

Male Attraction to Virgin Females in the Almond Seed Wasp, *Eurytoma amygdali* Enderlein (Hymenoptera, Eurytomidae)¹

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ABSTRACT

Laboratory observations showed that adults of the almond seed wasp, *Eurytoma amygdali* Enderlein (Hymenoptera: Eurytomidae) were sexually mature on the first day of emergence. Experiments with an olfactometer showed that 1 to 12 day-old virgin females attracted the males. This attraction was found to be due to an odor, apparently a pheromone, released by the virgin females. The responsiveness of the males was higher the first part of the photophase. Under field conditions too, virgin females were attractive to males. "Delta" traps, containing 5-20 virgin females, suspended on almond trees, attracted and captured males released among the same trees. On the contrary, traps containing mated females or empty cages were not attractive to males.

Introduction

The almond seed wasp, *Eurytoma amygdali* Enderlein, is a serious pest of almonds in the eastern Mediterranean region. It has one generation per year but a proportion of the population completes a generation every two years. In northern Greece, the female oviposits during April and May into the nucellar tissue of the developing seed of the almond. The larva develops in the young seed and at the end of June completes its growth. The larva overwinters in diapause and pupates in February-March. The wasp emerges in April or May (Mentjelos and Atjemis 1970). Little is known about the mating behavior of this insect. Observations however, reported by Plaut (1971), indicated that males were attracted to females exposed in cages in the orchard. This suggests that the females may produce a sex pheromone attracting males. The aim of this study was to verify the suspected male attraction to females in this species and to gain information on the factors affecting this phenomenon.

Materials and Methods

a. Wasps used and holding conditions

Wasps emerged from pupae obtained from infested almonds collected in the area of Thessaloniki in autumn and kept in a refrigerator. Next spring, these almonds were transferred in the laboratory where the wasps emerged. Upon emergence, the sexes were segregated and held until testing in groups of 10-20 individuals in one liter cylindrical plastic cages, provided with a tulle-window and water. A piece of cotton soaked in a sucrose solution was used as food. To obtain mated wasps, a number of females was caged together with males upon emergence until testing. The room conditions were $25 \pm 2^\circ \text{C}$, 60-70% R.H. and a 14 L: 10 D photoperiod, with the photophase between 06.00 and 20.00. Light was provided by 9 fluorescent tubes of the daylight type and during most of the photophase also by natural daylight entering from two windows. The light intensity at the level of the cages varied between 500-1000 Lux, according to the outdoor conditions, but usually was ca. 700 Lux.

b. Laboratory experiments

All laboratory experiments were conducted between March and April 1984 and 1985. For the bioassays an olfactometer developed and described by Katsoyan-

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nos et al (1980) was used. In brief, the olfactometer consisted of four 7 cm diam. 10 cm long polyethylene tubes, one of which was the "test-cage" holding the males for response to the pheromone and another was the "bait-cage" containing the odorous source of attraction (virgin females). During the bioassay an air stream generated by a ventilator passes through the tube, picks up the odor from the bait-cage, then enters the test-cage containing the males. Responding males enter the tube through a wire-screen funnel connected between the test-cage and the tube and are captured in a chamber, where they are counted on the termination of the test.

Ten virgin females were placed into each bait-cage containing food and water and 10 males of the same age into the test-cage. As control, empty bait-cages were used, containing only food and water. The bioassays were carried out between the 3rd and 7th hour of the 14-hour photophase and each one lasted 15 minutes. In most cases, four units of the olfactometer were run simultaneously, one or two of which were controls. When the same wasps were used more than once, bioassays were carried out at least 30 minutes after the termination of the previous ones and the bait-cages containing virgin females were interchanged with the control cages. Also, the position of the test-cages was rotated.

c. Field experiments

Field experiments were conducted in an orchard at the University Farm of Thessaloniki in April and May 1984 and in May 1985. The orchard consisted of 12 fruiting large almond trees arranged in three rows of four trees each. Cardboard "Delta" traps (9.5×23 cm base, 10 cm high) were used, into which 5-20 virgin females (1 to 3 day-old) were caged in a wire-screen cylinder (5 cm diam., 6 cm high) provided with food and water. In the bottom of the trap, a sticky-coated cardboard with Bird Tanglefoot® (The Tanglefoot Company, Grand Rapids, Michigan 49504, USA) was placed to capture the responding wasps. The traps were suspended 1.5 m from the ground on the branches of the almond trees. For each test, the one to five day-old male wasps were released during the morning hours, in the same day in which the traps were installed. The release took place from one cage put on the ground, at the center of the 12 almond trees. The captured males were counted once, five to seven days after the release.

Results and Discussion

a. Laboratory experiments

As shown in Table 1, male wasps, 1-12 days old, responded strongly to bait-cages containing virgin females, while no response was observed to cages containing mated females or control

empty cages. As the olfactometer excludes visual contact between the test and the bait insects, the observed attraction is obviously due to an odor, apparently a volatile sex pheromone,

TABLE 1. Male *E. amygdali* responses to virgin and mated females in the olfactometer (10 insects/cage, 1-12 days old).

Source of attraction	No. of replic.	% male response	
		Mean	S.D.
Virgin females	25	47.0	24.1
Mated females	20	0.0	0.0
Control	18	0.5	2.4

emitted by virgin females. Males tested a few hours after emergence responded to virgin females of the same age, which indicates that the wasps were sexually mature upon their emergence. The results of Table 2 show that the male response in the laboratory was very strong during the first part of the photophase and gradually diminished during the rest of it.

TABLE 2. Male *E. amygdali* response to virgin females in the olfactometer, in relation to the hour of day.

Time of day*	No. of replic.	% male response	
		Mean	S.D.
09.00-11.00	9	67.8	19.9
11.00-13.00	16	35.2	17.6
13.00-15.00	21	31.3	24.3
15.00-17.00	16	18.7	18.0
17.00-19.00	10	11.0	18.5

* Photophase between 06.00 and 20.00.

b. Field experiments

The results of field experiments are shown in Table 3. Due to shortage of wasps, a limited number of traps and a limited number of released males was used during 1984. That year, 49 out of 110 released males (i.e. 44.5%) were captured in the traps containing virgin females. On the contrary, no males were captured in the traps containing mated females. The males captured in the traps were the released ones, because the native population had not yet emerged, as shown by checking mummified almonds of the orchard which contained the overwintered population. In 1985, although the number of males captured in the traps was rather low, the results confirm the attraction of males to virgin females. The reason for the low cap-

TABLE 3. Attraction of released *E. amygdali* males to traps containing living females.

Exp.	Date of release	Number of released males	Sex pheromone traps		Total no. of captured males
			Number	Content	
1	30/IV/1984	65	1	10 virgin ♀♀	9
			1	10 mated ♀♀	0
2	5/V/1984	45	1	5 virgin ♀♀	15
			1	10 virgin ♀♀	25
			1	10 mated ♀♀	0
3	13/V/1985	170	8	5-20 virgin ♀♀	13
			4	control (no wasps)	0

tures in 1985 in comparison with those of the previous year was not identified with precision. However, we have indications that the sticky-coated cardboards of the traps used that year, were less effective in capturing the responding males than those used in 1984. In similar experiments conducted in April 1986 in the same orchard, more than 200 males of the natural population were captured in 6 days by 4 traps baited with 25 - 50 virgin females (B.I. Katsoyannos and A. Vassiliou, unpubl.).

In conclusion, our results demonstrated that adults of the almond seed wasp, *Eurytoma amygdali*, were sexually mature on the first day of emergence and that the virgin females attracted the males, which responded more the first part of the photophase. This attraction was apparently due to a sex pheromone which when identified and synthesized it may prove useful for monitoring the adult population and timing the control of this serious almond pest. Adult monitoring experiments, using living females to bait sex pheromone traps, were very successfully initiated in spring 1986 (B.I. Katsoyannos and A. Vassiliou, unpubl.).

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KEY WORDS: *Eurytoma amygdali*, Hymenoptera, Eurytomidae, Almond seed wasp, Sex pheromone

**Προσέλκυση Αρσενικών από Θηλυκά του Ευρυτόμου της Μυγδαλιάς,
Eurytoma amygdali Enderlein (Hymenoptera: Eurytomidae)**

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ΠΕΡΙΛΗΨΗ

Παρατηρήσεις που έγιναν στο εργαστήριο έδειξαν ότι τα ενήλικα του εντόμου *Eurytoma amygdali* Enderlein (Hymenoptera: Eurytomidae) είναι σεξουαλικά δραστήρια από την πρώτη ημέρα της εξόδου τους από τα προσβεβλημένα αμύγδαλα. Πειράματα που έγιναν με την βοήθεια ολφακτομέτρου έδειξαν ότι τα παρθένα θηλυκά του εντόμου ηλικίας μιας ως δώδεκα ημερών προσελκύουν από κάποια απόσταση τα αρσενικά. Η ανταπόκριση των αρσενικών ήταν ισχυρότερη τις πρωινές ώρες της ημέρας. Τα παρθένα θηλυκά ήταν ελκυστικά και σε συνθήκες υπαίθρου. Παγίδες τύπου «Δέλτα», που περιείχαν εγκλωβισμένα 5-20 παρθένα θηλυκά η καθεμιά και αναρτήθηκαν σε μυγδαλιές, προσέλκυσαν και έπιασαν ικανοποιητικό αριθμό αρσενικών που εξαπολύθηκαν. Αντίθετα, παγίδες που περιείχαν συζευγμένα θηλυκά ή κενά κλουβιά δεν ήταν ελκυστικές για τα αρσενικά έντομα.