Priming *Aphytis*: behavioral modification of host selection by exposure to a synthetic contact kairomone

J. D. Hare

*Department of Entomology, University of California, Riverside, CA, 92521, USA*

Accepted: August 4, 1995

**Key words:** *Aphytis melinus*, kairomone, O-caffeoyltyrosine, host recognition, learning

**Abstract**

The introduced parasitoid, *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), utilizes a kairomone, O-caffeoyltyrosine, to recognize California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae). Wasps used in augmentative release programs for California red scale on California citrus are reared on oleander scale, *Aspidiotus nerii* Bouché (Homoptera: Diaspididae), themselves reared on squash. The goals of this study were: 1) to determine if long-term rearing on oleander scale had caused *A. melinus* to develop a preference for oleander scale, and 2) to determine if the preference of oleander-reared *A. melinus* for California red scale might be enhanced by exposing them to synthetic O-caffeoyltyrosine prior to release. Wasps that were removed from their hosts as pupae and allowed to emerge as adults isolated from their hosts retained a strong preference for California red scale regardless of rearing host. This preference was reduced if wasps were allowed to emerge from oleander scale, thus acquiring early adult experience with oleander scale. The preference for California red scale was restored, however, by exposing wasps reared on oleander scale to synthetic O-caffeoyltyrosine prior to bioassay. Exposure of *A. melinus* reared in commercial insectaries to O-caffeoyltyrosine prior to release may be a means to improve the effectiveness of such wasps in augmentative release programs to control California red scale.

**Introduction**

One of the more interesting recent areas of research on host-parasitoid systems involves the ability of parasitoids to modify their foraging behavior on the basis of prior experience (recently reviewed by Vet & Dicke, 1992; Turlings *et al.*, 1993; Vet *et al.*, 1995). Current models of foraging behavior of parasitoids state that parasitoids, especially those that utilize multiple hosts, themselves distributed over multiple plant species, will improve their abilities to recognize and exploit certain host-host plant associations through associative learning (Vet & Dicke, 1992). Associative learning has several implications for biological control by parasitoids (Lewis & Martin, 1990; Prokopy & Lewis, 1993). Among these are that parasitoids imprinted to the volatile cues from the particular combination of host plant and host that the parasitoid is expected to suppress should cause the parasitoid to locate its host more efficiently than parasitoids imprinted on a different host-host plant complex (Vet & Papaj, 1992; Prokopy & Lewis, 1993).

Another aspect of early adult experience on parasitoid foraging behavior is that innate responses of parasitoids to hosts may be enhanced by exposing or 'priming' wasps to specific host products prior to release. Priming is the process by which insects come in contact with an innately recognized stimulus and become more receptive to other cues, not necessarily present during the experience, to which they already show some response (Turlings *et al.*, 1993). Priming as well as associative learning may be very important parts of improving the host-seeking behavior of insectary-reared parasitoids when such parasitoids are reared on different hosts than they are expected to control in the field.

Here, I seek to determine the significance of the host from which a parasitic wasp emerged on subsequent host preference, and whether such preference could be modified by subsequent exposure to a synthet-
ic contact kairomone. For the experimental system, I chose *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae), a parasitoid introduced to control California red scale, *Aonidiella aurantii* (Maskell) (Homoptera: Diaspidiidae), an important pest of citrus in arid and semi-arid regions throughout the world (Bodenheimer, 1951; Ebeling, 1959) and the most important citrus pest in California (Luck, 1981). *Aphytis melinus* was first released in California in 1957 and quickly became established in southern California (DeBach, 1974). It has provided economic biological control of California red scale in southern California ever since. In contrast, *A. melinus* can provide economically acceptable suppression of California red scale in the San Joaquin Valley only through an annual series of approximately 15 augmentative releases, spread over seven months, of approximately 2000 wasps per hectare per release (Haney et al., 1992.) These wasps are reared on a uniparental strain of oleander scale, *Aspidiotus nerii* Bouché (Homoptera: Diaspididae), because oleander scale is larger than California red scale and provides a higher proportion of female progeny. The oleander scales are also reared on squash (*Cucurbita maxima* Duch.) because squash are cheaper and more readily available than lemons (DeBach & White, 1960).

As part of a larger study to determine the factors that may contribute to the variation in the physiological suitability and behavioral acceptability of California red scales reared on different citrus cultivars and substrates within cultivars (Hare et al., 1990; Hare & Luck, 1991), we determined that oviposition behavior in *A. melinus* females is elicited by *O*-caffeoyltyrosine (hereafter OCT), a compound found in highest concentration in covers of third-instar California red scale (Millar & Hare, 1993; Hare & Luck, 1994), the scale life stage most attractive and susceptible to *A. melinus* (Luck & Podoler, 1985; Opp & Luck, 1986; Yu et al., 1990). Moreover, wasps respond in a dosage-dependent manner to increasing OCT concentrations when applied to filter paper disks up to 30 ng per disk, a concentration similar to the maximum concentration found in California red scale covers (Hare et al., 1993). We have been unable to detect OCT in oleander scale.

The system involving *A. melinus*, California red scale, and OCT provides several important advantages over other host-parasitoid systems for studies on host selection. First, OCT is easily synthesized from relatively inexpensive precursors (Millar & Hare, 1993), thus OCT is available in large quantities for experimentation. Second, *A. melinus* is an ectoparasitoid that feeds on the scale body but beneath the scale cover. Larvae of *A. melinus* consume the entire scale body then pupate beneath their host's cover. To obtain adult wasps that will have no adult experience with their hosts, all that is necessary is to lift the scale covers and collect the pupae with a fine brush or probe. No dissection of the host is necessary. Moreover, this system has important practical considerations because *A. melinus* is routinely reared commercially, albeit on a different host species than it is expected to control in the field, and a substantial augmentative release program is already in place for this biological control agent. Here, I determine: 1) if long-term rearing of *A. melinus* on oleander scale in insectaries has resulted in inadvertent selection for wasps that preferentially recognize oleander scale over California red scale, 2) if the scale host species from which *A. melinus* females emerge affects host preference, and, if so, 3) if wasps that emerge from oleander scale will more readily accept California red scale if they were exposed to OCT prior to bioassay.

**Materials and methods**

*Aphytis melinus* was reared on California red scale and oleander scale (26 °C., 40–60% r.h. L16:D8 photoperiod) as previously described (Opp & Luck, 1986). For scale reared on detached lemons, fresh lemons with scale were placed in a cage with *A. melinus* adults twice a week to maintain the colonies. For oleander scale reared on squash, a squash fruit infested with oleander scale was placed in a cage with adult *A. melinus*. There was no need to add to or replace the squash due to the far larger scale population that could be reared on a single squash compared to a single lemon. For the production of ‘naive’ adult wasps, i.e., those that did not emerge from beneath their host’s cover, thus had no early adult experience with that host species, wasp pupae were collected from beneath the covers of parasitized scales prior to emergence as follows. Scale were examined under a microscope and their covers were lifted. Each wasp pupa found beneath a cover was manually transferred individually with a fine brush to a 3 ml glass vial. A drop of honey was added as a food source for the adult when it emerged, then the vial was closed with a cotton plug and held for adult emergence. Wasps were sexed upon emergence, paired for 24 h, then held for an additional 24 h prior to use in bioassays. Because *A. melinus* has delayed egg maturation (Opp & Luck, 1986), we allow a minimum
of 48 h for eggs to develop after emergence prior to assessing oviposition behavior.

For the production of ‘experienced’ wasps, a lemon or squash, as appropriate, with parasitized scale was removed from the wasp colony and placed in an emergence cage constructed from a paper container with a clear plastic top. Lemons or squash with scale were selected on the basis of the age of wasp pupae beneath the covers of parasitized scales. Wasp pupae can be aged on the basis of eye color (Rosen & DeBach, 1979), and pupae with green eyes will emerge within the next 24 h under our rearing conditions. These wasps were allowed to emerge naturally from beneath the covers of their hosts and acquire early adult experience with the covers of their host species (either California red scale or oleander scale) and the scale’s host plant (lemon in the case of California red scale, or either lemon or squash in the case of oleander scale).

Wasp were collected the day of emergence by aspiration, transferred individually to glass vials containing only a drop of honey, then sexed, and held 48 h for egg development as described above. Because most adult wasps mate shortly after emergence when reared in groups (Rosen & DeBach, 1979), I did not need to individually pair these wasps to ensure mating. Thus, the difference between naive and experienced wasps was that naive adults eclosed into an environment that was devoid of all host tissue, non-living host products, and host plants, while experienced adults acquired early adult experience with its former host and host plant by eclosing beneath the host’s cover and emerging from beneath it on to the host plant substrate.

A second experiment was carried out to determine the effect of priming with synthetic OCT on host choice of wasps experienced with oleander scale. Oleander-experienced wasps were collected from lemon-reared oleander scale as above. After collecting and isolating as individuals, a filter paper disk treated either with OCT or solvent was placed in each vial containing a single female wasp for the 48-h egg development period prior to bioassay.

For ‘Primed’ wasps, filter paper disks (Whatman No. 2, 2.0 mm in diameter) were treated with 4 µl of a 7.5 µg per ml solution of OCT in a 1:1 mixture of acetone:dimethoxypropane. 0.075% trifluoroacetic acid in water, and the solvent was allowed to evaporate. All of the liquid was absorbed by the filter paper disk. This resulted in each disk being impregnated with 30 ng of synthetic OCT. (Millar & Hare, 1993). This is an amount found to be optimal to elicit oviposition by A. melinus in previous studies and near the maximum concentration observed in laboratory-reared scales to date (Hare et al., 1993).

Oleander-experienced wasps for the ‘Control’ treatment were collected and isolated individually in glass vials at the same time. For these wasps, however, the filter paper disks were treated only with 4 µl of solvent. Thus both Primed and Control wasps had acquired early adult experience with oleander scale, but the Primed wasps were exposed subsequently to OCT during the 48-h development period, while the Control wasps were exposed only to the disks but not OCT during their egg development period. The priming experiment only was performed with wasps from oleander scale reared on lemon because the effect of rearing oleander scale on squash had no additional effect on A. melinus’ host preference compared to rearing oleander scale on lemon (see Results).

All bioassays were conducted in Petri dish arenas (60 mm dia x 15 mm high) and observed under a microscope (12 x). Bioassays were usually conducted between 0900 and 1530 h at 24–26 °C, and test arenas were illuminated by fiber optic quartz lighting on either side of the test arena. All studies were designed to determine the relative preference for covers of California red scale compared to covers of oleander scale. Two covers each from the highly preferred third instar of both California red scale and oleander scale were collected and placed at opposite corners of a 1 cm square pattern within the arena. A single female wasp was introduced into the arena and observed for 10 min. The number of times a wasp contacted each scale cover, the number of drumming and turning behaviors, and the number of times a wasp probed a scale cover with her ovipositor was recorded. Wasps were used only once. A fresh set of scale covers were prepared for each wasp to avoid any effect of host marking on cover acceptability. Two or four replicate trials of 20 wasps per treatment were performed for each experiment.

**Innate preference of naive wasps.** Two groups of 20 naive wasps reared on California red scale and two groups each of 20 naive wasps reared on either oleander scale on lemon, or oleander scale on squash, were bioassayed as above. The number of oviposition probes during each 10-min bioassay ranged from 1 to 32. The proportion of oviposition probes on California red scale then were calculated for each wasp. Wasps that did not probe covers from either host (24 of 120) were excluded from the analysis because they
were assumed not to have matured eggs for deposition.

Preference of experienced wasps. Two groups each of 20 wasps that were allowed to emerge from beneath the covers of the hosts on which they were reared, either California red scale on lemon, oleander scale on lemon, or oleander scale on squash, were collected and bioassayed as described above. The number of oviposition probes during each 10-min bioassay ranged from 1 to 23. Wasps that did not probe covers from either host (14 of 120) were excluded from the analysis, as above.

Data from these experiments were analyzed by a two-factor analysis of variance (ANOVA) with replication (host by treatment, trials within [host by treatment] combinations) after employing the arcsin square root transformation to the proportion of oviposition probes on California red scale covers. The variation among hosts, among treatments, and the host by treatment interaction were tested over the variation among trials within host by treatment combinations, and the variation among trials was tested over the pooled within trial error. Separate ANOVAS were performed subsequently on naive wasps and experienced wasps to decompose the host by treatment interaction.

Effect of priming. Four groups each of 20 wasps that emerged from beneath the covers of oleander scale were primed either to filter-paper disks treated with solvent or disks treated with OCT and bioassayed as described above. For comparison, two groups of 20 naive wasps from pupae that were reared on oleander scale on lemon were also bioassayed at the same time. Wasps that did not probe covers from either host (40 of 180) were excluded from the analysis, as above. The total number of oviposition probes ranged from 1 to 22. Data were analyzed by a nested ANOVA (treatments, trials within treatments) after employing the arcsin square root transformation to the proportion of oviposition probes observed on California red scale covers. Significant differences among treatment means were determined by using Tukey's Honestly Significant Difference. All statistical analyses were performed using the SAS PROC GLM procedure (SAS Institute, 1988).

Results

Effect of rearing host and experience on scale cover preference. Host preference of *A. melinus* differed significantly only due to the host by treatment interaction (*F*$_{2,6}$ = 10.66, *P* = 0.011, Fig. 1). Host preference did not differ significantly overall due to the rearing host (*F*$_{2,6}$ = 3.66, *P* = 0.09) nor consistently due to treatments (*F*$_{1,6}$ = 0.57, *P* = 0.48), nor due to variation among trials within host by treatment combinations (*F*$_{6,187}$ = 0.39, *P* = 0.60).

Fig. 1. Oviposition probes (Mean % ± s.e.) by *A. melinus* on California red scale covers when reared on California red scale on lemons (black bars), or oleander scale reared either on lemons (grey bars), or squash (open bars). 'Naive' wasps were isolated from their hosts as pupae, thus they had no early adult experience with their rearing host. ' Experienced' wasps were allowed to emerge naturally from their host and therefore acquired early adult experience with their host.

The significant interaction was decomposed through separate analyses on naive and experienced wasps. Where a significant treatment effect occurred, this was decomposed by two *a priori* single degree of freedom orthogonal tests. The first contrasted host scale species (California red scale vs. oleander scale pooled over its two host plants), while the second contrasted the two host plants of oleander scale (squash vs. lemon). Naive *A. melinus* showed a strong, overall innate preference (89.95 ± 2.02%) for California red scale covers compared to oleander scale covers from either host (Fig. 1). Rearing host did not significantly affect this preference (*F*$_{2,3}$ = 1.14, *P* = 0.43) thus no orthogonal tests were justified.

In contrast, rearing host has a strong influence on host cover preference for experienced wasps (i.e., wasps allowed to emerge naturally from beneath the covers of their host) (*F*$_{2,3}$ = 23.33, *P* = 0.015). Wasps
emerging from beneath California red scale covers showed a stronger preference for California red scale covers than wasps emerging from beneath oleander scale covers from either squash or lemon ($F_{1,3} = 40.66$, $P = 0.008$). The preference of wasps emerging from beneath covers of oleander scale reared on squash did not differ significantly from that of wasps emerging from covers of oleander scale reared on lemon, however ($F_{1,3} = 0.81$, $P = 0.43$). Overall, wasps emerging from beneath California red scale covers showed a stronger preference for California red scale covers ($99.19 ± 0.46\%$) than naive wasps, while the preference for California red scale covers was diminished ($80.40 ± 3.34\%$) if wasps were allowed to emerge from beneath covers of oleander scale reared either on lemon ($78.71 ± 4.43\%$) or squash ($83.25 ± 4.97\%$, Fig. 1).

**Effect of priming.** The preference of oleander-experienced wasps was modified by exposure to OCT ($F_{2,7} = 16.43$, $P = 0.002$, Fig. 2). The preference of oleander-experienced wasps for California red scale was significantly greater when primed to disks treated with OCT than when primed to control disks ($93.93 ± 2.77\%$ vs. $75.97 ± 1.73\%$), and not significantly different from the preference of naive wasps for California red scale covers ($94.82 ± 1.48\%$). Therefore, the effect of early adult experience with oleander scale covers diminishing *A. melinus*’ innate preference for California red scale was overcome by exposing oleander-experienced wasps subsequently to the synthetic kairomone, OCT.

**Discussion**

Despite having been reared on oleander scale for about 300 generations, the original laboratory *A. melinus* population still retains a strong preference for California red scale covers compared to oleander scale covers. The four original collections of *A. melinus* were from India, three of which were from California red scale on rose, while the fourth was from yellow scale (*Aonidiella citrina* [Coquillett]) on citrus (DeBach, 1959).

Apparently these collections harbored little genetic variation for an innate ovipositional preference for oleander scale that could have been enhanced through continuous rearing on oleander scale in the laboratory. Nevertheless, early adult experience with their host can modify this innate preference; the preference for California red scale is reduced when wasps emerge from under oleander scale covers and is enhanced nearly to unity when wasps emerge from under California red scale covers.

One might hypothesize that wasps reared on the oleander scales that were themselves reared on squash would have a lower preference for California red scale than wasps reared on the oleander scales that were reared on lemons. This was not observed, however. Early experience with oleander scale reared either on squash or lemon reduced the preference of those wasps for California red scale similarly. It would be premature to conclude, however, that the host plant has no influence in host selection by *A. melinus*, because my experimental design only tested host acceptance behavior in the absence of any host plant cues. Long-range host location behavior, which often is strongly influenced by host plant cues (Vet & Dicke, 1992), was not examined. Thus, the effect of rearing *A. melinus* on squash-reared oleander scale on the ability of those wasps to locate California red scale on citrus prior to host acceptance remains undetermined.

The priming experiment involved an innately recognized unconditional stimulus, OCT, presented on filter-paper disks. The subsequent receptivity of wasps
to California red scale covers, which contain natural OCT as well as other potential chemical and physical cues, was then assayed. Associative learning probably did not occur in this experiment, because there were no 'conditioned' or surrounding stimuli associated with the filter paper disks that were also present when the wasps were subsequently assayed with intact covers. It is not clear, however, whether the prior experience to synthetic OCT simply enhanced the abilities of wasps to detect and respond to the natural OCT in intact California red scale covers, or if the experience caused a more general enhanced responsiveness not only to OCT but also to other potential innate cues provided by the cover, such as size, color, or texture. Such questions remain to be investigated. Because OCT concentration can be manipulated independently of other cues by washing it out of covers (e.g., Quednau & Hübisch, 1964) and/or by adding controlled quantities of synthetic OCT to the covers of hosts of varying suitability to A. melinus, these questions may be relatively easily addressed in this system.

Previously, Hare & Luck (1994) suggested that, by virtue of their lower OCT concentration, California red scale reared on the leaves or bark of orange cultivars may be less conspicuous to A. melinus than scale reared on the same substrates of lemon, and therefore may escape discovery by A. melinus. Results of experiments reported here suggest an additional explanation for the perceived reduced effectiveness of insectary-reared wasps to provide biological control of California red scale on orange compared to lemon. The nearly 20% reduction in the acceptance of California red scale covers by A. melinus when reared on oleander scale compared to California red scale suggests that California red scale are likely to escape discovery by insectary-reared A. melinus. Both the reduced conspicuousness of California red scale reared on substrates of orange cultivars as well as the reduced ability of oleander-reared A. melinus to recognize California red scale may contribute to the reduced effectiveness of commercial A. melinus to control California red scale on orange cultivars. While little may be able to be done to overcome the former problem, the latter may be alleviated by exposing commercially-reared A. melinus to OCT prior to release.

Acknowledgments

I thank Adrian Stull for technical assistance and R. F. Luck for comments on an earlier draft of the manuscript. This research was supported in part by grants from the California Citrus Research Board.

References