METAPHYCUS SPECIES NEAR *FLAVUS* RESPONSES TO SEMIOCHEMICALS RELEASED FROM A SCALE HOST, *COCCUS HESPERIDUM*

P. Lo Bue,[1,2] L. D. Forster,[2] J. G. Millar,[2] S. Colazza[1], and R. F. Luck[2]*
[1] University of Palermo, S. En. Fi. Mi. Zo. Department, Entomology, Acarology and Zoology, Viale delle Scienze, 13, 90128 Palermo, Italy[2] University of California, Department of Entomology, Riverside, California, 92521 USA

ABSTRACT

Metaphycus sp. nr. *flavus* (Encyrtidae: Hymenoptera) is a Mediterranean parasitoid species which oviposits in the immature stages of several economically important soft scale insects (Hemiptera: Coccidae), including brown soft scale *Coccus hesperidum* L. and *C. pseudomagnolium* (Kuwana). In Y-olfactometer bioassays measuring wasp choices and residency times, naïve parasitoids were significantly more attracted to yucca leaves infested with 26, 27, 28, and 29 d-old scale than to uninfested leaves, whereas leaves with 30 d-old scale were no more attractive than uninfested leaves. Parasitoids also spent significantly more time in the arm with yucca leaves infested with 26, 27, 28, and 29 d-old scale than in the arm with uninfested leaves, but they spent substantially more time in the arm with a yucca leaf infested 26 d-old scale than in the arm of any other age class of scale. Further, yucca leaves that had been infested with 26 d-old scale but from which the scales had been removed were as attractive as infested leaves. However, yucca leaves that had been infested with 26 d-old scales but from which the scales had been removed and the leaves washed with distilled water were less attractive than infested leaves. When the wash water containing scale residues was used to impregnate a filter paper disk, the impregnated disk was more attractive than a control disk impregnated with distilled water. In sum, these results suggest that *Metaphycus* sp. nr. *flavus* females utilize volatile, water soluble compounds produced by brown soft scale as cues to locate suitable hosts. These results also suggest that 26-28 d-old scales are more attractive than older scales. Significantly, these are the scale ages on which *M.* sp. nr. *flavus* develops most successfully and suffers the least mortality from encapsulation.

INTRODUCTION

Brown soft scale, *Coccus hesperidum* Linnaeus (Hemiptera: Coccidae), is a cosmopolitan pest of agricultural and ornamental plants (Saakyan-Baranova 1964; Gill and Kosztrab 1997), and of plants in indoor plantscapes (Stauffer and Rose 1997). Dense scale populations feeding on these plants reduce plant vigor, kill twigs, reduce yields, and decrease visual appeal. The scale also produces honeydew on which sooty mould grows, which can further downgrade plant and fruit appearance. *C. hesperidum* often develops as colonies on a plant's leaves and twigs because ants tend the scale to obtain honeydew, while protecting the scale from its natural enemies (Ebeling 1959; Gill 1988). In California, *C. hesperidum* usually occurs at low densities in citrus groves and on outdoor ornamental plants because several biological control agents suppress it in the absence of ants (Bartlett 1978, Bernal et al. 1998). *Metaphycus* species near *flavus* (Hymenoptera: Encyrtidae) was imported into California from Kosan, Turkey, in 1996 for release against a closely related scale, citricola scale, *Coccus pseudomagnoliarum* (Kuwana), which is a periodic pest of citrus in the San Joaquin Valley of California (Bernal et al. 1999). However, citricola scale has an annual life cycle and is difficult to rear in large numbers, so we mass produce *M.* sp. nr. *flavus* on brown soft scale (L. D. Forster, P. Pacheco, R. F. Luck, and J. Sinclair, in prep), a natural host for this parasitoid (Guerrieri and Noyes 2000). This strategy works well in providing large numbers of wasps for release but it remains unclear whether *M.* sp. nr. *flavus* reared from brown soft scale recognizes and utilizes citricola scale effectively. Thus, we sought: 1) to determine whether *M.* sp. nr. *flavus* uses volatile cues associated with *C. hesperidum* to locate this host and 2), to determine whether these cues are produced directly by the scale, by the plant in response to a scale infestation, or by some combination of the two if volatile cues are involved.

MATERIALS AND METHODS

Scale and parasitoid cultures

A brown soft scale colony was established with scale obtained from a pineapple guava plant, *Feijoa sellowiana* O. Berg (Myrtaceae), located on the UCR, CA campus. We reared the scales on excised leaves of *Yucca* sp. (Lilliaceae) maintained hydroponically in the UCR insectary at 27-28°C, 60% R.H., and a 21L: 3D photoperiod (L. D. Forster, P. Pacheco, R. F. Luck, and J. Sinclair, unpubl. data). The excised leaves were obtained from yucca plants maintained on the Ag. Exp. Sta., UCR. We maintained the parasitoid colonies by introducing mated females into plastic tubes (7.5 cm diam. x 50 cm long) containing 1-2 scale-infested yucca leaves, streaked the inside of the tubes with honey, closed them with nylon-vented plastic caps, and maintained them at 25 ± 1°C and 50-70% R.H. under continuous light. Scale size was standardized at ca. 2.0 mm in width. After ca. 14 d, individual scales containing parasitoid pupae were isolated to obtain naive females (= no encounters with healthy scales). Newly

emerged wasps were collected, each female was paired with a male, and the pair was placed in a 1 cm diam. glass vial with a drop of honey and stoppered with a cotton plug. Vials were stored at 25 ± 1°C and 50-70% R.H. at 14L: 10D photoperiod for 24 h prior to testing to allow egg maturation. *M.* sp. nr. *flavus* are synovigenic (Clausen 1940) and only search for hosts when they have mature eggs. Each female wasp was used only once in an experiment and then discarded.

Bioassay protocol

24 h-old parasitoids were bioassayed using a Y-olfactometer constructed from a transparent, 15 mm thick polycarbonate sheet (190 x 200 mm) with a Y-shaped space milled into its center, consisting of a central arm 90 mm long and two 80 mm long lateral arms, each at an angle of 130º to the central arm. Holes were drilled horizontally into the base of the sheet and into the end of each arm, through which we introduced test insects or stimulus airflows. The olfactometer was laid horizontally, and sandwiched between two, 15 x 20 cm glass sheets. We used 25 cm long Tygon® tubing to connect each of the two, 8 cm long arms to parafilm-sealed, 800 ml glass aeration chambers, one chamber per arm. The Tygon® tubes were changed every fifth experimental run. Humidified medical air was passed through each aeration chamber at a flow rate of 300 ml/min per chamber.

Bioassays

We first tested whether a wasp spent a similar amount of time in each arm of the Y-olfactometer in the absence of yucca leaves and scale to verify the absence of a positional bias. For all subsequent bioassays, we tested whether *M*. sp. nr. *flavus* responded to a leaf infested with *C. hesperidum* or its residues. We first tested leaves infested with 26, 27, 28, 29, and 30 d-old scale (ca. 300 scale per leaf) versus clean leaves which had never been exposed to scale. We ran 28 reps (a rep = an individual female parasitoid) with 26 d-old scale; 28 reps with 27 d-old scale; 28 reps with 28 d-old scale; 31 reps with 29 d-old scale, and 32 reps with 30 d-old scale. Infested leaves were randomly assigned to each arm at the beginning of the bioassays and, we replaced and reversed their position after testing three wasps.

A second series of bioassays tested the responses of female *M*. sp. nr. *flavus* to scale residues left on the yucca leaves after the scale (ca. 150, 26 d-old scale per leaf) had been removed, and to previously infested leaves with both scale and residues removed. We washed a subset of leaves from which the scale had been removed with 20 ml of warm distilled water to obtain leaves without scale residues. All leaves with and without residues were tested against noninfested leaves (30 reps per trial), with the infested yucca leaves assigned randomly to one of the two arms. We replaced the infested or treated leaf and reversed their positions every fifth replicate.

A third series of bioassays tested whether female *M*. sp. nr. *flavus* were attracted to 7 cm diam. filter paper discs treated with 0.5 ml (= 3 scale equivalent) of the 20 ml of wash-water containing scale residues from the previous trials. Treated discs were placed in the 800 ml glass chambers, and the chambers were attached to the arm of the Y-olfactometer as described above. The second chamber held a disk impregnated with 0.5 ml of distilled water as a control. The treated discs were replaced after testing 3 wasps. We ran 35 reps comparing residue-impregnated disks versus distilled water-impregnated control disks.

In all bioassays, each time we reversed the test stimulus positions, we changed the olfactometer glass plates and wiped the walls of the Y-shaped arena with solvent and air dried them before reuse. We disassembled the whole system and thoroughly washed and rinsed the glass parts in acetone at day's end and baked them overnight at 200ºC. The Y-shaped olfactometer was cleaned by wiping it with pentane-wetted tissues, and then air-dried. Bioassays were conducted at 23º C between 0900 and 1500 hrs. In each experimental replicate, a single, naïve female parasitoid was gently introduced into the hole at the base of the Y-olfactometer's central arm. The parasitoid's behavior was recorded for 5 min with a charge coupled device camera (Sanyo VCB 3512T with Sony TV zoom lens F12.5 mm to 75 mm) mounted above the olfactometer. The camera was fitted with an infrared filter, and connected to both a video monitor (Sony Trinitron) and a computer via a video frame grabber board (Pinnacle Studio PCTV; Pinnacle Systems, Mountain View, CA). The analog video signal from the camera was transformed to a digital signal and processed by video tracking and motion analysis software (Xbug; Colazza et al. 1999). To record and calculate the total seconds that a wasp spent in each olfactometer arm and the final choice made by the wasp for one of the two odors, we defined a hypothetical line in each arm, 5 mm distal to the central Y-junction of the olfactometer. A wasp was recorded as choosing a particular odor if it crossed the hypothetical line and remained in one arm for at least 40s.

Statistical analysis

We first tested the hypothesis that a wasp spent a similar amount of time in each arm in the absence of a scale-infested yucca leaf (*t*-test). We then tested several hypotheses concerning the response of wasps to an arm with

a scale-infested yucca leaf vs. the other arm with an uninfested leaf. We first tested whether the proportion of wasps choosing an arm was independent of the presence or absence of scale and their residues on a leaf, for each of five scale age classes (Ho: $P = 0.5$). We then tested whether the choice of an arm by the wasps was the same for each of the five age classes of scale (Ho: $P_1 = P_2 = P_3 = P_4 = P_5$; χ^2 goodness-of-fit test; Sokal and Rohlf, 1997). We also tested whether a wasp's residence time in the arm with odors from a scale-infested yucca leaf differed from that in the arm with odors from an uninfested yucca leaf lacking scale residue. We then tested whether a wasp's difference in residence times between the arm with an infested leaf and that with an uninfested leaf was independent of scale age (PROC GLM, SAS 2001, Little et al. 2002). We used *t*-tests in the series of bioassays testing responses of wasps to odors from scale-infested yucca leaves vs. unwashed leaves from which all scale had been removed or washed leaves from which all scale had been removed, and the wash water vs. distilled water.

RESULTS

Scale age experiment

Female *M*. sp. nr. *flavu*s spent a similar amount of time in each olfactometer arm when only humidified medical air was passed through the empty olfactometer (48.9 ± 7.0 sec left vs. 46.3 ± 10.4 sec right, $t = 0.18$, $P = 0.86$, $N = 29$), indicating no bias for one arm over the other. However, higher proportions of female wasps chose the olfactometer arm with odors from leaves infested with 26, 27, 28, or 29 d-old scale than the arm with odors from uninfested leaves lacking scale residues (Fig. 1). Odors from 30 d-old scale on leaves were not significantly more attractive to wasps than uninfested leaves (Fig. 1). Female wasps also spent significantly more time in the olfactometer arm with odors from a leaf infested with brown soft scale than in the control arm with volatiles from an uninfested leaf lacking scale residues ($F_{[1, 142]} < 0.0001$) (Fig 2.). The wasps residency times differed with scale age ($F_{[4, 142]} = 0.003$). It was longest in the arm with odors from the youngest scale (26 and 27 d-old scale), shortest in the arm with the oldest scale (30 d-old scale), and of intermediate length for 28-29 d-old scale (Fig. 2). Interaction between scale age and residence time in an arm with odors from a scale-infested leaf was not significant ($F_{[4, 142]} = 0.09$).

Scale residue experiment

Similar proportions of female *M.* sp. nr. *flavus* were attracted to odors from both scale-infested leaves and infested leaves from which the scale but not their residues had been removed (Fig. 3a). In contrast, a significantly higher proportion of female wasps were attracted to scale-infested leaves than to infested leaves from which both the scale and their residues had been removed by rinsing the leaves with water after scale removal (Fig. 3a). Subsequent bioassays revealed that the rinse water was significantly more attractive to wasps than distilled water controls (Fig. 3a), suggesting that wasps were attracted by odors from the scale residue solution. Further, female wasps spent similar amounts of time in each olfactometer arm when one arm contained volatiles from a scale-infested leaf (= positive control) and the other arm contained a leaf with only scale residue (i.e. scale removed) (Fig. 3b; $t = 0.73$, $P = 0.47$, $N = 30$). In contrast, female *M.* sp. nr. *flavus* spent significantly more time in the olfactometer arm with volatiles from a leaf infested with 26 d-old scale (= positive control) than in the arm with volatiles from a leaf that had been washed after the 26 d-old scale had been removed (Fig. 3b; $t = 3.83$, $P = 0.0005$, $N = 30$). When we used the wash water from this experiment to treat a filter paper disc, female parasitoids spent significantly more time in the olfactometer arm containing the volatiles from the treated filter paper disc than in the arm containing volatiles from a control disc treated only with distilled water (Fig. 3b; $t = 2.97$, $P = 0.005$, $N = 35$).

DISCUSSION

Parasitoids often exploit semiochemical cues to locate and recognize their hosts, using chemicals directly associated with their host (e.g. host or frass odors), or indirect cues resulting from their host's activities (e.g. plant volatiles released or induced by feeding damage) (Vinson 1976, Vet and Dicke 1992, Vet 2001). Our results show that *M.* sp. nr. *flavus* is more attracted to odors associated with brown soft scale and its water soluble residues than to odors produced by the uninfested host plant, or odors induced in the host plant by scale infestation. A higher proportion of female *M*. sp. nr. *flavu*s were attracted to yucca leaves infested with 26 to 29 d-old brown soft scale than to yucca leaves lacking scale. Similar proportions of females were attracted to leaves infested with 30 d-old scale and to uninfested leaves. Thus, the semiochemical to which *M.* sp. nr. *flavus* responds appears to be host age dependent. More dramatically, female parasitoids spent significantly more time in the Y-tube olfactometer arm with scale infested leaves than in the arm with uninfested leaves. This difference was most pronounced with the youngest scale (26 and 27 d-old scale). The wasps spent only slightly more time in the arm with odors of infested leaves than in the arm with odors of uninfested leaves when they were offered leaves with older scale (28 to 30 d-old scale) versus uninfested leaves.

That the kairomone was associated with the scale itself and not the leaf was clearly indicated by the loss of attraction when both scale and their residues were removed (washed) from a yucca leaf, and by the presence of the kairomones in the wash water. These results suggest the existence of a transitory, age dependent, semiochemical cue that attracts *M.* sp. nr. *flavus* females to relatively young brown soft scale infestations. Furthermore, these are the scale ages (= size) in which *M.* sp. nr. *flavus* offspring (eggs) are most likely to survive. Brown soft scale is known to encapsulate *M.* sp. nr. *flavu*s eggs; Blumberg (1997) and Kapranas (2002) found that egg survival is correlated with scale age: older (larger) brown soft scale are more likely to encapsulate parasitoid eggs. This suggests that the parasitoid's attraction to younger brown soft scales is adaptive, particularly as *Metaphycus* sp. nr. *flavus* is one of the more commonly occurring parasitoids emerging from brown soft scale (Guerrieri and Noyes 2000).

ACKNOWLEDGEMENTS

We thank P. Pacheco and R. Trautman for providing the scale, host plant, and parasitoid material to conduct these experiments; J. S. McElfresh for assistance in setting up the bioassay equipment; D. Jeske, Statistical Collaboratory Group, Dept Statistics, UCR for assistance in the analysis of the experimental results, and A. Kapranas for discussion of parasitoid- brown soft scale interactions. A portion of this research was supported by Citrus Research Board Grant 5500-144 awarded to RFL, Regional Research Project CA-R*ENT-5711-RR to RFL, and AES Experiment Station Projects CA-R*-ENT-7179-H to RFL and #CAR-5181-H to JGM.

REFERENCES

Bartlett, B.R. (1978) Coccidae. In: Clausen, C.P. (Ed) Introduced Parasites and Predators of Insect Pests and Weeds: A World Review. USDA Agricultural Handbook, U.S. Gov. Printing Office. Washington D. C. 480 pp. 57-74.

Bernal, J.S., Luck, R.F., Morse J.G., 1998. Sex ratios in field populations of two parasitoids (Hymenoptera: Chalcidoidea) of *Coccus hesperidum* L. (Homoptera: Coccidae). Oecologia 116, 510-518

Bernal, J.S., Luck, R.F., Morse, J.G., 1999. Augmentative release trials with *Metaphycus* spp. (Hymenoptera: Encyrtidae) against citricola scale (Homoptera: Coccidae) in California's San Joaquin Valley. J. Econ. Entomol. 92, 1099-1107.

Blumberg, D., 1997. Parasitoid encapsulation as a defense mechanism in the Coccoidea (Homoptera) and its importance in biological control. Biol. Control 8, 225-236.

Clausen, C.P., 1940. Entomophagous Insects. McGraw-Hill, New York.

Colazza, S., Peri, D., Peri, E., Lo Pinto, M., Liotta, G., 1999. A video tracking and motion analysis system for LINUX. XII Int. Entomoph. Insect Workshop, Asilomar CA, U.S.A. Sept. 26-30

Ebeling, W., 1959. Subtropical Fruit Pests. Univ. Calif. Div. Agric. Sci., Berkeley CA.

Gill, R.J., 1988. The Scale Insects of California I. The Soft Scales (Homoptera: Coccoidea: Coccidae). Calif. Dept. Food Agric., Sacramento, CA.

Gill, R.J., Kosztrab, M., 1997. Economic importance. In Ben-Dov Y and Hodgson CJ (Eds.), Soft Scale Insects-Their Biology, Natural Enemies and Control. Vol. 7B, Elsevier. Amsterdam. pp. 161-3

Guerrieri, E., Noyes, J.S., 2000. Revision of European species of genus *Metaphycus* Mercet (Hymenoptera: Chalcidoidea: Encyrtidae), parasitoids of scale insects (Homoptera: Coccoidea). System. Entomol. 25, 147-222.

Kapranas, A., 2002. Clutch size, pattern of sex allocation and encapsulation of *Metaphycus* sp. nr. *flavus* (Hymenoptera: Encyrtidae) eggs by brown soft scale, *Coccus hesperidum* L. (Homoptera: Coccidae). MS Thesis, Depart. Entomol., Univ. Calif., Riverside.

Little, R.C., Stroup, W.W., Freund, R.J., 2002. SAS for Linear Models (4th Ed.), SAS Institute Cary, North Carolina.

SAS (2001) SAS Institute Inc., Release 8.2, Cary, North Carolina USA.

Saakyan-Baranova, A.A., 1964. On the biology of the soft scale *Coccus hesperidum* L. (Homoptera, Coccoidea). Entomol. Rev. 43, 135-147.

Sokal, R.R,, Rohlf, F.J., 1997. Biometry. W H Freeman and Comp., New York,

Stauffer, S., Rose, M., 1997. Biological control of soft scale insects in interior plantscapes in the USA. In: Ben-Dov, Y., Hodgson, C.J., (Eds.) Soft Scale Insects-Their Biology, Natural Enemies and Control. Elsevier, Amsterdam, pp. 183-205.

Vet, L.E.M., 2001. Parasitoid searching efficiency links behaviour to population processes. Appl. Entomol. Zool. 36, 399-408.

Vet, L.E.M., Dicke, M., 1992. Ecology of infochemical use by natural enemies in a tritrophic context. Ann. Rev. Entomol. 42, 141-172.

Vinson, S.B., 1976. Host selection by insect parasitoids. Annu. Rev. Entomol. 21, 109-33.

Fig. 1. Responses of naïve *Metaphycus* sp. nr. *flavus* females in a Y-olfactometer to odors from *Yucca* sp. leaves infested with different ages of *Coccus hesperidum* females (26 – 30 d-old) (dark bars) vs. uninfested leaves (open bars.). All wasps tested chose one arm or the other during the 300 s assay. Data were analyzed using a χ^2 goodness-of-fit test with Yates correction for continuity involving two hypotheses: 1) parasitoids were equally likely to respond to the two arms ($P = 0.5$) and 2) their responses to each arm did not differ with scale age ($P_{26} = P_{27} = P_{28} = P_{29} = P_{30}$). Asterisks (*) denote significant differences at the 5% level in the proportion of parasitoids responding to the two arms. Experiment-wise significance: bars connect scale age treatments that do not differ at the 5% level of significance.

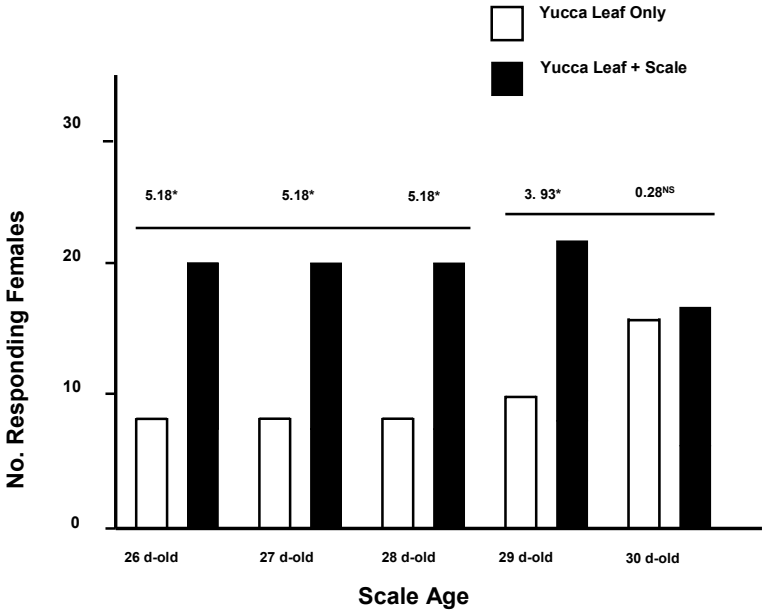

Fig. 2. Time spent in each arm of the olfactometer during a 300 s observation period for *M.* sp. nr. *flavus* females challenged with odors of *Yucca* sp. leaves infested with different ages of *C. hesperidum* females (26 – 30 d-old) (dark bars) vs. uninfested leaves (open bars). Within each age class, a *t*-test (PROC TTEST) was used to determine significance within an age class of scale. Asterisks (*,**) denote significant differences at the 5% and 1% level in the mean difference in times spent in arms with odors of scale-infested leaves vs. that in arms with odors of uninfested leaves. For experiment-wise significance, data were analyzed using multivariate statistics (SAS PROC GLM, MANOVA option) to construct the relevant matrices and PROC ANOVA OPTION MEANS /Duncan to determine differences among treatment means. The bars connect scale age treatments in which the mean difference in residency times between the two arms do not differ among treatments at the 5% level of significance.

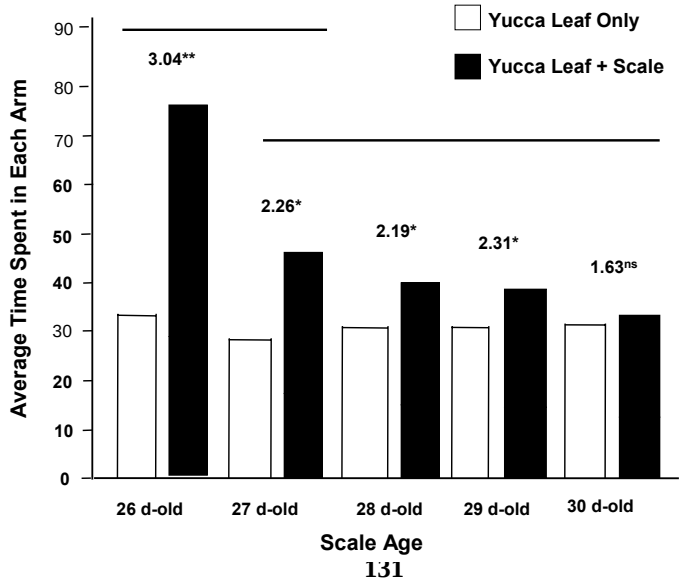

Fig. 3. The average time spent by (Fig. 3a) and the proportion of (Fig. 3b) naïve *M.* sp. nr. *flavus* females responding in a Y-olfactometer to odors in three experiments: 1) scale-infested yucca leaves vs. unwashed leaves from which all scales had been removed; 2) scale-infested yucca leaves vs. scale-infested leaves from which all scales had been removed and the leaves washed with distilled water and 3) wash-water from scale-infested leaves (≈ 3 scale equivalents) applied to filter paper vs. distilled water applied to filter paper. Differences in the proportion of wasps responding between treatments were determined with *t*-tests (Fig. 3a). Differences in average time spent by wasps between treatments were analyzed using Wilcoxon matched-pairs tests (Fig. 3b). Asterisks (*,**,***) denote significant differences at the 5%, 1%, and 0.1% level in the proportion of females parasitoids (Fig. 3a) or the average time spent by the female parasitoids (Fig. 3b) in the two arms.