

Responses of *Aphytis chilensis* to the synthetic sex pheromone of the Oleander scale.

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Abstract: The role of the synthetic sex pheromone of the Oleander Scale, *Aspidiotus nerii* Bouché (Homoptera: Diaspididae), in the long-range host-searching behaviour of the specialist parasitoid *Aphytis chilensis* Howard (Hymenoptera: Aphelinidae) was studied. Different concentrations in hexane of the (-) and (+) enantiomers of the synthetic host sex-pheromone were compared for their attractiveness in dual choice tests in an Y-olfactometer. Wasp females were significantly attracted from the synthetic sex pheromone at the concentrations from 3×10^{-4} up to 6×10^{-3} ($\mu\text{g}/\mu\text{l}$) of the (+) enantiomer, and at a concentration of 3×10^{-3} ($\mu\text{g}/\mu\text{l}$) of the (-) enantiomer. At the concentrations less than 6×10^{-5} ($\mu\text{g}/\mu\text{l}$), for the (-) enantiomer, or less than 3×10^{-4} ($\mu\text{g}/\mu\text{l}$) for (+) enantiomer female wasps did not show preferences. These results indicate that in searching behaviour of *A. nerii* *A. chilensis* is orientated towards areas likely to contain suitable host stages by host-derived information.

Key words: parasitoid, Y-olfactometer, kairomone, sex-pheromone, *A. nerii*, *A. chilensis*

Introduction

The Oleander scale, *Aspidiotus nerii* Bouché (Homoptera: Diaspididae), is a cosmopolitan pest mainly spread in the Mediterranean basin. This scale insect is polyphagous and attacks citrus and olive trees and ornamental plants. In Sicily, the population level of this pest is generally below the economic threshold, nevertheless its density could increase causing serious damages on fruits in some areas and in lemon orchards heavily sprayed (Liotta *et al.*, 1973).

One of the most important biological control agents of Oleander scale in Sicily is *Aphytis chilensis* Howard (Hymenoptera: Aphelinidae) (Liotta, 1972) that develops as ectophagous on second and third instar larvae of host with a cycle of development of 21 ± 1 °C (personal observations).

In the field of our studies on foraging behaviour regarding the host-parasitoid system *Aspidiotus nerii* – *Aphytis chilensis* we are investigating on the possible role played by volatile cues from host. Parasitoids often use chemical stimuli, called info-

chemicals (Nordlund & Lewis, 1976), as information from their environment while are searching for their hosts, and these infochemicals can be associated to their host, such in the case with the host sex pheromone (reviewed in Godfray, 1994). Parasitoids can orientate towards these cues over a moderate distance that they associate in some way with the host. For many parasitoids species this means flying to the general source of the odors perceived (Vinson, 1991). For this reason Y-tube olfactometers have been used to behaviour analysis of parasitoids (Colazza *et al.*, 1997; Greany *et al.*, 1977; Lecomte & Thibout, 1986; Moneith, 1955; Morgan & Hare, 1998). Host finding process in the *A. chilensis*-*A. nerii* system might involve some degree in infochemicals-mediated behaviour: it was observed that *A. chilensis* respond to host-associated kairomones as water extracts of Oleander scale covers (Luck & Uygun, 1986). We thought be interesting evaluate the possible effects of volatile cues as the Oleander scale sex pheromone. We addressed a question: does *A. chilensis* is attracted from Oleander scale sex pheromone?

In order to give a response to the question we analysed the behaviour of the parasitoid females in Y-olfactometer using in tests the synthetic sex pheromone of *A. nerii*, recently isolated (Einhorn *et al.*, 1998) and synthesised (Boyer & Ducrot, 1998).

The aim of this work was to examine the response of *A. chilensis* to (+) and (-) enantiomers of the synthetic sex pheromone of *A. nerii* at different concentrations.

Materials and methods

Insects. The colony of *A. nerii* was reared on fruits of lemon (*Citrus limon* L.) in cages kept in laboratory at 25 ± 1 °C, 60-70 % RH, and a photoperiod 16 L: 8 D. The colony of *A. chilensis* was established from pupae collected in the field and reared on *A. nerii* and kept in the same laboratory condition.

Tested parasitoids were obtained from pupae removed from their rearing host, individually isolated in glass vials (=1 cm) and supplied with a drop of honey solution. The females used in the experiment were 2-3 days old, had no oviposited previously, or been in contact with the host, and were used only once.

Host sex pheromone. We used the synthetic sex pheromone of the Oleander scale diluted in hexane (1.5 g/l), provided by Dr. Einhorn and Dr. Ducrot of the Unité de Phytopharmacie et médiateurs chimiques, INRA, Versailles (France). We tested both (-) and (+) enantiomers in different concentrations in hexane as following: (-) enantiomer ($\mu\text{g}/\mu\text{l}$): 6×10^{-3} , 3×10^{-3} , 3×10^{-4} , 6×10^{-5} , 6×10^{-6} , 3×10^{-6}
(+) enantiomer ($\mu\text{g}/\mu\text{l}$): 3×10^{-3} , 3×10^{-4} .

Test protocol. Wasps were bioassayed using a Y- olfactometer chamber made of transparent Plexiglas (two arms 8 cm long and common stem 8.5 cm long) sandwiched between two glass sheets (15 x 20 cm). Medical-grade compressed air flowed through both arms creating an airstream of 30 ml/min per arm: the flow was regulated

by flometers. Both arms were connected to two glass vials (350 ml) holding the odour source sealed with Teflon tape. In each experiment as test we used a diskette of filter paper ($\phi = 8,5$ cm) impregnate with 500 μl of sex pheromone in the concentrations as above mentioned and as control a diskette of filter paper ($\phi = 8,5$ cm) impregnate with 500 μl of hexane. The diskettes were changed any 3 tests.

For each bioassay, a single female was introduced into the Y-olfactometer at the entrance of the common stem and observed for 10 min. Adults were observed under infrared light illumination (homogenous emission of wave-lengths at 950 nm provided by 108 LEDs) with a CCD camera connected with a video monitor. Analog video signals from the camera were digitalized by a video frame grabber and data was processed by a video tracking a motion analysis system for "Xbug" (Colazza *et al.*, 1999). Measurements were made of time spent by wasps in each arm of the olfactometer. Female wasps were tested singly. The temperature in the bioassay room was 26°C at all times and all tests were carried out from 9:00 to 15:00.

Choices between test and control arm within a test were analysed using Wilcoxon matched-pairs test (Statistica 5.1. Statsoft, inc. 1997).

Results

With only clean air passing through Y- olfactometer females spent an equal proportion of their time in each arm (42.13 ± 11.43 left vs. 52.98 ± 13.76 right, $n= 15$ $t=0.51$ $p= 0.61$).

The responses of *A. chilensis* to the synthetic host sex pheromone are presented in figures 1 and 2. The (-) enantiomer (fig. 1) of synthetic sex pheromone at the concentrations of 6×10^{-3} $\mu\text{g}/\mu\text{l}$, 3×10^{-3} $\mu\text{g}/\mu\text{l}$ and 3×10^{-4} $\mu\text{g}/\mu\text{l}$ induced the wasp parasitoids to spend significantly more time in the test arm than in the control arm ($n=21$, $t=5.71$, $p=0.000013$, $n=34$, $t=2.41$, $p= 0.0212$ and $n=40$, $t=3.15$, $p=0.0030$ respectively). With concentrations of 6×10^{-5} $\mu\text{g}/\mu\text{l}$, 6×10^{-6} $\mu\text{g}/\mu\text{l}$, and 3×10^{-6} $\mu\text{g}/\mu\text{l}$ wasps showed no significant preference for one of the two arms ($n=33$, $t=1.48$, n.s., $n=38$, $t=1.79$, n.s. and $n=23$, $t=0.19$, n.s. respectively).

In the experiments with the (+) enantiomer (fig. 2) of synthetic sex pheromone *A. chilensis* spent significantly more time in arm test with a concentration of 3×10^{-3} $\mu\text{g}/\mu\text{l}$ ($n=17$, $t=2.47$, $p=0.02$) and it does not show any preference for the two arms with a concentration of 3×10^{-4} $\mu\text{g}/\mu\text{l}$ ($n=17$, $t=-1.01$, n.s.).

Discussion

Successful parasitism by parasitoids of herbivorous insect hosts is preceded by several phase of searching that lead females into the close vicinity of their potential hosts (Vinson, 1991; Godfray, 1994). Since successful foraging is directly linked with

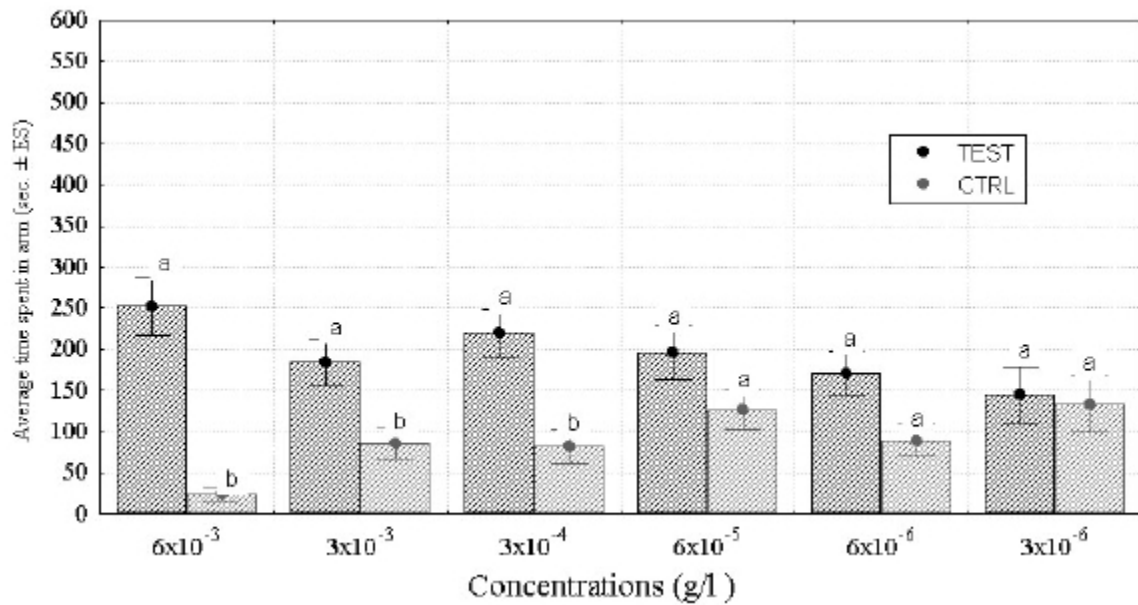


Figure 1. Response of *A. chilensis* in Y-olfactometer to (-) enantiomer of synthetic *A. nerii* sex pheromone

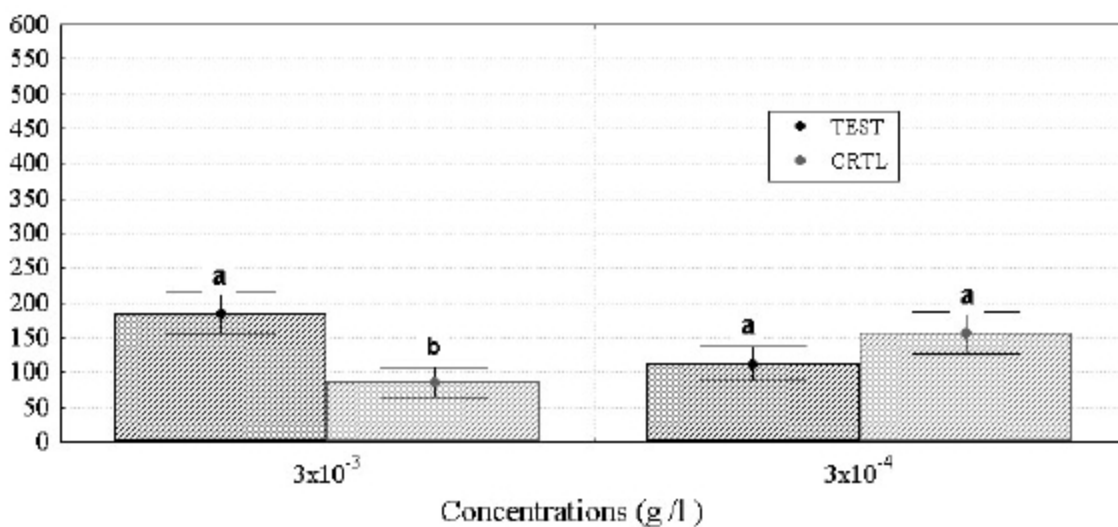


Figure 2. Response of *A. chilensis* in Y-olfactometer to (+) enantiomer of synthetic *A. nerii* sex pheromone

their reproductive success, natural selection will favour animals that make optimal use of foraging cues. Vinson (1991) organised these various chemical cues in different groups in relation to the chemical characteristics of the compounds and the host selection level they effect. After parasitoid locates the host-habitat, it may initiate a host search for cues eliciting a behavioural response (named Group I). These cues are volatile, thus acting over a distance and they arise from the host's food (plant

volatiles), volatiles as a consequence of associated organism, or volatile from a pre-host stage (female sex pheromones). These chemical cues result in the female parasitoid arriving at a potential host community or the microhabitat of the host rather than the host (Vinson, 1991).

Some parasitoid species use stimuli produced by host adults to help in the location of the immature stages that they attack and these cues can be sex pheromones of the hosts. Cues directly from the host tend to be non-volatile contact kairomones (Hare *et al.*, 1993), although some parasitoids exploit volatile pheromones of their host as kairomones (Hardie *et al.*, 1994; Feener *et al.*, 1996; Vet *et al.*, 1991). Sex pheromones, often, are not directly associated with the attacked host stages but serve to reduce host searching area (reviewed in Vinson, 1985; Godfray, 1994).

Our results suggest that synthetic *A. nerii* sex pheromone is used by *A. chilensis* as cue eliciting a positive response in the females. Both (-) and (+) enantiomers of sex pheromone attracted female wasps but the (-) enantiomer was attractive at three different values of concentration tested while the (+) enantiomer at only one concentration value. *A. chilensis* attacks immature male and II and III instar female scales; sex pheromone is produced by III instar female scale. Therefore these volatile cues could be used by *A. chilensis* as indirect cue to locate suitable search area or as direct cue to attach host stage producing it.

In monitoring of *A. nerii* population by mean of pheromone traps to capture males it could be interesting evaluate any possible effect that the attraction of the synthetic sex pheromone might have on parasitism efficacy of *A. chilensis*, even if we have to consider that more complex cues depending on scale- host plant system could be important for foraging parasitoids (Morgan & Hare ,1998).

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