Entomologia Experimentalis et Applicata **98:** 49–57, 2001.

Oviposition behavior of *Coccidoxenoides peregrinus*, a parasitoid of *Planococcus ficus*

A. L. Joyce, M. S. Hoddle*, T. S. Bellows & D. González
*Deptarment of Entomology, University of California, Riverside, CA 92521, USA; *Author for correspondence (E-mail: mark.hoddle@ucr.edu)*

Accepted: July 26, 2000

Key words: oviposition preference, behavior, acridine orange, grape, parasitoids, host size selection, Encyrtidae, Pseudococcidae

Abstract

The encyrtid parasitoid, *Coccidoxenoides peregrinus* has been used as a biological control agent against the mealybugs *Planococcus citri* and *Planococcus ficus*. This study examined the behavior and host selection of *C. peregrinus* attacking *P. ficus*. Adult parasitoids were fed a 0.1% solution of acridine orange, a DNA binding dye used to label *C. peregrinus* eggs. In a choice test, adult parasitoids were offered equal numbers of first through fourth instars of *P. ficus* and behavior of *C. peregrinus* was filmed and analyzed. Acridine orange labeled ova of the parasitoids found within mealybug hosts fluoresced green under fluorescence microscopy and presence of fluorescing eggs in hosts was used to determine oviposition events. A time budget prepared for *C. peregrinus* indicated that this parasitoid spent the majority of its time searching (71.64%) and grooming (15.06%). The average probing duration over all instars which led to oviposition from single visits was 4.93 ± 0.62 s. A total of 35.51% of probes from all attacks led to ovipositions, whereas 33.72% of single visits to hosts resulted in ovipositions. Detection of fluorescing acridine orange labeled eggs showed all instars of *P. ficus* were acceptable for oviposition by *C. peregrinus*. There was a significant preference to probe second, third, and fourth instars rather than first instars of *P. ficus*. Host feeding was not observed for this parasitoid.

Introduction

Planococcus ficus (Signoret) (Homoptera: Pseudococcidae) is an important mealybug pest infesting grapes, that has caused substantial economic losses in California, Europe, the Middle East, South America, Pakistan (CPPDR, 1994), South Africa, and the Mediterranean (Engelbrecht & Kasdorf, 1990; Rosciglione & Gugerli, 1989; Tanne et al., 1989). *Planococcus ficus* has been shown to transmit the grapevine leafroll disease and an associated closterovirus, which causes redness and rolling of the leaves, leading to delayed ripening of fruit, reductions in yield, and reduced accumulation of sugar (Engelbrecht & Kasdorf, 1990; Monis & Bestwick, 1997; Rosciglione & Gugerli, 1989). Corky-bark, a possible viral disease of grapevine also transmitted *P. ficus*, causes abnormal swelling at the basal internodes of canes (Tanne et al., 1989). Honeydew from *P. ficus* nymphs feeding in clusters of maturing fruit promotes disfiguring sooty mold growth and feeding by mealybug nymphs can cause weakening of grape vines (Berlinger, 1977). *Planococcus ficus* is difficult to control with insecticides as it hides in bark crevices and secretes thick layers of protective wax (Meyerdick et al., 1981).

The parasitoid *Coccidoxenoides peregrinus* (Timberlake) (= *Pauridia peregrina* Timberlake) (Hymenoptera: Encyrtidae), which attacks *P. ficus*, is being studied as part of a larger biological control program for this pest which attacks grapes in the Coachella Valley, California, U.S.A (Katz, 2000). *Coccidoxenoides peregrinus* has been used previously in biological control programs against *Planococcus citri* (Risso) in California, Bermuda, Chile and Italy (Bartlett, 1977; Bennett, 1959; Zinna, 1960). This parasitoid is a solitary, thelytokous, proovigenic endopar-

asitoid which completes its life cycle in approximately four weeks (Bartlett, 1977). Limited information on the reproductive biology of *C. peregrinus* is available (Gol'berg, 1982; Krishnamoorthy & Mani, 1989; Zinna, 1960), however, studies to quantify the host selection and oviposition behavior of *C. peregrinus* have not been conducted.

Our objective was to quantify the oviposition behavior of *C. peregrinus* and to determine if *C. peregrinus* exhibits host stage preferences when selecting *P. ficus* nymphs for oviposition. In this study, we fed *C. peregrinus* a solution of acridine orange in honey water and used fluorescence microscopy to locate labeled eggs in dissected mealybugs to determine oviposition preferences of *C. peregrinus* for different developmental stages of *P. ficus*. Knowledge of oviposition behavior and preferences may improve the culturing of this parasitoid for augmentative releases against *P. ficus* in California grape growing areas and assist with insectary rearing efforts.

Materials and methods

Colonies. *Planococcus ficus* colonies were cultured on Red Lasoda potato (*Solanum tuberosum* L.) stems (AgriEmpire, Hemet, California, USA) at the Insectary Facility at the University of California, Riverside at 27 ± 1 °C, 50% r.h. (L14:D10) in plastic trays measuring 30 cm in length ×20 cm in width containing UC Soil Mix Type 4 (Matkin & Chandler, 1957). *Planococcus ficus* were originally collected from infested table grapes grown in the Coachella Valley, California, USA. Mealybug identification was confirmed by Dr Ray Gill at the California Department of Food and Agriculture (CDFA) and mealybug voucher specimens have been deposited at CDFA in Sacramento, California.

The colony of *C. peregrinus* was reared on *P. ficus* infesting Red Lasoda potato. The parasitoids used in this study were originally collected from mealybugs infesting citrus at Kibbutz Givat near Afula, Israel. *Coccidoxenoides peregrinus* were reared by offering adult parasitoids potato stems infested with all instars of *P. ficus* nymphs. A parasitoid emergence cage was checked every 24 h for newly emerged adult females which were removed and used for behavioral experiments. *Coccidoxenoides peregrinus* voucher specimens have been deposited in the Entomology Museum at the University of California, Riverside, U.S.A.

Acridine orange stain preparation and ingestion by C. peregrinus

The eggs of *C. peregrinus* are minute and difficult to locate inside their hosts. Parasitoid eggs have previously been labeled with acridine orange, which binds to DNA, to aid identification of parasitoid ova within hosts (Strand et al., 1990). A solution of 0.1% acridine orange (Sigma®, St. Louis, MO, USA) was prepared by mixing 5 ml of honey and 5 ml of deionized water together to obtain a honey/water solution. From this stock solution, a 100-microliter aliquot was taken and mixed with 0.1 mg of acridine orange to produce a 0.1% solution.

Adult female parasitoids used in this behavioral study were newly emerged (0–24 h) and individually isolated in 2 ml glass vials with a cotton lint stopper. A streak of honey/water containing 0.1% acridine orange was placed inside the vial. Parasitoids in vials were then placed in the dark to protect the dye from photodegradation and *C. peregrinus* was left to feed on the honey and dye mixture for 24 h. Acridine orange binds to DNA, and labeled DNA fluoresces green under an Fitc fluorescent filter (497 nm) (Kasten, 1973). The Fitc fluorescence filter we used was attached to a Nikon compound microscope with fluorescence attachment blue filter (Nikon B-2A, excitation at 475-495 nm, emission at 520 nm).

Autofluorescence tests and acridine orange toxicity tests. To test for autofluorescence, ten each of first through fourth instar nymphs of *P. ficus* were dissected and examined using the fluorescence microscope described above. Autofluorescence determination was necessary as it would confound the results of green fluorescence emitted from dye-labeled parasitoid eggs that had been oviposited in the mealybug nymphs by *C. peregrinus*. The abdomens of ten female parasitoids were individually slide mounted in physiological saline, and crushed to determine if green autofluorescence was present.

A toxicity test was conducted at three concentrations, 0.01%, 0.1%, and 1.0%, to determine if acridine orange solution was toxic to *C. peregrinus*. At each concentration of dye, eight newly emerged adult female *C. peregrinus* were isolated individually for 24 h in 2 ml glass vials with a streak of honey that contained the dye. Survivorship of parasitoids fed dye was compared with newly emerged adult female parasitoids that were fed honey/water alone for 24 h. χ^2 analyses were used to compare survivorship of

C. peregrinus fed different concentrations of acridine orange to parasitoids fed honey water only.

Filming adult parasitoids searching in artificial arenas. Experimental arenas consisted of plastic petri dishes measuring 12 cm in diameter, and contained one grape leaf each (*Vitis vinifera* L.) ('Flame variety'). Grape leaves were placed abaxial side up in the dish and leaf edges were sealed to the dish with Scotch Tape® to prevent *P. ficus* from settling under leaves.

Twelve hours prior to filming oviposition behavior, mealybug nymphs were placed into arenas with grape leaves that each had five plastic cover slips over leaf veins to encourage mealybugs to settle. At the time of filming, an arena consisted of ten mealybugs in each stage (total of 40 *P. ficus* in an arena) for ovipositional preference experiments. Settled nymphs on leaves were circled with a Sharpie® nontoxic black pen and numbered (1–40) for subsequent identification on video recordings and identification numbers were correlated with mealybug instar.

A single adult female parasitoid that had been fed 0.1% acridine solution in the previous 24 h was placed in the petri dish arena on which a clear plastic petri dish lid was then secured. The behavior of searching females was recorded. A video camera (Javelin JE33620) was mounted in place of one ocular on a Leica MZ12 microscope. During recording, the behavior of each female was observed on a video monitor, and times of events were recorded directly onto the video tape. The video recording started when the first probe by *C. peregrinus* on *P. ficus* was observed. Each female that exhibited searching behavior was filmed for 30 min. At the end of the 30-min session, the abdomen of the female parasitoid was removed, slide mounted in physiological saline, and crushed to observe the ova and confirm that eggs fluoresced green from acridine orange. Following the confirmation of labeled parasitoid ova, all probed mealybug nymphs were removed from the leaf with forceps and were individually slide mounted in physiological saline. Slide-mounted mealybug nymphs were dissected and examined within 1 h using the Fitc filter and fluorescent microscope. Presence or absence of fluorescent *C. peregrinus* eggs was noted for each nymph. Ten female *C. peregrinus* were filmed in this manner for this study.

Data analysis. The video tape recording of each female parasitoid was reviewed and the start time of each behavior was recorded. Oviposition events identified from dye-labeled eggs found within slide mounted mealybug nymphs were matched with data obtained during filming of parasitoid searching behavior. Video-taped events were used to calculate the frequency and duration of searching, antennating, probing, grooming, mealybug encounters, resting, and oviposition by *C. peregrinus*. An ethogram was constructed showing the behavioral frequencies of all events observed on the recordings. Mean duration of types of behavior were calculated and a time budget constructed. The time budget consisted of a tally of the proportion of total filming time spent in each individual behavior.

Analyses of variance were run using SAS (1988) to determine if the mean duration of antennation prior to probing was significantly different than antennation time that led to mealybug rejection and continued searching by *C. peregrinus*. These analyses were used to determine if parasitoids spend disparate quantities of time evaluating the surface of hosts for size or chemical cues prior to probing or host rejection. Chi-squared analyzes were performed on the total number of antennations that resulted in probing and compared to the frequency which resumed in searching to determine if there was a probing preference based on mealybug instar. A similar analysis was conducted on the frequency of probes which led to ovipositon and compared with the frequency of probes which resulted in rejection of hosts. Only hosts that were visited once, which we termed 'single visits' were used for the oviposition preference analysis. Mean probing duration for probes that led to oviposition or did not lead to oviposition were calculated. The average number of probes per female leading to an oviposition event was also calculated.

Results

Autofluorescence and acridine orange toxicity test. No green autofluorescence was observed in unlabeled *C. peregrinus* eggs, or in dissected *P. ficus* nymphs. The toxicity test for adult parasitoids showed that the percentage survival over a 24-h period in the presence of honey containing dye at 0.01–1.0% did not differ from the percentage survival in the presence of honey alone ($\chi^2 = 1.93$, df $= 2$, P $= 0.17$).

Dissection of *C. peregrinus* abdomens revealed $>$ 100 fluorescent ova. Fluorescence of parasitoid ova at 0.01% was faint and difficult to detect. Parasitoid eggs fluoresced strongly when females were fed acri-

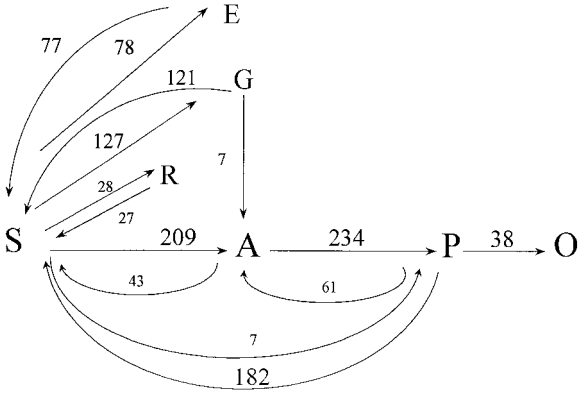

Figure 1. The frequency of all behavioral events for *Coccidoxenoides peregrinus* with mealybug host *Planococcus ficus* for events with frequency of > 6. Behavioral types included on the ethogram include encounters (E), grooming (G), resting (R), searching (S), antennations (A), probing (P), and ovipositions (O).

dine orange solutions of 0.1% and 1.0%, but parasitoid searching appeared to be affected following ingestion of a 1.0% solution. Searching behavior appeared 'normal' for parasitoids fed 0.1% dye and this concentration was subsequently used for the remainder of the studies.

Behavior. A total of 1,251 behavioral events for *C. peregrinus* were recorded from the ten females. Filmed behavioral events included searching, antennating, probing, grooming, mealybug encounters, resting, and oviposition (Figure 1). Host feeding was not observed. 'Host defense' (violent twitching by mealybugs following parasitoid probing) described by Cadée & van Alphen (1997) was observed with *P. ficus* following encounters with *C. peregrinus* but was not quantified. Ovipositing *C. peregrinus* females did not appear to 'pump' the abdomen when ovipositing in *P. ficus* as was described for *Leptomastidea abnormalis* (Girault) when ovipositing in *P. citri* (Cadée & van Alphen, 1997).

Ethogram construction. Females began their behavioral pathway by searching (S), which was characterized by walking across the leaf and drumming their antennae on the leaf surface (Figure 1). Searching was interrupted by several events including encountering hosts (E), grooming (G), resting (R), or antennation (A) of mealybug hosts (Figure 1). Of the 458 searching events, 78 terminated in a momentary encounter of a host (E) (17.03%), where the parasitoid contacted the host momentarily and appeared to randomly collide with the nymph, but did not further antennate the host or attempt to utilize or evaluate the host and continued searching. Searching was followed by grooming (G) 127 events (27.73%), characterized by parasitoids using their foretarsi to remove wax and debris from their bodies. Searching for hosts led to antennation (A) 209 times (45.63%), where parasitoids tapped their antennae across the dorsal or lateral surfaces of mealybug nymphs (Figure 1). A behavioral loop was recognized where searching could lead to resting (R), but the majority of these 28 resting events (96.43%) resumed in searching.

Adult parasitoids antennated 289 hosts, of which *P. ficus* nymphs were probed 234 times (80.97%), while 43 probes resulted in resumed searching (14.88%) (Figure 1). Of the 289 hosts antennated, 10 nymphs were not correlated with an instar, and the remaining 279 were used for analysis. Five antennations were followed by an antennation of a different mealybug individual, one antennation was followed by grooming, and a single antennation led to an encounter. One antennation that led to probing of a fourth instar, and one that led to searching in the fourth instar were censored as the duration of the events were outliers. This left a total of 270 antennations, 232 which resulted in probes, and 38 antennations followed by searching which we used for analysis of probing preference by instar and analysis of the duration of antennation. Figure 1 illustrates the behavioral pathway for the parasitoid, but excludes events with a frequency of < 6 in order to show the primary behavior sequence. Mealybug hosts that were visited more than once during filming were termed 'repeat visits', while those visited once were called 'single visits'. *Coccidoxenoides peregrinus* had a total of 38 (35.51%) probes of the 107 repeat and single visits which led to ovipositions (Figure 1). Single visits alone had 29 of 86 probes (33.72%) result in oviposition.

Time budget construction. Searching and grooming by *C. peregrinus* occupied the majority of the time budget (71.64% and 15.06%), while probing, resting, antennating and encounters, respectively, all occupied less than 6.0% each of the total time in events (5.59%, 4.09%, 3.01%, and 0.61%, respectively) (Figure 2). The more time consuming, uninterrupted events of searching, resting and grooming events averaged 28.16 ± 1.52 s, 21.03 ± 3.89 s, and 19.64 ± 2.53 s, respectively (Table 1). Probing averaged 4.07 ± 0.15 s per event, while antennating and encounters each averaged 1.87 ± 0.11 s and 1.31 ± 0.16 s, respectively (Table 1).

Table 1. The frequency and duration of all behavioral events for *Coccidoxenoides peregrinus* ($n = 10$ females) with its mealybug host, *Planococcus ficus* ($n = 40$ nymphs), on grape leaves

Behavior	Total frequency	Proportion of total events	Total time in behavior (s)	Mean duration ± S. E. (s)	Percentage of total time (%)
Search	458	36.61	12895	28.16 ± 1.52	71.64
Antennate	289	23.1	541	1.87 ± 0.11	3.01
Probe	247	19.74	1006	4.07 ± 0.15	5.59
Groom	138	11.03	2711	19.64 ± 2.53	15.06
Encounter	84	6.71	110	1.31 ± 0.16	0.61
Rest	35	2.8	736	21.03 ± 3.89	4.09
Totals	1251	100	17999		

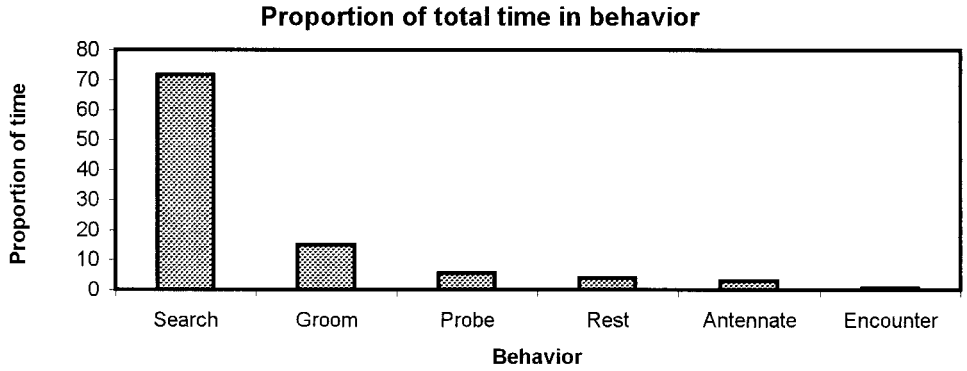

Figure 2. The proportion of time spent in each quantified behavior for *Coccidoxenoides peregrinus* (n = 10 females) attacking *Planococcus ficus* on grape leaves.

Mean duration of antennation by *C. peregrinus* on each *P. ficus* instar encountered prior to probing and searching is shown in Table 2. A two-way analysis of variance over all instars comparing the average duration of antennation prior to probing (1.64 ± 0.09 s) versus the average duration of antennation prior to searching (2.24 ± 0.26 s) found that the duration of antennation prior to probing was significantly shorter than antennation duration prior to abandoning a potential host ($F = 8.49$, $df = 1$, $P = 0.004$) (Table 2). The differences in antennation times between instars were not significant ($F = 1.34$, $df = 3$, $P = 0.262$) (Table 2) and there were no interactions.

Chi squared analysis of the percentage of antennations leading to probing compared with the percentage of antennations that led to resumed searching for all instars combined showed that *C. peregrinus* was equally likely to probe all *P. ficus* instars ($\chi^2 = 6.267$, $df = 3$, $P = 0.099$). However, a chi sqared analysis that grouped second, third, and fourth instars and compared them with first instars found that first instars were probed significantly less than larger instars ($\chi^2 = 5.189$, $df = 1$, $P = 0.023$) (Table 3). Of the 270 antennations, 232 were followed by probing (85.93%), indicating the majority of antennations led to probes with the ovipositor. A chi squared analysis of the observed frequency of all antennations (regardless of subsequent behavioral event) was determined to investigate if one instar was encountered more frequently than by chance. The analysis found there was a significant preference to antennate a particular instar ($\chi^2 = 15.52$, $df = 3$, $P = 0.001$). Further analysis by chi square removing first instars and only comparing second, third, and fourth instars found no difference in antennation frequencies for larger instars ($\chi^2 = 3.41$, $df = 2$, $P = 0.182$). Thus, first instars were antennated more frequently than expected.

Oviposition preference. Parasitoid eggs labeled with acridine orange dye were observed in all instars of *P. ficus*. Although *C. peregrinus* parasitoids repeatedly visited individual hosts, and probed hosts more

Table 2. Analysis of the durations of antennation time when followed by two subsequent types of behavior by instar of *Coccidoxenoides peregrinus* with mealybug host *Planococcus ficus* on grape leaves. A two-way analysis of variance found antennation time prior to probing to be significantly shorter than prior to abandoning the host and continued searching ($F = 8.49$, $df = 1$, $P = 0.004$). Antennation duration by instar was not significant ($F = 1.34$, $df = 3$, $P = 0.262$) and the interaction was not significant

Behavior	Subsequent behavior	*n*	Mealybug instar	Mean duration (s)	Significant at $P < 0.05$
Antennation	Probe	232	1–4 (all)	1.64 ± 0.09	Yes
		33	1	1.21 ± 0.11	No
		74	2	1.49 ± 0.12	No
		68	3	1.85 ± 0.19	No
		57	4	1.82 ± 0.19	No
Antennation	Search	38	1–4 (all)	2.24 ± 0.26	Yes
		11	1	2.36 ± 0.62	No
		13	2	2.00 ± 0.34	No
		8	3	1.88 ± 0.4	No
		6	4	3.00 ± 0.86	No

Table 3. The frequency and percentage of all antennations resulting in probes, and the frequencies and percentages of probes from single visits resulting in oviposition by instar for *Coccidoxenoides peregrinus* (n = 10 females) and mealybug host *Planococcus ficus* on grape. Parasitoids preferred to probe second, third, and fourth instars significantly more than first instars ($\chi^2 = 5.189$, $df = 1$, $P = 0.023$). There was no significant preference by instar for oviposition ($\chi^2 = 1.23$, $df = 3$, $P = 0.75$)

Mealybug instar	Frequency of antennations to probes	Percentage of antennations to probes	Frequency of probes to ovipositions	Percentage of probes to ovipositions
1st	33	73.33	3	23.08
2nd	74	82.22	9	31.03
3rd	68	88.31	7	38.89
4th	57	87.69	10	38.46

than once in a single visit, only one egg per host was observed. Eggs were located in various regions of the host body, and there did not appear to be a pattern to oviposit in a particular location of the host. This however may have been an artifact resulting from host dissections which could have displaced parasitoid eggs.

Analyses of oviposition preference and probing duration only used data from single visits. If a mealybug nymph had more than one visit from a parasitoid (repeat visit), it could not be determined which visit resulted in oviposition. The proportion of probes which led to an oviposition for single visits showed no oviposition preference by instar ($\chi^2 = 1.23$, $df = 3$, $P = 0.75$) (Table 3). Pooling first and second instars from single visits as 'young' hosts compared with 'old' (third and fourth instars) hosts showed no significant oviposition preference for young versus older instars ($\chi^2 = 0.97$, $df = 1$, $P = 0.32$).

The mean probing time for probes in a single visit which led to oviposition over all instars was 4.93 ± 0.62 s ($n = 28$, range = 1–14). One probing duration for a third instar of 39 s was censored from the data set. Mean probing time that did not lead to oviposition was 5.86 ± 0.54 s ($n = 57$, range = 1–17). Two-way analysis of variance of duration across instars detected that mean probing times that resulted in oviposition for first through fourth instars were not significantly different from the mean probing time that resumed in searching ($F = 0.70$, $df = 1$, $P = 0.407$), respectively

Table 4. The mean probing time by *Coccidoxenoides peregrinus* for single visits that resulted in oviposition or rejection of mealybug nymphs of *Planococcus ficus*. A two-way analysis of variance found probing duration leading to oviposition not significantly different than probing duration leading to rejection ($F = 0.70$, $df = 1$, $P = 0.407$). There were no significant effects by instar ($F = 1.57$, $df = 3$, $P = 0.205$) and no significant interactions

Instar	Mean duration for oviposition (s) ± S.E.	*n*	Mean duration for rejection (s) ± S.E.	*n*
1st	5.00 ± 1.53	3	4.90 ± 1.48	10
2nd	3.67 ± 0.58	9	5.50 ± 0.65	20
3rd	5.00 ± 1.29	6	4.91 ± 1.12	11
4th	6.00 ± 1.40	10	7.56 ± 1.23	16

(Table 4). There was no effect by instar ($F = 1.57$, $df = 3$, $P = 0.205$) nor any interactions (Table 4).

The mean number of probes by an individual female's (that resulted in an oviposition for single visits) across all instars was 1.52±0.288 ($n = 29$, range 1–9). The mean number of probes resulting in oviposition for first through fourth instars were 1.7±0.7, 1.0±0.0, 2.3 ± 1.1, and 1.4 ± 0.2, respectively.

Discussion

The toxicity tests showed no difference in mortality for parasitoids that were fed a honey/water solution that contained between 0.01–1.0% acridine orange versus parasitoids fed honey/water only. Acridine orange successfully labeled the eggs of *C. peregrinus*, and facilitated their location within the host. The potential for use of this compound to find parasitoid eggs is discussed by Strand et al. (1990), who also found no difference in the mortality of parasitoids in the families Encyrtidae and Braconidae that were fed a 0.001–1.0% solution of acridine orange compared to controls fed only honey or water.

Searching and grooming occupied the majority of the time budget prepared for *C. peregrinus* at 71.64% and 15.06% of time, respectively. For *Gyranusoidea tebygi* Noyes (Hymenoptera: Encyrtidae) attacking *Rastrococcus invadens* Williams (Homoptera: Pseudococcidae), parasitoid searching and grooming occupied 20.38% and 36.76% of time, respectively (Boavida et al., 1995). Grooming ranks in the top two time expenditures for both encyrtid parasitoids that attack mealybugs. The time spent grooming indicates the importance of removing mealybug waxes and other debris from the body and possibly from olfactory receptors, in order to receive chemical information about hosts and the environment.

The mean time antennating by *C. peregrinus* prior to probing with the ovipositor was significantly shorter than the mean time antennating prior to rejecting hosts. Examination of the surface of mealybug nymphs by *C. peregrinus* may be to search for chemical cues that identify host suitability for oviposition. Our results suggest that detection of cues leading to oviposition are rapid and the longer antennation time that led to rejection of hosts may indicate that cues were not sufficient in large enough quantities to be detected, thus leading to host rejection by *C. peregrinus*.

Coccidoxenoides peregrinus antennated and probed all instars of *P. ficus*, and exhibited a probing preference for second, third, and fourth instar mealybug nymphs over the smaller first instars ($P = 0.023$). The nonpreference for first instars of *P. ficus* may relate to the mobility of the nymphs, which appears to make oviposition in them more difficult than in larger nymphs. All instar mealybugs were present in equal numbers on the leaf in the arena, and *C. peregrinus* encountered all instars on numerous occasions during filming. First instars were antennated significantly more frequently than larger instars, and when they were antennated they were probed significantly less frequently than larger instars. Filming several more females may have revealed a more specific host size preference between 2nd, 3rd, and 4th instar nymphs over 1st instar mealybugs. Host stage preferences by *C. peregrinus* towards other species of mealybugs (i.e., *P. citri*) that are used for reproduction have not been determined. This warrants further research to determine if results observed with *P. ficus* are consistent with other mealybug hosts.

We found that *C. peregrinus* oviposited in all nymphal instars of *P. ficus* as confirmed by the presence of acridine labeled eggs in all immature stages. Zinna (1960) observed *C. peregrinus* to oviposit in first, second, and third instars, but not fourth instars of *P. ficus*. Krishnamoorthy & Mani (1989) found that all instars of *P. ficus* were attacked by *C. peregrinus*, but they did not confirm that oviposition had taken place. There may be a chemical cue rather than a structural or physical one such as size that motivates the *C. peregrinus* to oviposit in *P. ficus*. While there was no oviposition preference by *C. peregrinus* by instar, there may be higher survival of immature para-

sitoids in selected mealybug hosts because of variation in host defenses such as encapsulation which lessen parasitoid survival rates. Lloyd (1958) found that the encyrtid *Leptomastix dactylopii* oviposited in mealybug hosts that it could not develop in and suggested that parasitoids may oviposit rather than resorb ova, to maintain a readily available supply of mature eggs for when suitable hosts are found. Egg load can affect host selection and may lead to selection of less than optimal hosts for oviposition (Minkenberg et al., 1992). Future studies investigating egg load and its influence on oviposition and host selection by *C. peregrinus* are warranted.

The oviposition preference of other encyrtid parasitoids of *Planococcus* mealybugs have been investigated. *Leptomastidea abnormis*, prefers to oviposit in second instar *P. citri*, although it would attack third and fourth instars with less frequency (Cadée et al., 1997). *Leptomastix dactylopii* prefers to oviposit in third and fourth instars of *P. citri*, and did not attack second instars (Cadée et al., 1997). *Anagyrus mangicola* Noyes, an encyrtid parasitoid of *Rastrococcus invadens* Williams (Homoptera: Pseudococcidae) oviposits more frequently in second and third instars of this host than first and fourth instars (Bokonon-Ganta et al., 1995). In contrast, *C. peregrinus* did not exhibit oviposition preferences for *P. ficus* of different stages.

Although hosts of *P. ficus* were often probed more than once by *C. peregrinus*, only one fluorescent ovum was visible in mealybug hosts that were successfully identified as being parasitized, indicating that this parasitoid may discriminate against previously parasitized hosts. *Anagyrus mangicola* also oviposits just one ovum in mealybug hosts that receive repeat visits (Bokonon-Ganta et al., 1995). Furthermore, work on the oviposition preferences of the encyrtid *Gyranusoidea tebygi* parasitizing *R. invadens* in a choice test found that 2.7% of mealybug nymphs that were attacked had two parasitoid ova in each (Boavida et al., 1995) suggesting that discrimination between parasitized and unparasitized hosts was not perfect.

The mean probing time 4.93 ± 0.62 s for *C. peregrinus* that leads to oviposition was longer than the findings of Zinna (1960), who estimated that *C. peregrinus* attacking *P. citri* had probes of approximately 2 s that were associated with oviposition. In comparison, *Anagyrus mangicola* attacking *R. invadens* was reported to take 5.4 s to oviposit, while *L. abnormis* attacking *P. citri*, took 142–341 s (Bokonon-Ganta et al., 1995, Cadée et al., 1997). In comparison to *L. abnormis*, egg laying by *C. peregrinus* is rapid, but is of similar time to *A. mangicola*, and slower than conspecifics attacking *P. citri*.

Acknowledgements

We thank Dr Ray Gill of the California Department of Food and Agriculture (CDFA) for mealybug identification, and Dr Serguei Triapitsyn, University of California Riverside Department of Entomology, for parasitoid identification. Colonies of mealybugs and parasitoids were maintained at the University of California, Riverside, by Marcella Waggoner, Kathryn McGiffen, and Ernesto David DeMoreno. Assistance with the fluorescent microscopy was provided by John Hermesmann, Dept. of Environmental Toxicology at University of California, Riverside. Dr Dave Eastman, Dept. of Environmental Toxicology at UCR, is thanked for use of the fluorescence microscope and laboratory.

References

Bartlett, B. R., 1977. Citrus mealybug. In: C. Clausen (ed.), Introduced Parasites and Predators of Arthropod Pests and Weeds: a World Review. US Department of Agriculture Handbook 480, pp. 150–155.

Bennett, F. D., 1959. Biological control of insect pests in Bermuda. Bulletin of Entomological Research 50: 423–436.

Berlinger, M. J., 1977. The Mediterranean vine mealybug and its natural enemies in Southern Israel. Phytoparasitica 5: 3–14.

Boavida, C., M. Ahounou, M. Vos, P. Neuenschwander & J. J. M. van Alphen, 1995. Host stage selection and sex allocation by *Gyranusoidea tebygi* (Hymenoptera: Encyrtidae), a parasitoid of the mango mealybug, *Rastrococcus invadens* (Homoptera: Pseudococcidae). Biological Control 5: 487–496.

Bokonon-Ganta, A. H., P. Neuenschwander, J. J. M. van Alphen & M. Vos, 1995. Host stage selection and sex allocation by *Anagyrus mangicola* (Hymenoptera: Encyrtidae), a parasitoid of the mango mealybug, *Rastrococcus invadens* (Homoptera: Pseudococcidae). Biological Control 5: 479–486.

Cadée, N. & J. J. M. van Alphen, 1997. Host selection and sex allocation in *Leptomastidea abnormis*, a parasitoid of the citrus mealybug *Planococcus citri*. Entomologia Experimentalis et Applicata 83: 277–284.

Calif. Plant Pest and Disease Report, 1994. Vine mealybug. California Department of Food and Agriculture 13: 8.

Engelbrecht, D. J. & G. G. F. Kasdorf, 1990. Transmission of grapevine leafroll disease and associated closteroviruses by the vine mealybug, *Planococcus ficus*. Phytophylactica 22: 341–346.

Gol'berg, A. M., 1982. Influence of temperature and relative humidity on survival and fecundity of *Pauridia peregrina*, a parasite of mealybugs and its interactions with *Planococcus citri*. Entomologia Experimentalis et Applicata 32: 86–90.

Kasten, F. H., 1973. Acridine Dyes. In: Gray, P. (ed.), The Encyclopedia of Microscopy and Microtechnique. Van Nostrand Reinhold Co., New York, pp. 4–6.

Katz, M., 2000. Vine mealybug presents new challenge to San Joaquin Valley grape growers. Agribusiness Fieldman January–February: 1–4.

Krishnamoorthy, A. & M. Mani, 1989. *Coccidoxenoides peregrina*: a new parasitoid of *Planococcus citri* in India. Current Science 58: 466.

Lloyd, D. C., 1958. Studies of the parasite oviposition behavior II. *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae). Canadian Entomologist 90: 450–461.

Matkin, O. H. & P. A. Chandler, 1957. The U.C. soil mixes. In: K. F. Baker (ed.), The U.C. System for Producing Healthy Container-Grown Plants Through the Use of Clean Soil, Clean Stock, and Sanitation. California Agricultural Experiment Station Manual 23. University of California, Berkeley, pp. 68–85.

Meyerdick, D. E., J. V. French, L. D. Chandler & W. G. Hart, 1981. Effect of commercially applied pesticides for control of the citrus mealybug. Southwestern Entomologist 6: 49–52.

Minkenberg, O. P. J. M., M. Tatar & J. A. Rosenheim, 1992. Egg load as a major source of variability in insect foraging and oviposition behavior. Oikos 65: 134–142.

Monis, J. & R. K. Bestwick, 1997. Serological detection of grapevine associated closteroviruses in infected grapevine cultivars. Plant Disease 81: 802–808.

Rosciglione, B. & P. Gugerli, 1989. Transmission of grapevine leafroll disease and an associated closterovirus to healthy grapevine by the mealybug *Planococcus ficus*. Phytoparasitica 17: 63.

SAS Institute, 1988. SAS/STAT User's Guide, Version 6, 4th ed. SAS Institute, Cary, N.C.

Strand, M. R., B. D. Roitberg & D. R. Papaj, 1990. Acridine orange: a potentially useful marker of Hymenoptera and Diptera. Journal of the Kansas Entomological Society 63: 634–637.

Tanne, E., Y. Ben-Dov & B. Raccah, 1989. Transmission of the corky-bark disease by the mealybug *Planococcus ficus*. Phytoparasitica 17: 55.

Zinna, G., 1960. Esperimenti di lotta biologica contro il cotonello degli agrumi (*Pseudococcus citri* (Risso)) nell' isola di procida mediante l'impiego di due parassiti esotici, *Pauridia peregrina* Timb. e *Leptomastix dactylopii* How. Bollettino del Laboratorio di Entomologia Agraria 'Filippo Silvestri' 18: 257–284.