained active mines while 201 (4.6%) contained old mines. Moreover, more mines were found on *S. terebinthifolius* during the austral winter (June 2008: $\chi^2 = 15.8$; df = 1; $P < 0.0001$; May 2009: $\chi^2 = 10.4$; df = 1; $P = 0.0013$ Table 3). Similarly, although active leaf mines were found during all four sampling dates, the highest percentages were found during June 2008 (44.3%) and May 2009 (28.6%). No blotch mimes were ever found on leaflets of *L. molleoides* or *S. molle* (Table 1).

Table 1. Field host range of *Leurocephala schinusae* determined from sampling of native South American Anacardiaceae.

Date of field survey	Plant species	Total number of mines from 10 sites	
		Active	Old
June 2008	*Astronium balansae*	1	1
	Lithrea molleoides	0	0
	Schinus lentiscifolius	0	3
	Schinus longifolius	0	1
	Schinus molle	0	0
	Schinus terebinthifolius	30	121
	Schinus weinmannifolius	0	2
September 2008	*A. balansae*	0	2
	L. molleoides	0	0
	S. lentiscifolius	0	11
	S. longifolius	3	2
	Schinus molle	0	0
	S. terebinthifolius	12	658
	S. weinmannifolius	0	22
December 2008	*A. balansae*	0	0
	L. molleoides	0	0
	S. lentiscifolius	0	0
	S. longifolius	0	0
	Schinus molle	0	0
	S. terebinthifolius	4	53
	S. weinmannifolius	0	2
May 2009	*A. balansae*	1	1
	L. molleoides	0	0
	S. lentiscifolius	0	0
	S. longifolius	0	0
	Schinus molle	0	0
	S. terebinthifolius	2	19
	S. weinmannifolius	0	0

Host range of L. schinusae

No-choice oviposition test – Argentina

Leurocephala schinusae showed a host range that included many of the tested species (Table 2). Eggs or incipient mines were found on all native South American Anacardiaceae, except *L. molleoides.* Eggs were also recorded on the exotic and economically important *P. vera* and *M. indica*, the latter with only one egg in one replicate. However, no eggs were found on *P. integerrima*.

Multiple-choice oviposition and larval development test-Argentina

Eggs and incipient mines of *L. schinusae* were found on the three potted *S. terebinthifolius* plants and on two of the closely related *S. lentiscifolius* plants. From a total of 109 eggs found on *S. terebinthifolius*, only 30 adults emerged. From a total of

Table 2. No-choice oviposition tests of *Leurocephala schinusae* undertaken in Argentina.

Test-plant species	Number of replicates	Status in USA	Number of eggs or incipient mines (Mean ± SE)[2]
Astronium balansae	7	Not recorded	4.8 ± 2.8^{abc}
Lithrea molleoides	8	I (CA)	0
Mangifera indica	6	I/C (FL, HI)	0.2 ± 0.2^{c}
Pistacia integerrima	7	C (CA)	0
Pistacia vera	5	C (CA)	12.8 ± 6.6^{abc}
Schinus fasciculatus	5	Not recorded	177.6 ± 45.8^{a}
Schinus lentiscifolius	10	Not recorded	11.7 ± 7.6^{abc}
Schinus molle	10	I (AZ, CA, FL, HI, TX)	8.6 ± 6.6^{bc}
Schinus terebinthifolius	15	I (CA, FL, HI, TX)	40.3 ± 11.1^{ab}

Note: Means followed by different letters in superscript are statistically different ($P < 0.05$); Means followed by the same letter in superscript are not statistically different ($P < 0.05$).
C, Cultivated; I, Introduced; CA, California; FL, Florida; HI, Hawaii; TX, Texas (USDA-NRCS).
[1]Number of eggs: One-way ANOVA: $F_{6, 51} = 6.7$; $P < 0.0001$. As no eggs were laid on *L. molleoides* or *P. integerrima* these data were not included in the analysis.

15 eggs found on *S. lentiscifolius*, only 12 adults emerged. Neither eggs nor incipient mines were found on *L. molleoides* or *M. indica* leaves.

No-choice oviposition and larval developmental test-USA

As with the tests conducted in Argentina, *L. schinusae* showed a host range that included several of the tested species. Except for *S. mombin*, incipient mines were found on all tested species and the highest preference occurred on *R. aromatica* (Table 3). The incipient mines successfully developed into complete mines, pupae and adults on *R. aromatica, R. copallinum*, *S. molle* and *S. terebinthifolius*. Among these species, little difference was found in insect use and success in terms of these life history parameters. However, the number of pupae was significantly greater on *R. aromatica* compared with *S. terebinthifolius*. The percentage aborted leaflets was significantly greater on *R. sandwicensis* than *S. terebinthifolius*. Possibly the increased shading caused by the sleeve cages induced increased leaf drop in this species. However, the percent aborted leaflets was not associated with incipient mines (Pearson Correlation $R = -0.10$; $P = 0.50$) or larval completion of development (Pearson Correlation $R = -0.17$; $P = 0.24$) (Table 3).

Discussion

The plant use of *L. schinusae* in the native range of *S. terebinthifolius* indicated that although *L. schinusae* displayed a clear preference for *S. terebinthifolius*, the species *S. lentiscifolius*, *S. longifolius, S. weinmannifolius* and *A. balansae* also served as natural hosts. The laboratory host-specificity tests revealed that *L. schinusae* was able to oviposit on the agricultural *Pistacia* species: *P. vera* and *P. terebinthus* and complete larval development on non-target plant species from South America (*S. lentiscifolius*, *S. molle*), and North America (*R. aromatica* and *R. copallinum*).

Table 3. No-choice oviposition and larval development test of *Leurocephala schinusae* undertaken in USA.

Test-plant species	Number of replicates	Status in USA	Number of incipient mines[1]	Number of complete mines[2]	Number of pupae developing[3]	Number of adults emerging[4]	Aborted leaves or leaflets (%)[5]
Schinus terebinthifolius	20	I (CA, FL, HI, TX)	115.8 ± 32.2^{b}	72.7 ± 11.3	68.8 ± 10.4^{b}	52.0 ± 9.2	4.3 ± 0.8^{b}
Schinus molle	4	I (CA, FL, HI, TX)	28.0 ± 10.0^{b}	34.3 ± 14.5	44.3 ± 11.9^{ab}	39.0 ± 11.0	9.5 ± 5.0^{b}
Rhus aromatica	4	N	397.75 ± 156.1^{a}	127.8 ± 50.3	159.8 ± 48.3^{a}	115.5 ± 41.3	10.6 ± 6.9^{b}
Rhus copallinum	4	N	45.5 ± 25.7^{b}	52.8 ± 32.5	62.3 ± 44.8^{ab}	31.75 ± 20.7	15 ± 6.7^{b}
Rhus sandwicensis	4	N (HI)	7.7 ± 1.2^{b}	0^{6}	0^{6}	0^{6}	41.5 ± 3.6^{a}
Rhus glabra	4	N	25.0 ± 0^{6}	0^{6}	0^{6}	0^{6}	5.2 ± 2.9^{b}
Metopium toxiferum	4	N	31.3 ± 20.3^{b}	0^{6}	0^{6}	0^{6}	5.3 ± 2.8^{b}
Toxicodendron radicans	4	N	2.0 ± 0.0^{6}	0^{6}	0^{6}	0^{6}	21.2 ± 2.9^{ab}
Spondias mombin	4	N	0^{6}	0^{6}	0^{6}	0^{6}	21.4 ± 10.8^{ab}
Pistacia terebinthus	4	C (CA)	7.5 ± 1.6^{b}	0^{6}	0^{6}	0^{6}	9.2 ± 9.1^{b}
Anacardium occidentale	4	I (PR, VI)	30.75 ± 10.0^{b}	0^{6}	0^{6}	0^{6}	20.6 ± 6.4^{ab}
Mangifera indica	4	I/C (CA, FL, HI)	10.0 ± 0^{6}	0^{6}	0^{6}	0^{6}	$25.4 \pm {}^{ab}$

Note: Statistical analyses were conducted on replicates where the response (e.g., number of complete mines) was greater than zero or one.
C, Cultivated; I, Introduced; N, Native; CA, California; FL, Florida; HI, Hawaii; PR, Puerto Rico; TX, Texas; VI, Virgin Islands (USDA-NRCS).
[1]One-way ANOVA: $F_{1,\ 51} = 5.57$; $P < 0.0001$.
[2]One-way ANOVA: $F_{3,26} = 1.56$; $P > 0.2$.
[3]One-way ANOVA: $F_{3,26} = 3.16$; $P = 0.0413$.
[4]One-way ANOVA: $F_{3,\ 26} = 2.77$; $P = 0.0616$.
[5]One-way ANOVA: $F_{11,\ 51} = 5.08$; $P < 0.0001$.
[6]Replicates omitted from analysis as no or too few observations were represented. Means within a column followed by the same letter in superscript are not statistically different ($P < 0.05$).

The acceptance of some non-target species for oviposition and development by *L. schinusae* in the laboratory tests could be a result of the artificial testing conditions (Harris and Zwölfer 1968; Dunn 1978; Shepherd 1990). In addition, it can be argued that false positives could result from laboratory tests and thus potential safe and useful agents could be rejected on the basis of no-choice tests alone (Marohasy 1998; Hill 1999). For example, the testing of *M. indica* (mango), a cultivated plant in California, Florida and Hawaii, resulted in only one egg found in oviposition tests, but no complete mines were found in the no-choice and multiple-choice larval development tests. To a lesser degree, a similar testing artefact could have resulted when testing *S. molle* (Peruvian peppertree), a valuable ornamental plant in western US. Although *L. schinusae* accepted *S. molle* for oviposition and larval development under no-choice tests, no mines were found on this plant in the field, indicating *S. molle* is only a host of *L. schinusae* under restricted testing conditions.

The two native species, *R. aromatica* and *R. copallinum*, which do not occur in South America, but grow sympatrically with *S. terebinthifolius* in the invaded areas of the USA, were accepted during the no-choice test. This apparent 'new association' of *L. schinusae* with these species, could also be interpreted as a result of restricted testing conditions and thus, a potential safe and useful agent (e. g. *L. schinusae*) would be rejected on the basis of no-choice tests alone (Marohasy 1998; Hill 1999). However, in our no-choice example, the potential danger posed by *L. schinusae* to these native species and other factors compelled us to cancel further testing.

Commonly no-choice results are followed by multiple-choice studies where the candidate agents are offered two or more plant species (Schaffner 2001). Such tests are thought to compliment no-choice tests as they aim to include components of the host finding behaviour of the candidate agent. In our example, choice tests would have been conducted on the insect's mobile stage, as adults choose oviposition sites. Our adult no-choice oviposition results indicated relatively non-discriminating behaviour as nearly all species were accepted. Thus, with the results presented above we question the added value of laboratory choice tests for this species. Moreover, regulating agencies throughout the world, and especially in the USA, have become increasingly risk averse (Sheppard et al. 2003; Dudley and Bean 2012). Results of no-choice studies showing use of valued plants are considered of greater significance by some regulators than the results of choice tests. Thus, considering our results of no-choice and field tests showing use of several non-target species and the especially cautious regulatory atmosphere we did not perform additional multiple-choice testing of this species.

In conclusion, we do not recommend *L. schinusae* for the biological control of *S. terebinthifolius* in the continental USA. However, the low performance displayed by *L. schinusae* on the only Hawaiian native Anacardiaceae, *R. sandwicensis* and the cultivated mango, justify further screening of this species as a potential biocontrol agent against *S. terebinthifolius* in Hawaii. In the case of Australia, where few endemic Anacardiaceae occur (Jessup 1985), *L. schinusae* should be considered if a project is initiated against this weed.

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