Comparison of Parasitic HymenopterA Captured in Malaise Traps Baited with Two Flowering Plants, Lobularia maritima (Brassicales: Brassicaceae) and Spermacoce verticillata (Gentianales: Rubiaceae)

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ABSTRACT

Many adult hymenopterous parasitoids feed on floral nectar, and occasionally pollen. However, flowers differ in both accessibility and attractiveness to these insects. Malaise traps, a type of "passive/interception" trap, were baited with potted flowering plants, *Lobularia maritima* L. (Brassicaceae) or *Spermacoce verticillata* L. (Rubiaceae), or left unbaited as controls. These plants have different floral structures, but both have been previously used as food-plants for biological control agents. In general, *L. maritima* captured more Braconidae, particularly Opiinae, than either alternative. Species of this subfamily attack Diptera and certain species are important natural enemies of pest Tephritidae. The roles of plant attractiveness (volatiles) and architecture (trap access) are discussed, as is the possibility of employing *L. maritima* and/or its products in monitoring or maintaining fruit fly parasitoids.

Key Words: Braconidae, Ichneumonidae, Chalcidoidea, conservation biological control

RESUMEN

Muchos adultos de parasitoides himenópteros se alimentan del néctar floral, y también de vez en cuando de polen. Sin embargo, las flores varían en su accesibilidad y atracción a estos insectos. Las trampas del Malaise, un tipo de trampa de "pasivo/intercepción", fueron proveídas con las plantas florecientes, *Lobularia maritima* L. (Brassicaceae) o *Spermacoce verticillata* L. (Rubiaceae) o dejada sin atrayente como testigo. Estas plantas tienen diversas estructuras florales, pero ambos se han utilizado previamente como fuentes de alimento para agentes de control biológico. En general, *L. maritima* capturó más Braconidae, particularmente Opiinae, comparado a cualquiera alternativa. Las especies de este subfamilia atacan Dipteros y ciertas especies son enemigos naturales importantes de plagas de Tephritidae. Dicutimos tambien los papeles respectivos de la atración de la planta (volátiles) y de la arquitectura (acceso a la trampa), al igual que sobre la posibilidad de emplear *L. maritima* y/o sus productos para el monitoreo o mantenimiento de parásitos de moscas de la fruta.

The identification of the food sources available to adult parasitoids could yield several benefits. These include: (1) obtaining kairomones that might be formulated as attractants to monitor parasitoid population dynamics, such as age-structure and dispersal; (2) characterizing sites capable of maintaining natural enemies after release; (3) using known food sources to modify agro-environments in order to better concentrate parasitoid populations; and (4) provide cues and nutrients to improve longevity, oviposition and mating in mass-rearing facilities.

Many species of adult hymenopteran parasitoids feed on the nectar of flowering plants (Jervis et al. 1993; Landis et al. 2000; Syme 1975). Nectar provides a valuable source of carbohydrates and is often the only source available (Wackers et al. 1996). Even host-feeding parasitoids consume additional floral foods (Jervis & Kidd 1986). Floral feeding increases the longevity, fecundity and parasitism rates of certain visiting wasps in the laboratory (Idris & Grafius 1995; Zhao et al. 1992), in field cages (Dyer & Landis 1996) and in the field (Zhao et al. 1992).

However, not all parasitic Hymenoptera are able to use the nutrients provided by specific flowers. Pollen feeding appears to be uncommon among parasitoids (Jervis et al. 1992), and in the case of nectar, the generally short mouthparts of most parasitic Hymenoptera restrict them to ex-

ploiting flowers with short corollas or with exposed nectaries (Patt et al. 1997). Some flower species possess guard hairs above nectaries, which only allow access to certain parasitoids (Beattie 1985; Jervis et al. 1992, 1993; Sivinski et al. 2006).

Just as not all flower-resources are accessible, neither are all flowers attractive. For example, volatile compounds vary greatly among plant species (Dudareva et al. 2000), and insects attracted to specific odor complexes also vary greatly at the level of species, family, and even order (Pellmyr 1986). Some plants such as *Ficus* sp. attract a single species of fig wasps, while at the other end of the spectrum, species of the family Apiaceae often attract Hymenoptera from multiple families.

Given the known variance in the attractiveness of flowers, and presumably in their ability to hold insects in their vicinity because of difference in nectar-accessibility, we used interception traps to evaluate the differences in the parasitic Hymenoptera associated with 2 species of flowering plants, *Spermacoce verticillata* L. and *Lobularia maritima* L. Both have been suggested for use in conservation biological control and natural enemy monitoring.

Flowering Plants used in the Experiments.

Spermacoce verticillata L. (=*Borreria verticillata* L.) (Rubiaceae) shrubby false buttonweed is a perennial dicot native to the West Indies, but introduced into Florida and Texas as well as west tropical Africa, the tropical Americas and the south Pacific. It grows in open or disturbed sandy zones and pinelands, and requires full to part sun and moderate amounts of water. *S. verticillata* will flower year round in frost-free areas. It bears small white flowers that form dense clusters at the upper stem nodes. Flowers, on average, have a 1.5-mm corolla depth, 1.0-mm corolla width and posses a honey guard at the interior base of the corolla (Sivinski et al. 2006). *Spermacoce verticillata* nectar is a major food source for the adult mole cricket ectoparasitoids *Larra bicolor* F. and *Larra analis* F. in Florida, and has been planted for monitoring purposes and to maintain populations (Frank & Parkman 1999).

Lobularia maritima L. (= alyssum) (Brassicaceae) *Lobularia. maritima* is a non-weedy dicot that was introduced from the Mediterranean and now ranges throughout most of the United States including Hawaii. It spreads as a ground cover and attracts large numbers of parasitic Hymenoptera (Chaney 1998). Small cruciform-stalked white flowers grow in clusters randomly throughout the plant. On average, corollas are 0.67 mm wide by 1.4 mm deep (Sivinski et al. 2006).

Johanowicz & Mitchell (2000) examined the use of *L. maritima* to increase the longevity of 2

augmentatively-released parasitoids, *Cotesia marginiventris* (Cresson) and *Diadegma insulare* (Cresson) in cabbage fields. In greenhouse experiments they found that both parasitoids lived significantly longer when provided with flowers over those provided with only water, and lived as long with flowers as when provided with honey. *Lobularia maritima* flowers increased female and male *Dolichogenidea tasmanica* (Cameron) longevity in cage studies (Berndt & Wratten 2005). Begum et al. (2004) found alyssum flowers to be the ideal flower food source for the egg parasitoid *Trichogramma carverae* (Oatman and Pinto), but only the white flower variety of alyssum increased *T. carverae* longevity and fecundity. This was the color used in the present experiments.

MATERIALS AND METHODS

Malaise Traps

The numbers and types of insect natural enemies attracted to *S. verticillata* and *L. maritima* were compared through flower-baited traps placed along a field margin. Insects were collected in 3 H-shaped Malaise traps (BioQuip Products, Inc. Rancho Dominguez, California, model 2875D) based on the Townes pattern (Ent. News 83:239-247, 1972). They were constructed of a dark green fine mesh (10 threads per cm) and measured 1.8 m long by 1.2 m wide. Collecting heads were located at the top of a 1.8-m aluminum pole on one end of the trap and this end was oriented to the west (2004 collecting season; see below) and southwest (2007 collecting season). These heads (model 2875 WDH) were opaque white and measured 140 mm wide by 215 mm tall with a 19-mm diameter opening for insect egress. Isopropyl alcohol (70%) was added to a depth of 2-3 cm in order to kill and preserve the trapped insects.

Plant Culture.

Spermacoce verticillata was grown in ~4-L plastic pots during the 2004 trapping season and in ~12-L pots during 2007. *Lobularia maritima* was grown in ~4-L pots during both seasons. Jungle Growth Professional Growers Mix potting soil (Piedmont Pacific, Inc. Statham GA) was the growing medium and plants were maintained in a greenhouse at the USDA, CMAVE complex in Gainesville, FL when not involved in an experiment. All flowerpots were watered daily in the greenhouse and every other day in the field.

Trap Sites and Flower Placement.

Trapping was done at 2 locations in Alachua County, FL. The first site was at the University of Florida Dairy research unit in Hague (2004 and

2007). From Apr 30 thru May 28, 2004, three traps were placed 30.5 m apart along a thin line of deciduous trees and associated undergrowth separating 2 cornfields (*Zea mays* L.; in the vicinity of 29° 47.333 N, 082° 25.047 W). From Nov 23 thru Dec 18, 2004 a similar trap line was run on the USDA-ARS Center for Medical, Agricultural and Veterinary Entomology, (29° 38.0884 N, 082° 21.640 W). Traps were placed 30.5 m apart in a straight line along a fence line with light, weedy vegetative growth, and replicated in the same manner as the traps at Hague. In 2007, the 3 traps were located along another densely vegetated margin of 1 of the above corn fields at 29° 47.332 N, 082° 25.012 W; 29° 47.328 N, 082° 24.969 W and 29° 47.311 N, 082° 24.880 W, respectively.

In both years, wild flowering plants were regularly removed from within 4 m of the traps. In 2004, 12 flowerpots, 6 per side, of any 1 species of full-flowering test-plant were placed under a particular trap. In 2007, 12 smaller pots of *L. maritima* and 6 larger pots of *S. verticillata* were used. In both years a third trap without flowerpots acted as a control.

Trapping Protocol.

The trap-treatments were randomly placed in their starting order and then rotated following each collection period. In 2004, each trap was set for 48 h, at the end of which the collecting jars were emptied and the flowerpots or "blank-control" were moved to the adjacent trap. This rotation was completed 4 times so that each treatment was exposed for 12 collection periods. In 2007, traps were set for 24 h and rotated through each position 5 times for a total of 15 exposures / treatment. All hymenopteran parasitoids were removed and preserved in 70% isopropyl alcohol in individual jars, one per collecting head, labeled and brought back to the laboratory for sorting and identification with the keys in Goulet & Huber (1993). Total Hymenoptera numbers per day and per family were recorded. In 2007, Ichneumonidae and Braconidae were further subdivided initially on the basis of body length (>1cm, 0.5-1cm and <0.5cm). In 2004, braconid subfamily identification was done by Lionel Stange, Florida Department of Agriculture, Division of Plant Industry, Gainesville, Florida, and Gregory Evans at the Systematic Entomology Laboratory, USDA/ARS/BARC Beltsville, MD. Although specimens were not identified to species, each morphotype was recorded separately and an example preserved in the authors' collection. In 2007, Braconidae were identified to subfamily by the authors and all individual parasitoids were retained in the authors' collections.

Data analysis was initially by Analysis of Variance (PROC ANOVA; SAS Inst.) with bait (plant) type, trap site, and the interaction of bait and site as variables. When statistically significant relationships were found, further comparisons of means were performed through one-way Analysis of Variance (PROC ANOVA) followed by Waller's separation tests.

RESULTS

In 2004, *L. maritima*- baited traps captured 688 Hymenoptera from 19 families, *S. verticillata* 517 individuals from 23 families and the control 534 specimens from 20 families (Tables 1 and 2). In 2007, *L. maritima* baited traps captured 918 hymenopteran parasitoids, *S. verticillata* 738 and the control 781.

Hague (2004): There were no differences in the total numbers of insects among trap-sites ($F = 1.5$, $df = 2$, $P = 0.24$) and no interactions between trap-site and flower type ($F = 0.3$, $df = 4$, $P = 0.84$). Nor was there a difference among the total numbers of insects caught on the various flower type- treatments ($F = 1.9$, $df = 2$, $P = 0.17$, Table 1). However, when the 2 families composing 88% of the capture, Ichneumonidae (53%) and Braconidae (35%), were considered separately a pattern of differential capture emerged.

Among the ichnuemonids there were differences among trap-sites ($F = 7.8$, $df = 2$, $P = 0.002$), but there were no differences among catches on flower type or any interaction between trap-site and flower type ($F = 0.01$, $df = 2$, $P = 0.99$). However, braconid numbers were influenced by flower type ($F = 3.3$, $df = 2$, $P = 0.05$), with a Waller grouping showing that *L. maritima*- traps captured significantly more insects than *S. verticillata* but not the unbaited controls. No differences were found among sites ($F = 0.7$, $df = 2$, $P = 0.50$), nor was there any interaction between site and flower type ($F = 0.03$, $df = 4$ $P = 0.99$). Of Braconidae captured in the alyssum baited malaise traps, 28% consisted of 2 unidentified "morphotypes" of Opiinae.

CMAVE (2004): The numbers of insects captured was much lower than previous samples taken at the Hague site (Table 2). There were neither significant effects of flower type nor any interactions between flower type and trap-site in ichneumonids ($F = 1.96$; $df = 2$; $P = 0.16$ and $F = 0.45$, $df = 4$, $P = 0.76$, respectively). However, there was a pronounced but insignificant effect of flower type on Braconidae ($F = 3.1$ $df = 2$; $P = 0.06$). A Waller separation test did find significantly more braconids captured in *L. maritima*-baited traps than in the empty controls. Eleven percent of the total braconids captured were opiines.

Hague (2007): While there was a difference in total numbers of parasitoids captured at the different trap sites ($F = 10.3$, $df = 2$, $P < 0.0003$), there were no differences among the trap-treat-

TABLE 1. THE NUMBERS (PERCENTAGES) OF VARIOUS FAMILIES OF HYMENOPTERA CAPTURED AT THE HAGUE, FLORIDA, SITE (2004) IN MALAISE TRAPS BAITED WITH *LOBULARIA MARITIMA* OR *SPERMACOCE VERTICILLATA* OR LEFT UNBAITED AS CONTROL.

Family	*L. maritima* Total specimens (%)	*S. verticillata* Total specimens (%)	Control Total specimens (%)
Braconidae	308 (44.9)	129 (25)	155 (29)
Ichneumonidae	256 (37.3)	261 (50.5)	253 (47.4)
Sphecidae	47 (6.8)	39 (7.5)	47 (8.8)
Halictidae	29 (4.2)	18 (3.3)	15 (2.8)
Pompilidae	20 (2.9)	17 (3.3)	25 (4.7)
Bethylidae	6 (0.87)	2 (0.39)	6 (1.1)
Proctotrupidae	5 (0.72)	4 (0.77)	2 (0.37)
Eupelmidae	3 (0.44)	2 (0.39)	3 (0.56)
Evanidae	2 (0.29)	1 (0.19)	2 (0.37)
Pteromalidae	2 (0.29)	6 (1.1)	3 (0.56)
Chalcididae	1 (0.14)	4 (0.77)	4 (0.75)
Chrysididae	1 (0.14)	0	0
Dryinidae	1 (0.14)	0	0
Embolembidae	1 (0.14)	3 (0.57)	0
Tenthredinidae	1 (0.14)	1 (0.19)	2 (0.37)
Torymidae	1 (0.14)	2 (0.39)	1 (0.19)
Figitidae	0	3 (0.57)	1 (0.19)
Vespidae	0	3 (0.57)	1 (0.19)
Eurytomidae	0	2 (0.39)	1 (0.19)
Ormyridae	0	2 (0.39)	2 (0.37)
Apidae	0	1 (0.19)	0
Mymaridae	0	1 (0.19)	0
Scelionidae	0	1 (0.19)	0
s.f. Chalcidoidea	2 (0.29)	12 (2.3)	8 (1.5)
s.f. Cynipoidea	1 (0.14)	3 (0.57)	2 (0.37)
s.f. Proctotrupoidea	1 (0.14)	0	1 (0.19)

ments ($F = 0.9$, $df = 2$, $P = 0.42$) or the interaction between site and treatment ($F = 0.6$, $df = 2$, $P = 0.64$, Table 3). This pattern of differences among sites but not treatments was true of Icnuemonidae in particular (respectively; $F = 12.7$, $df = 2$, p< 0.0001; $F = 0.03$, $df = 2$, $P = 0.97$; $F = 0.6$, $df = 4$, $P = 0.70$). Among the Braconidae, the situation was reversed. There was no difference among trap-site ($F = 1.3$, $df = 2$, $P = 0.29$), but there was a difference among trap-treatments ($F = 7.7$, $df = 2$, $P = 0.002$) with *L. maritima* baited traps capturing more insects than either *S. verticillata* or controls. The difference among the treatments was due to the differential capture of relatively small individuals, those < 0.5 cm in length ($F = 8.3$, $df = 2$, $P = 0.001$). Of the 19 braconid subfamilies represented in the 2007 trap-captures, Opiinae and Microgasterinae, were exceptionally abundant (Table 3). Opiines were more likely to be captured in Malaise traps baited with *L. maritima* than in unbaited traps or those baited with *S. verticillata* ($F = 8.6$, $df = 2$, $P = 0.0009$) This was not the case with Microgasterinae ($F = 0.2$, $df = 2$, $P = 0.82$). In neither subfamily was there a significant effect of trap-site or any interaction between flower-baits and trap-site. There was a significant interaction between treatment and trap-site in the relatively small numbers of chalcidoids captured ($F = 2.8$, $df = 4$, $P = 0.04$). A Waller means separation test found *L. maritima* – baited traps to have caught more insects than those with *S. verticillata*, but neither flower differed from the unbaited control.

DISCUSSION

Although nectar feeding, and to a lesser extent pollen consumption, is common in the parasitic Hymenoptera (Jervis et al. 1993), the baited-Malaise trap experiment could not irrefutably demonstrate that the Hymenoptera captured were feeding on flowers. Direct observations of feeding were not recorded. However, there are at least 2 likely explanations for the differences in the numbers of Braconidae, particularly Opiinae, captured in the various traps: (1) that there are differences in floral attractiveness, and (2) that there were differences in floral food quality / accessibility so that particular insects spent less

TABLE 2. THE NUMBERS (PERCENTAGES) OF VARIOUS FAMILIES OF HYMENOPTERA CAPTURED AT THE CENTER FOR MEDICAL, AGRICULTURAL AND VETERINARY ENTOMOLOGY, GAINESVILLE, FLORIDA, SITE (2004) IN MALAISE TRAPS BAITED WITH *LOBULARIA MARITIMA* OR *SPERMACOCE VERTICILLATA* OR LEFT UNBAITED AS CONTROL.

Family	*L. maritima* Total specimens (%)	*S. verticillata* Total specimens (%)	Control Total specimens (%)
Ichneumonidae	45 (45)	28 (32.2)	24 (48)
Braconidae	27 (27)	24 (27.6)	11 (22)
Pteromalidae	6 (6)	4 (4.6)	2 (4)
Sphecidae	5 (5)	3 (3.4)	6 (12)
Torymidae	5 (5)	11 (12.6)	3 (6)
Chalcididae	3 (3)	2 (2.3)	0
Halictidae	3 (3)	1 (1.1)	0
Apidae	2 (2)	1 (1.1)	0
Eurytomidae	2 (2)	0	0
Scelionidae	1 (1)	1 (1.1)	0
Vespidae	1 (1)	6 (6.9)	4 (8)
Encyrtidae	0	1 (1.1)	0
s.f. Chalcidoidea	0	3 (3.4)	0
s.f. Cynopoidea	0	2 (2.3)	0

time in the vicinity *S. verticillata*-baited (and unbaited) traps and were less likely to be captured.

Sivinski et al. (2006) examined several flowering plants, including *L. maritima* and *S. verticillata,* as food sources for the opiine braconid *Diachasmimorpha longicaudata* (Ashmead). Individuals were observed flying to and apparently feeding upon the flowers of *L. maritima* in a greenhouse. Male and female maximum longevities in cages with flowers were greater than those with access to water only, but there was no difference in mean longevities.

Under laboratory conditions, *D. longicaudata* responded in flight tunnels to *L. maritima* but not to *S. verticillata*. Volatiles of the 2 flowers were collected and analyzed by capillary gas liquid chromatography (GC) and mass spectral analysis (MS). Acetophenone was isolated from *L. maritima* but not from *S. verticillata*. In flight tunnels, female *D. longicaudata* significantly attracted to acetophenone but no odor source, either floral or floral-derived, was attractive to male parasitoids. The demonstration of an attractive floral volatile-compound suggests that it was not foliage or any associated insect hosts / honeydew producers that were responsible for the attractiveness of *L. maritima* in the field.

Flower morphology is known to influence insect parasitoid access to nectar and pollen (Patt et al. 1997; Wäckers 2004). The characters considered to be most likely to effect access to nectar are corolla width at the bottom of the flower where nectar was present and corolla depth. The first because a relatively narrow corollar diameter might block the entrance of an insects head and the later because the typically short tongues of Braconidae, particularly small species, might be better suited to feeding on shallow flowers (see Jervis 1998). *Lobularia maritima* and *S. verticillata* have similar corollar widths and depths, but the later has what appears to have a honey guard, filaments positioned above the interior nectar source, that might restrict the access of an insect too small and weak to displace them (Sivinski et al. 2006).

Not only flower, but whole plant architecture might play a role in accessibility as well. *Spermacocae verticillata* is a higher growing plant and was potted in larger/taller pots than was *L. maritima*. It is possible that low-lying insects might have been blocked from the upper reaches of the Malaise trap by *S. verticillata*, or simply do not forage far enough above the ground to exploit its flowers. If the former (blocking) but not the latter (foraging height) were the case, unbaited traps would be expected to capture more insects than those baited with low growing plants, which in turn would capture more than high growing plants. This was generally not the case, although partially consistent with the pattern of 2007 chalcidoid and 2004 braconid captures, in which *L. maritima*- traps captured more insects than those baited with *S. verticillata* but not more than the unbaited control. Such complications make us very reluctant to extrapolate from negative results that certain taxa, e.g., ichneumonids and microgasterine braconids, did not feed on either flower. For example, there is laboratory evidence that at least certain microgasterine parasitoids feed on *L. maritima* (Johanowicz & Mitchell 2000).

The most consistent result of our trapping was the greater numbers of opiine braconids caught in *L. maritima* –baited traps. Species of this subfamily oviposit in the larvae and eggs of Diptera and complete development in the hosts' prepupae (Wharton & Marsh 1978; Shaw & Huddleston 1991; Wharton 1997). Many important biological control agents of pest Tephritidae are opiines and

TABLE 3. THE NUMBERS OF THE VARIOUS BRACONID SUBFAMILIES CAPTURED AT THE HAGUE, FLORIDA, SITE DURING MAR AND APR 2007 IN MALAISE TRAPS BAITED WITH *LOBULARIA MARITIMA* OR *SPERMACOCE VERTICILLATA* OR LEFT EMPTY AS A CONTROL.

Subfamily	*L. maritima*	*S. verticillata*	*Control*
Agathidinae	1	0	0
Alysiinae	23	12	23
Aphidiinae	7	3	2
Blacinae	3	2	1
Braconinae	7	2	5
Cardiochilinae	1	0	0
Cheloninae	10	5	5
Doryctinae	2	1	1
Euphorinae	23	10	9
Helconinae	0	1	1
Homolobinae	7	8	13
Hormiinae	1	1	1
Ichneutinae	0	2	0
Macrocentrinae	2	1	1
Microgasterinae	120	69	87
Opiinae	100	29	52
Orgilinae	23	11	23
Roganiae	1	1	2
Sigalphinae	1	0	0

some of these are mass-reared for inundative area-wide releases (Sivinski et al. 1996). Of the opiines captured in 2007 in traps baited with *L. maritima,* 35% were from various ill-defined species of *Utetes,* a genus that includes *U. anastrephae* (Viereck), a wide spread and sometimes abundant natural enemy of tropical and subtropical fruit infesting tephritids, and *U. canaliculatus* (Gahan), which with related species attacks various pest species in the genus *Rhagoletis* (Lopez et al. 1999). Given this and the previously mentioned attraction of another tephritid biological control agent, *D. longicaudta,* to a *L. maritima* volatile constituent, it might be profitable to examine *L. maritima* and/or its odors as means of attracting, concentrating and maintaining tephritd fly natural enemies in fruit agroecosystems.

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