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NECTAR SOURCES FOR *LARRA BICOLOR* (HYMENOPTERA: SPHECIDAE), A PARASITOID OF *SCAPTERISCUS* MOLE CRICKETS (ORTHOPTERA: GRYLLOTALPIDAE), IN NORTHERN FLORIDA

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ABSTRACT

Larra bicolor (F.) (Hymenoptera: Sphecidae) is an introduced biological control agent of pest *Scapteriscus* mole crickets (Orthoptera: Gryllotalpidae) in northern Florida. The pests are of southern South American origin. *Larra bicolor* is widespread in South America; the imported stock is from Bolivia. Its adults seem to require nectar sources. In South America, Puerto Rico (where it was also introduced from Brazil), and southern Florida (a separate introduction from Puerto Rico), the neotropical wildflower *Spermacoce verticillata* L. (Rubiaceae) has been observed to be a favored nectar source. In northern Florida (29°N) this wildflower is uncommon, freezes to the ground at first winter frost, and does not flower again until April-May. Nevertheless, where it has been planted in northern Florida, the wasps feed on it throughout the warmer months. Wasps were observed to feed at nectaries of 10 other plant species in northern Florida. Four of these other plants were compared experimentally with *S. verticillata*, but all received fewer visits from the wasps. Known disadvantages to the use of *S. verticillata* to augment *L. bicolor* are that it is not native to Florida, and that it grows vigorously in full sun when its roots are not immersed in water. It has been reported as a minor weed in southern Florida. However, it is the best alternative to attract *L. bicolor* to places where mole cricket control is needed.

Key Words: Nectar-feeding, nectar source, biocontrol, wasp-gardening, butterfly-gardening, turfgrass, *Larra bicolor*, mole crickets

RESUMEN

Larra bicolor (F.) (Hymenoptera: Sphecidae) es un agente de control biológico de grillotopos del género *Scapteriscus* (Orthoptera: Gryllotalpidae) en el norte de la Florida. Esta plaga es originaria de Sur América. *Larra bicolor* se encuentra en varias partes de Sur América; las avispas que se encuentran al norte de la Florida se importaron desde Bolivia. En Puerto Rico (la cual se introdujo desde Brasil), y en el sur de la Florida (introducida desde Puerto Rico), *Spermacoce verticillata* L. (Rubiaceae), una planta silvestre neotropical ha sido la principal fuente de néctar para adultos de esta avispa. En el norte de la Florida (29°N) esta flor es comúnmente encontrada, se congela en el invierno con la primera helada y comienza a florecer nuevamente en abril o mayo. No obstante, la avispa se alimenta de esta flor en el norte de la Florida durante los meses calidos. La avispa fue observada alimentándose de los nectarios de otras 10 especies de plantas en el norte de la Florida. Cuatro de estas plantas fueron experimentalmente comparadas con *S. verticillata*, pero todas recibieron menos visitas de esta avispa. Algunos argumentos en contra del uso de *S. verticillata* para aumentar las poblaciones de *L. bicolor* son: la planta no es nativa a la Florida, crece vigorosamente bajo exposición total al sol siempre y cuando las raíces no se encuentren bajo el agua y se ha reportado como una maleza menor en el sur de la Florida. Sin embargo, esta planta es la mejor alternativa para atraer *L. bicolor* a lugares donde el control de grillotopos es necesario.

Translation provided by the authors.

Larra bicolor (F.) is a koinobiont ectoparasitoid of *Scapteriscus* spp. mole crickets in its native range in South America (Menke 1992). In 1936-1938 stock was imported from Belém, Pará, Brazil, to Puerto Rico, and established as a classical biological control agent of *S. didactylus* (Latreille) (Wolcott 1938, 1941a). In 1981, stock was imported from Puerto Rico by J. A. Reinert and established at Ft. Lauderdale, Florida, as a classical biological control agent of *S. abbreviatus* Scudder, *S. borellii* Giglio-Tos, and *S. vicinus* Scudder, all pests of South American origin (Sailer 1985). Stock of the same species was imported in 1988-89 from Santa Cruz, Bolivia, released and became established in and near Gainesville in northern Florida (Frank et al. 1995). The population established at the first Ft. Lauderdale site spread no more than 3 km, and attempts to redistribute it failed (Castner 1988). The stock established at Gainesville spread naturally, and has now been recorded in many counties in northern Florida, to a distance of >220 km

NW and S (J.H. Frank, unpublished). It was assumed simply that stock obtained from Bolivia was a more cold-hardy biotype of *L. bicolor* (Frank et al. 1995) because Menke (1992) could not distinguish them at the species level from the Belém/ Puerto Rico stock by morphological methods. The possibility of cryptic species has not yet been investigated. However, Menke (1992) observed and illustrated what he believed to be intraspecific variation in punctation of the head capsule of the adults of *L. bicolor* from Belém/Puerto Rico and those from Bolivia. The *L. bicolor* established in northern Florida has the punctation of the latter stock (Frank et al. 1995).

Because of its assumed cold-hardiness, it seems likely that the Bolivian stock will spread far more widely in northern Florida. Knowledge of the nectar sources of *L. bicolor* is needed to devise methods of improving the rate of spread both state-wide and locally. Encouraging the establishment of plants that serve as nectar sources (wasp-gardening) could be used to enhance wasp populations, as has been done to manage other biological control agents (Jervis 1988; Jervis & Kidd 1996).

Wolcott (1941a) collected *L. bicolor* adults from flowers of *Spermacoce verticillata* L. (Rubiaceae) in Belém, and imported them into Puerto Rico, where this plant also grows (Liogier 1980). Wolcott (1941b) considered *S. spermacoce* essential to the survival of *L. bicolor* in Puerto Rico. In Florida, all sites where *L. bicolor* was released were prepared in advance with plantings of *S. verticillata*, which was already widespread in southern Florida, but very sparse farther north, reported only in Alachua and St. Johns counties (Wunderlin 1979, 1998). Wolcott (1941b) also mentioned *Hyptis atrorubens* Poit. (Lamiaceae) as a nectar source for *L. bicolor* in Brazil and Puerto Rico. This plant is not established in Florida (Wunderlin 1998). In southern Florida, Castner (1988) observed that *S. verticillata* outperformed various native and ornamental plants in attracting adult *L. bicolor* of the Belém/Puerto Rico stock.

This paper reports research to explore several questions regarding nectar sources of *L. bicolor* in northern Florida. Does this plant have weedy characteristics? What other plant species provide useful nectar sources for *L. bicolor* and might these further its range expansion throughout Florida? What other plant species may be used to encourage local buildup of *L. bicolor* populations? How does *L. bicolor* access nectar from *S. verticillata*, and can it do the same from other plant species?

Wunderlin (1998) states that *S. verticillata* is not native to Florida, but is native to the Neotropical region, including Cuba, Haiti, Jamaica, Puerto Rico, and the Bahamas. It was not detected as established in Florida until the 1960s (B. F. Hansen, pers. comm.).

In southern Florida *S. verticillata* flowers all year (Bryan Steinberg, pers. comm.). However, in northern Florida it does not flower all year; in Gainesville (29°N) it freezes to the ground at the first frost (typically in early December), and does not flower again until late April or early May of the following year (JHF, observations). This limits its availability as a nectar source in northern Florida. The limitation is of little consequence for *Larra bicolor*, whose pupae diapause underground in winter, and whose adults have been observed to be killed by frost (Cabrera-Mireles 2000).

Spermacoce verticillata contains a low level (0.2%) of alkaloids which would be toxic if present in higher concentrations, but it serves as a non-preferred forage plant for cattle (Francis 2002 and references therein).

Materials and Methods

Movement of Plants and Seeds (Weediness)

In 1990, one of us (JHF) planted a plot of *S. verticillata* plants (obtained from roadside waste land in Miami) in the grounds of the Entomology & Nematology Department, University of Florida and, in 1997-1998 planted five other plots (progeny of the first plot) on University of Florida property in the Gainesville area. These five were all planted by the same method; each had 25-26 plants installed in a single line, on 60 cm centers through a 2.6×16 m sheet of black polyethylene, 0.15 mm thick. Plantings were variously destroyed by prolonged flooding, maintenance crews, or a construction crew, so not all were constantly available. In 2000, a seventh was planted without mulch and in several rows by USDA collaborators at the USDA, Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) garden. The seven plots were installed to allow study of the seasonality of the plant and the wasp in northern Florida, as well as to harvest wasps for distribution to distant localities. Collaborators were recruited to monitor for presence of wasps in distant counties in 2002 and 2003, and we supplied them with some of the plants. We know of no easier way of observing and collecting the wasps than their attraction to *S. verticillata* flowers, although they can be collected at traps baited with phenylacetaldehyde (Meagher & Frank 1998).

What other plant species provide useful nectar sources for *L. bicolor* and might these further its range expansion throughout Florida?

Three of the *Spermacoce verticillata* plots installed in 1997-1998 in the Gainesville, FL area were used for this 2001 study. They were at the Beef Cattle Research Unit, the Horse Teaching Unit, and the Fisheries and Aquatic Sciences Department. A fourth, at the USDA-CMAVE garden had many more plants, in several rows, without plastic mulch. The plots constantly (during the

warmer daylight hours) had feeding adult *L. bicolor* in September-November 2001.

Once every two weeks in September-November 2001, one of us (HAA) walked transects in the four cardinal and four secondary compass directions away from those four plots, until he was impeded by structures (buildings, fences, roads) or water bodies. Plants on which adult *L. bicolor* were seen feeding were identified and recorded. No attempt was made to analyze frequency of wasp-feeding observations because the observations were not random. The sole purpose was to compile a list of the plant species other than *S. verticillata* on which one or more *L. bicolor* adults were observed feeding in areas where *S. verticillata* maintained a wasp population.

What plant species may be used to encourage local buildup of *L. bicolor* populations?

Sites used were the Beef Cattle Research Unit, the G. C. Horn Turfgrass Research Laboratory, the Plant Sciences Unit at Citra, and the USDA-CMAVE garden. Four of the plant species identified as providing nectar to *L. bicolor* (*Conoclinium coelestinum* (L.) DC, *Elephantopus elatus* Bertol., *Passiflora coccinea* Aubl., and *Solidago fistulosa* Mill., see below) were available from local nurseries, and 32 of each were purchased in pots. Plants of *S. verticillata* were already in culture. All were planted in a completely randomized block design with four blocks. Each block was adjacent to one of the existing plots of *S. verticillata* to ensure that *L. bicolor* adults were present. The plants were removed from pots, planted through cuts made through a sheet of black polyethylene in the required block design, and watered daily for 5 days. Each plant was again watered once each 15 d with about 0.4 L of a 0.4% N10-P52-K10 fertilizer solution/suspension to promote flowering. Each of the blocks had five treatments (plant species), with eight plants per treatment, with each treatment in two lines of four. The separation between plants was 0.6 m within each treatment, and between treatments was 2.4 m, giving a block size of 92.8 m^2. Observations were made weekly between late July and early November 2002. Repetitions were at 10, 11, and 12 AM (GMT-5). Data recorded were the total number of adult *L. bicolor* observed, when about 20 s were spent examining each plant. The routine used was for one observer (HAA) to move left to right and clockwise among the treatments, beginning with the plant in the southwest corner of the treatment and of the block. This was a repeated measures experiment with a completely randomized block design. Analysis was made by a χ^2 pairwise comparison with the least square means (LSM) procedure with one degree of freedom. The data were adjusted to a Poisson distribution for analysis in the SAS (2000) program.

This experimental design was discussed with several researchers before it was put into operation. All recognized that each plant species has a different floral size and architecture, that the number of flowers produced by each plant varies in time, and perhaps nectar production varies within each plant. However, it was the time spent by *L. bicolor* at each plant that was to be compared, so it was not appropriate to try to control for interplant species differences that were inherent in the comparison—they are not flaws in the design. Our methods are described so that readers may accept the results or reject them, or repeat them.

How does *L. bicolor* access nectar from *S. verticillata* and other plants?

Adult *L. bicolor* were observed feeding at nectaries in the field. For 40 flowers of each of the four species (*C. coelestinum*, *E. elatus*, *S. fistulosa*, and *S. verticillata*) having floral nectaries, the distance from the rim of the corolla to the nectaries was measured in the laboratory under a dissecting microscope, as was the length of the glossa of 20 adult male and 20 female wasps.

RESULTS

Movement of Seeds and Plants (Weediness)

An unrestrained plot of *S. verticillata*, planted in 1990 on the grounds of the Entomology/Nematology Department, University of Florida, Gainesville, Florida by the end of 2003 had produced infrequent seedlings in adjacent, occasionally-mowed Bahiagrass turf, <1 m to the east, 2 m to the north, 2 m to the south, and ≈ 25 m to the southwest. Mowing of adjacent turf was by rotary mower, which, we suspect (1) prevented nearby seedlings from flowering and (2) dispersed seeds around a corner of a building only in a southwesterly direction and to a much greater distance. In other words, a plot of the plant produced seedlings to a distance of up to 2 m in 13 years, but use of a rotary mower discharged a few seeds up to ≈ 25 m in one direction, which was presumably due to the track of the mowing crew. Later plots were established in 1997-1998 through ≈ 2.6 × 16 m sheets of black polyethylene, whose original purpose was to allow establishment of the wildflower without competition from other plants. At the Beef Cattle Research Unit, after almost six years (fall 2003), there was only one seedling plant outside (by 5 cm) the confines of the original plot (outside the edge of the now-damaged plastic mulch). Mowing was done by a tractor-drawn reel mower, which may not have dispersed seeds. At the Horse Teaching Unit (after six years), there was spread by about 1 m to the south in places, but this probably was due to partial redistribution of the plot by a bulldozer moving it from its original line and destroying the

plastic mulch. Evidently *S. verticillata* is not highly 'invasive.' Seedlings it produces in adjacent turf may be controlled by occasional mowing. These are appropriate characteristics for a nectar-source plant for a beneficial insect: it may spread, and, once established, it does not demand constant care for its survival. Furthermore, *S. verticillata* plants installed in 2000 at Tifton, Georgia, were killed outright, by inadvertent application of glyphosate (Roundup®) (W. G. Hudson, pers. comm.), suggesting that the plant is easily controlled by application of this chemical herbicide. In many places, its vigorous growth is desirable.

What other plant species provide useful nectar sources for *L. bicolor* and might these further its range expansion throughout Florida?

Larra adults were observed feeding at nectaries of 10 species of plants in addition to *S. verticillata* (Table 1). The number of Florida counties shown by Wunderlin & Hansen (2003) to be occupied by the plant in question is also shown in Table 1.

What other plant species may be used to encourage local buildup of *L. bicolor* populations?

Results of the experiment are shown in Table 2 and Fig. 1. It is clear that *L. bicolor* adults spent much more time at *S. verticillata* plants than at any of the other four tested. We here assume this was due to its superiority as a nectar source. There were significant differences in all pairwise comparisons between plant species except between *P. coccinea* and *S. fistulosa* (where $P = 0.7232$).

How does *L. bicolor* access nectar from *S. verticillata* and other plants?

The length of the glossa in relation to the floral depth is shown in Fig. 2. There was no difference between length of the glossa of males and females ($F = 0.20$; $df = 1,19$; $P = 0.6631$). For two of the plant species, *S. fistulosa* ($F = 80.54$; $df = 1,39$; $P < 0.001$), *C. coelestinum* ($F = 81.86$; $df = 1,39$; $P < 0.0001$), the floral depth is less than the length of the glossa. In *P. coccinea*, the principal nectaries are extrafloral, and the measurement is irrelevant. The floral depth of *S. verticillata* matches the length of the glossa ($F = 1.46$; $df = 1, 39$; $P = 0.2341$). The floral depth of *E. elatus* seems too great to allow access by the wasp to nectaries ($F = 498.02$; $df = 1,39$; $P < 0.0001$). However, wasps were observed to extend mandibles, push the head into the flower, move the head from side to side, and thus access nectaries with the glossa. The petals are loosened from the corolla and typically fall as the wasp removes its head or leaves the flower.

Conclusion and Discussion

Although *S. verticillata* is not native to Florida, it is now widely distributed in the south of the peninsula (Table 1). Its floral nectaries are highly attractive to adult *Larra bicolor* wasps. It flowers, and presumably provides nectar, throughout the year in southern Florida, and for at least seven months of the year near Gainesville (29°N) in northern Florida. No other plant has yet been shown to rival it in Florida or Puerto Rico as an attractant for these wasps. It has potential for use in wasp-gardening, in which it is planted in plots intended to enhance local populations of *Larra bicolor* wasps to help control pest mole crickets. Its planting in areas not yet occupied by the wasp will pave the way for arrival, establishment, and beneficial effects of the wasp. Areas not yet occupied by the wasp are most likely (a) most of southern Florida, in part of which *S. verticillata* already is widespread, and (b) most of the Florida panhandle. Beneficial effects of this wasp may also be experienced in southern Georgia.

Table 1. List of plants on which *Larra bicolor* adults were observed feeding in the Gainesville, Florida, area in 2001.

Species	Family	Status	Distribution in Florida[1]
Aralia spinosa L.	Araliaceae	native	30 counties
Conoclinium coelestinum (L.) DC	Asteraceae	native	58 counties
Elaphantopus elatus Bertol.	Asteraceae	native	58 counties
Heliotropum angiospermum Murray	Boraginaceae	native	18 counties
Heliotropum curassavicum L.	Boraginaceae	not native	17 counties
Lobularia maritima (L.) Desv.	Brassicaceae	not native	3 counties
Melilotus albus Medik	Brassicaceae	not native	40 counties
Passiflora coccinea Aubl.[2]	Passifloraceae	not native	3 counties
Richardia brasiliensis Gomes	Rubiaceae	not native	51 counties
Solidago fistulosa Mill.	Asteraceae	native	55 counties
Spermacoce verticillata L.	Rubiaceae	not native	12 counties

[1]From Wunderlin & Hansen 2003.
[2]Observation by Craig Welch, graduate student, Entomology/Nematology Dept., University of Florida. It has extra-floral nectaries on which *L. bicolor* feeds.

TABLE 2. RESULTS OF PAIRWISE COMPARISONS WITH THE χ^2 TEST TO SHOW FREQUENCY OF FEEDING BY ADULT *L. BICOLOR* AT NECTARIES OF THE FIVE PLANT SPECIES.

Plant species 1	Plant species 2	*df*	χ^2	Pr > χ^2
C. coelestinum	*E. elatus*	1,326	30.14	<0.0001
C. coelestinum	*P. coccinea*	1,326	13.48	0.0002
C. coelestinum	*S. fistulosa*	1,326	21.57	<0.0001
C. colestinum	*S. verticillata*	1,326	178.13	<0.0001
E. elatus	*P. coccinea*	1,326	8.95	0.0028
E. elatus	*S. fistulosa*	1,326	8.20	0.0042
E. elatus	*S. verticillata*	1,326	98.40	<0.0001
P. coccinea	*S. fistulosa*	1,326	0.13	0.7232
P. coccinea	*S. verticillata*	1,326	122.45	<0.0001
S. fistulosa	*S. verticillata*	1,326	169.96	<0.0001

Vernacular names assigned to *S. verticillata* include 'whitehead broom' (Murphy et al. 1998, said to have been assigned by the Weed Science Society of America) and 'shrubby false buttonweed' assigned by Wunderlin (1998). The Puerto Rican common name *botón blanco* (= white button) was used by Francis (2002). The native *Spermacoce assurgens* Ruiz and Pavon ('bushy buttonweed') and non-native *S. verticillata* ('whitehead broom') are reported as weeds in turfgrass in the southern USA (Murphy et al. 1998). No golf course superintendent, extension agent, or rancher with whom we spoke recognized either of these two names (nor did they recognize the name shrubby false buttonweed). However, that publication alerts us to the 'weediness', somewhere, of *S. verticillata*.

We tried to find a native plant in northern Florida as attractive as *S. verticillata* to the wasp. This was done by searching the vicinity of established plots of *S. verticillata* for evidence of feeding on other plants, and then by experimental evaluation of relative attractiveness. We did not test the plants (mostly non-native) on which the native wasp *Larra analis* (F.) was reported by Smith (1935) to feed in Louisiana. That wasp attacks only the native mole cricket *Neocurtilla hexadactyla* (Perty). Further tests should be made of a wider range of plants, including those on which *L. analis* has been observed to feed, others on which *L. bicolor* has been observed to feed (Table 1), and native Florida species of *Spermacoce*.

Butterfly-gardeners routinely promote some non-native weedy plants such as *Buddleia* and *Lantana* species as nectar sources for butterflies, as well as others (*Asclepias*, *Aristolochia*, etc.) for host plants to draw interesting butterfly species. The crops protected are *Cynodon dactylon* (L.). Pers. and hybrids with *C. transvaalensis* Burtt-Davey (Bermudagrass, the major turfgrass in southern Florida), *Paspalum notatum* Fluegge (Bahiagrass, the major pasturegrass in Florida, and also used widely as a turfgrass), and numerous kinds of vegetable seedlings, none of which is native to Florida. We suggest that using a small percentage of the area of these crop plants to grow *S. verticillata*, another non-native plant, is much more sensible than using broad-spectrum chemical pesticides as the sole means of control of non-native pest mole crickets.

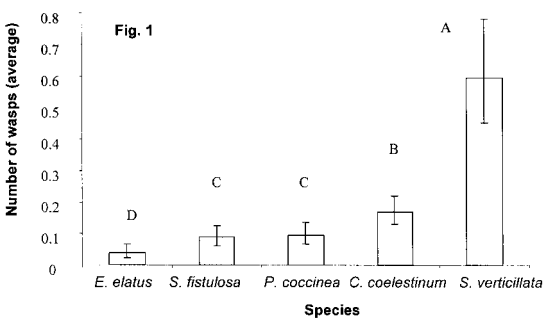

Fig. 1. Mean comparison for plant selection test made by *Larra bicolor* in the field. Data represent the average number of wasps per treatment per sampling ± SE. Bars with different letters are significantly different ($P \leq 0.005$) according to chi-square pairwise comparisons under Poisson distribution.

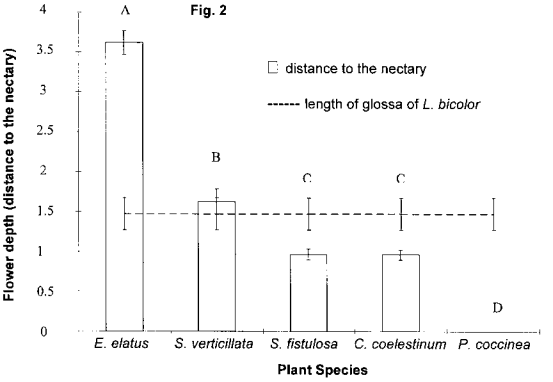

Fig. 2. Mean comparisons of floral depth (distance to the nectaries) and the length of *L. bicolor*'s glossa. Data with the same letter do not differ ($\alpha = 0.05$) according to Duncan's test. Nectaries of *P. coccinea* are extra-floral.

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