

NOTE:

EVIDENCE FOR LEARNING OF VISUAL HOST-ASSOCIATED CUES IN
THE PARASITOID WASP *TRICHOGRAMMA THALENSE*

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Parasitoid wasps, which lay their eggs and develop within other insects, often modify their parasitization behavior according to their past experience. In particular, the presence of hosts (or host-related plant substrates) on which the parasitoid had oviposited before, often affects foraging behavior. For example, *Leptopilina* wasps choose substrates on which they had previously oviposited rather than novel ones (Vet, 1988; Vet and Schoonman, 1988; Papaj and Vet, 1990; Poolman Simons et al., 1992). *Trichogramma pretiosum* remain longer in host patches after a single pre-release oviposition than without previous experience (Gross et al., 1981). *Trichogramma maidis* increase their affinity to a given host species after oviposition on the same host (Kaiser et al., 1989). Early exposure of *Trichogramma principium* to young hosts decreases the likelihood of later host rejection (Reznik et al., 1997). It has been demonstrated that parasitoids learn to associate the presence of hosts (an unconditioned stimulus, US) with related environment cues. After conditioning had taken place, the environmental cue serves as a conditioned stimulus (CS), which elicits the response (R) of searching and oviposition (Vet and Groenewold, 1990).

What types of CS cues do parasitoid wasps associate with the presence of hosts? Most studies of associative learning in parasitoids demonstrate perception and learning of chemical signals (Turlings et al., 1993). Such signals include kairomones secreted by the hosts (e.g., Shaltiel and Ayal, 1998; Hoffmeister et al., 2000) or by their substrate plants (e.g., Turlings et al., 1995), or artificial odors (e.g., Vet and Groenewold, 1990). Recently, the role of vision in the foraging of parasitoids has also received increasing attention. The use of visual information for host location was demonstrated in the parasitoids *Microplitis croceipes* (Wäckers and Lewis, 1994, 1999), *Campoletis sonorensis* (McAuslane et al., 1991), *Cotesia rubecula* (Wäckers, 1994), and *Aphidius ervi* (Battaglia et al., 2000). Moreover, *Microplitis croceipes*, *Exeristes roborator*, and *Trybliographa rapae* can learn to associate the presence of their hosts with a visual cue. These experiments included conditioning to color, shape and pattern cues in *Microplitis*, and to color cues in *Trybliographa* and *Exeristes*. A spectral sensitivity analysis of

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Accepted September 2000.

Trybliographa revealed peaks of sensitivity in the UV, blue, and green-yellow regions of the spectrum, suggesting a three-receptor system of color vision (Wardle, 1990; Wäckers, 1994; Wäckers and Lewis, 1994, 1999; Brown et al., 1998).

Trichogramma is a gregarious parasitoid wasp that oviposits and feeds on a wide range of moth eggs. The genus has a worldwide distribution, and several species exist in Israel. Various species of *Trichogramma* are reared commercially in insectaries for use in biocontrol of agricultural pests. One motivation for studying the learning abilities of *Trichogramma* was to assess the potential for improving their biocontrol efficiency through conditioning. *Trichogramma pretiosum* prefers young corn borer eggs over old eggs as hosts, but this discrimination is much reduced under conditions of darkness. This suggests that *Trichogramma* uses visual cues for foraging (Calvin et al., 1997). In the present experiment we studied conditioning to color cues in the species *Trichogramma thalense* Pinto & Oatman (Hymenoptera:Trichogrammatidae).

Trichogramma thalense parasitoids were collected in Santa Cruz County, California in 1997 and reared in the insectary of the University of California, Santa Cruz Center for Agroecology and Sustainable Food Systems (UCSC-CASFS). The generation time of *T. thalense* is 10.1 ± 0.13 d, and the mean lifetime fecundity is 36.9 ± 1.9 at 25 °C (Abbinanti, 1994). We used mated females that were 24–48 h old for experiments. Preliminary experiments showed that females of this age group parasitize more readily than younger and older wasps. *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) moths were obtained from Beneficial Insects Inc., Guelph, ON, Canada, and were supplemented by collections from Santa Cruz County. They were reared, mated, and allowed to oviposit in the insectary of the UCSC-CASFS. Their eggs were collected daily and were used as 0–24 h old hosts for the experiments since *T. thalense* prefer fresh *Anagasta* eggs to older eggs (Kearar et al., in press).

The experiment was conducted at 25 °C in a laboratory under constant illumination by D-65 neon light tubes. Hosts were prepared on egg cards in petri dishes. The egg-cards were made out of thick, non-glossy paper, and were 2 × 1 cm in size. Parasitoids were chilled briefly (<10 min at 5 °C) prior to manipulation, and were then placed on the hosts. About 10% of the parasitoids, those that did not start inspecting the hosts immediately, were replaced. The experiment consisted of two phases. In the first phase, which lasted four hours, we assigned each of 250 single parasitoids to one of the following treatments: (1) exposure to 10 hosts on a black egg card (black + hosts), (2) exposure to 10 hosts on a green egg card (green + hosts), (3) exposure to a black egg card with no hosts (black – hosts), (4) exposure to a green egg card with no hosts (green – hosts), (5) Exposure to an empty petri dish with no egg card or host (control). In the second phase, which immediately followed the first phase, we moved each parasitoid to an egg card that was half black and half green, and which carried >25 hosts on each half (Table 1). We removed the parasitoid seven hours later, and incubated the Phase-I and Phase-II hosts at 25 °C. Preliminary observations showed that *T. thalense* can oviposit their whole egg complement (20–30 eggs) within 4 hours. Therefore parasitoids were not host- or time-limited (but probably egg-limited) in the second phase. We identified parasitized hosts by their black color six days after exposure to the parasitoid.

Table 1

Design of the experiment. Phase-I lasted 4 hours. Phase-II was identical for all treatments and consisted of a 7-hour exposure to >50 hosts, glued to an egg card that was half black and half green

Treatment	N	Phase-I visual cue	Phase-I hosts
Black + hosts	60	black	10
Black - hosts	40	black	none
Green + hosts	60	green	10
Green - hosts	40	green	none
Control	50	white	none

Parasitoids that were allowed to oviposit on a green background in the first phase of the experiment preferred hosts on a green background in the second-phase choice test. Similarly, parasitoids that were exposed to hosts on a black background in Phase I preferred them in Phase II. Wasps that were not exposed to hosts in the first phase of the experiment did not show a preference for background color in the second phase (Fig. 1).

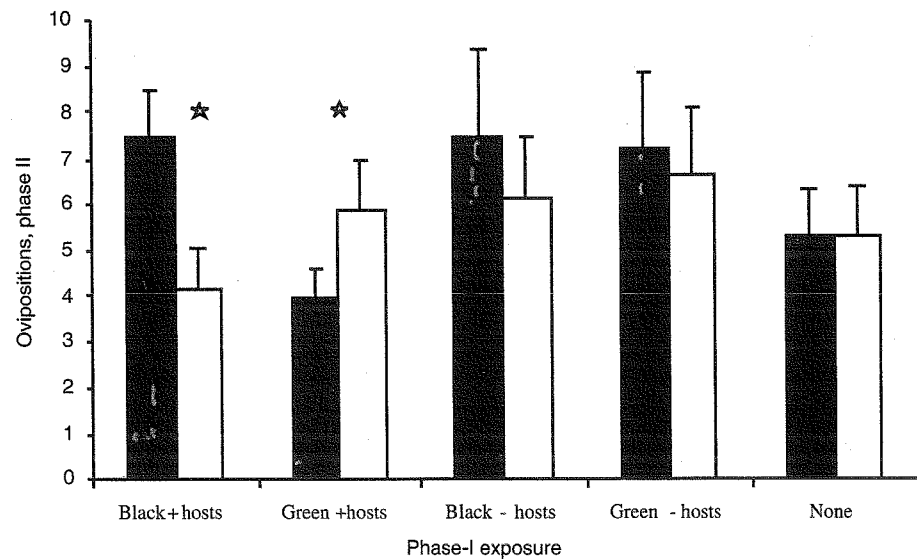


Fig. 1. Mean number of ovipositions on black (black bars) and green (white bars) background in the second phase of the experiment. Error bars are 1 SE. Asterisks denote a statistically significant difference between the number of parasitized hosts on black and green backgrounds (one-tailed, paired *t*-tests, $p < 0.05$).

The results demonstrate the ability of *Trichogramma* to associate the presence of hosts with a visual cue (green or black background). The results also suggest that oviposition in a host is required for learning to take place because wasps from the "black – hosts" and "green – hosts" treatments did not show color preferences in Phase II. We do not know whether the *Trichogramma* differentiates between green and black on the basis of spectral differences, or whether it responded to some other visual quality, such as differences in brightness or saturation between our green and black background.

The mass rearing protocol of *Trichogramma* in our insectary involved exposure to hosts on black egg cards for many generations. We suspected that this procedure may have selected for preference for black background in the insectary *Trichogramma* line. Our results show no evidence for such selection, since wasps that had no Phase I exposure did not prefer the black over the green background. Nevertheless, it is rather unusual that the parasitoids were not deterred by black (many parasitoids are). This may be attributed to the rearing method.

ACKNOWLEDGMENTS

We thank Cathy Carlson for technical assistance. This work was partially supported by a USDA grant to Marc Mangel.

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