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FIELD EVALUATION AND EAG MEASUREMENTS OF SEX PHEROMONE COMPONENTS OF THE TOMATO LOOPER, *CHRYSODEIXIS CHALCITES* (ESPER) (LEPIDOPTERA: NOCTUIDAE)*

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ABSTRACT

Mixtures containing pheromonal components: 12:Ac, Z7-12:Ac, Z9-12Ac, ll-12:Ac, Z8-13:Ac, Z9-14:Ac and Z7-12:OH identified from gland volatiles of the tomato looper, *Chrysodeixis chalcites*, were tested in the field. Night observations on the behaviour of flying males towards baits containing Z7-12:Ac and/or Z9-14:Ac, separated and combined, were performed. The major pheromone component Z7-12:Ac induced some long-range attraction but not landing on the bait. A combined plume of both components induced males to land. The attractiveness of 15 blends containing two to seven components was assessed in the field. Several blends gave statistically the same trap catches, however, one ternary and a five component blend gave generally the highest catches. For practical use the ternary blend containing 1 mg of Z7-12:Ac + 0.2 mg Z9-12: Ac + 0.2 mg Z9-14:Ac is recommended.

The EAG dose response curves of all the components, identified in pheromone gland volatiles of *C. chalcites*, were determined. Comparison of the EAG responses at a loading of 100 µg per paper strip, showed that the major pheromone component Z7-12:Ac elicited the highest response of 13.8 mV, whereas the other acetates caused responses of 4.1-8.3 mV. The alcohols Z7-12:OH, Z-9-12:OH and Z9-14:OH revealed a lower EAG response. *KEY WORDS:* Tomato Looper, *Chrysodeixis chalcites*, sex pheromone, field observations, trapping tests, EAG measurements,

INTRODUCTION

The tomato looper, *chrysodeixis chalcites* (Esper) (Lepidoptera:Noctuidae), previously referred to as *Plusia chalcites* by Dunkelblum *et al.*, 1987, is a polyphagous pest throughout the world (Rivnay, 1962). In Israel and Egypt it is an important pest of solanaceous plants (Yathom & Rivnay, 1968; Harakly, 1974). Recently, it has been found in increasing numbers in cotton fields in Israel. Two main sex pheromone components from gland extract, (Z)-7-dodecenyl acetate (Z7-12:Ac) and

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(*Z*)-9-teradecenyl acetate (*Z*9-14:Ac), were identified previously (Dunkelblum *et al.*, 1981). In field tests a blend of *Z*7-12:Ac and *Z*9-14:Ac in 5:1 ratio was found to be an effective bait for *C. chalcites* males (Dunkelblum *et al.*, 1981). Recently six components were identified in the volatiles emitted from the pheromone glands and preliminary flight tunnel experiments with males indicated that five components have pheromonal activity (Dunkelblum *et al.*, 1987). In view of these results we performed more detailed field tests with these chemicals in order to obtain a more efficient bait than the two component used before (Dunkelblum *et al.*, 1981). EAG measurements of the components found in the pheromone gland volatiles were also conducted.

MATERIALS AND METHODS

Insects. The insects were reared on an artificial medium (Shorey and Hale, 1965). Pupae were sexed and males, fed on a 10% sugar solution, were kept in screen cages (30x30x30 cm).

Chemicals. All chemicals were from our collection (97-99% pure). For field observations and tests, rubber septa (Maavit Products, Tel-Aviv, Israel) were impregnated with the hexane solutions of the pheromonal blends. Baits were dried for 5 hours in a fume-hood before use.

Field observations. For the study of the behaviour of males in the field, baits were hung at 1 m height on poles, 100 m between each treatment. When two baits were tested, they were positioned in line either parallel or perpendicular to the wind direction. In the parallel test, the three arrangements were: 1. the two baits, *Z*7-12:Ac and *Z*9-14:Ac, hanging close together (~ 1 cm apart) on one pole; 2. *Z*7-12:Ac in front of the wind direction and *Z*9-14:Ac in the rear 50 cm apart; 3. *Z*9-14:Ac in front and *Z*7-12:Ac in the rear. In the perpendicular test, the three arrangements were: 1. both baits hanging close on one pole; 2. and 3. the two baits hanging on two separate poles at a distance of 5 cm and 30 cm apart respectively. The behaviour of males approaching and landing on three baits was recorded simultaneously by three observers in each test. The observers were sitting on the ground, 2 m from the bait, looking in a direction perpendicular to that of the wind direction. Each observation (four in Tests 1 & 2 and six in Test 3; Table 1) lasted 10 min and then the observers exchanged places. Mixed baits contained *Z*7-12:Ac and *Z*9-14:Ac in one septum.

Field tests. Tests were conducted in tomato and cotton plots. Large funnel traps were located 25 m apart, hung about 10 cm above the plant canopy. The trap was made of a protective wooden roof fixed 5-7 cm above a plastic funnel of 20 cm diameter to which a 2 litre plastic container was attached. The baits were hung from the centre of the roof. The catches were recorded every 3-5 days, and the baits were rotated by one position at that time (Dunkelblum *et al.*, 1981).

Electroantenogram (EAG) tests. The instrumentation and technique of biological amplification by connecting the antennae in series were as described previously (Moore, 1981), but modified through the use of a small gauge pheromone/moist air dispensing tube (Moore, 1984).

Four to six antennal preparations (replicates) per test were used. All the preparations consisted of four antennae of 7-10 day old males, each with the distal 2-3 annuli excised. The antennae were laid out tip to base on a stage with their ventral sides facing the pheromone stream, in a way to form a 3-cm-wide letter M. symmetric to the dispensing tube's axis. The midpoint of the preparation was 4 cm from the tube's aperture.

The stage was a rectangle of polypropylene covered with graph paper, itself overlaid with unstretched parafilm. This arrangement made it possible to maintain the above geometry as constant as possible.

Pasteur pipettes loaded with the required amounts of the chemicals (serial dilutions were prepared from stock solutions) absorbed into 5 x 0.2 cm paper strips (Whatman No. 1), were prepared and stored as described previously (Moore, 1981). The chemicals tested were: dodecyl acetate (12:Ac), (*Z*)-7-dodecenyl acetate (*Z*7-12:Ac), (*Z*)-9-dodecenyl acetate (*Z*9-12:Ac), 11-dodecenyl acetate (11-12:Ac), (*Z*)-8-tridecenyl acetate (*Z*8-13:Ac), tetradecyl acetate (14:Ac), (*Z*)-9-tetradecenyl acetate (*Z*9-14:Ac), hexadecyl acetate (16:Ac), (*Z*)-7-dodecenyl alcohol (*Z*7-12:OH), (*Z*)-9-dodecenyl alcohol (*Z*9-12:OH) and (*Z*)-9-tetradecenyl alcohol (*Z*9-14:OH).

A constant 1 litre/min air stream and a 2-3 min interval between stimulations were used in the dose response tests. In these tests each preparation was stimulated according to an increasing dose gradient.

RESULTS AND DISCUSSION

Field observations and trapping tests

A field programme to evaluate all components of the sex pheromone of *C. chalcites* was begun in 1984. The following compounds were included in the programme: 12:Ac, *Z*7-12:Ac, *Z*9-12:Ac, 11-12:Ac, *Z*8-13:Ac, *Z*9-14:Ac and *Z*7-12:OH. As observed in previous field tests (Dunkelblum *et al.*, 1981), the major sex pheromone component, *Z*7-12:Ac, did not catch males. The addition of *Z*9-14:Ac, which is the second most abundant component, in a 5:1 ratio (blend C1) resulted in high catches. In addition to the trapping tests, night observations were conducted on the behaviour of males approaching and landing on baits containing these two compounds. The observations were performed at the beginning of night, when males flew most actively (Snir *et al.*, 1986). The mixture of *Z*7-12:Ac + *Z*9-14:Ac (C1) and the two separate components were tested in different arrangements (Table 1). Whereas *Z*7-12:Ac induced some long range attraction, *Z*9-14:Ac had no effect on flying males. Mixing the two components induced numerous males to fly upwind and to land on the bait (Test 1, Table 1). Some of the males were observed performing copulation attempts with the bait. However, it was difficult to quantify this behavioural stage at the night conditions. Loading the two components into separate septa parallel to the wind direction did not affect the number of males landing on the baits (Test 2, Table 1). On the other hand, separating the two components perpendicularly to the wind direction showed that only the combined plume of *Z*7-12:Ac + *Z*9-14:Ac induced approaching males to land. As the distance between the two compounds increased, the number of landing males decreased (Test 3, Table 1). These results confirm preliminary flight experiments in a wind tunnel, which indicate that *Z*7-12:Ac induces directed flight but not landing; addition of *Z*9-14:Ac, or some of the other pheromone components, induced males to perform the whole courtship sequence (Dunkelblum *et al.*, 1987).

TABLE 1. FIELD OBSERVATIONS ON THE RESPONSE OF *CHRYSODEIXIS CHALCITES* MALES TO BAITS CONTAINING 1 mg OF *Z*7-12:Ac AND 0.2 mg OF *Z*-9-14:Ac[1]

Test	Bait[2] Z7-12:Ac Z9-14:Ac Arrangement	Distance between baits[2]	Position of baits[2]	No. of males approaching bait	No. of males landing on bait[3]
1	+	One bait	One bait	17a	0b
	+			1b	0b
	Mixed			Not counted	264a
2	Close	1 cm	Parallel to wind direction	Not counted	83a
	Front Rear	50 cm		"	85a
	Rear Front	50 cm		"	79a
3	Close	1 cm	Perpendiclar to wind dir.	104a	95a
	+ +	5 cm		90a	50ab
	+ +	30 cm		93a	13b

[1]Numbers followed by same letter do not differ at 5% level of significance (ANOVA and Duncan's Multiple Range Test).

[2]Mixed bait means that both compounds were loaded onto the same septum. Close baits refers to two separate septa, each loaded with one compound, hanging together on one pole. For details, see Materials and Methods.

[3]When the bait consisted of two separated septa, the number of landing males is the sum of moths which landed on the two septa.

TABLE 2. COMPOSITION OF THE BLENDS TESTED IN THE FIELD FOR TRAPPING OF *CHRYSODEIXIS CHALCITES* MALES; BAIT LOADINGS ARE GIVEN IN μg.

Blend	Z7-12:Ac	Z9-12:Ac	11-12:Ac	12:Ac	Z8-13:Ac	Z9-14:Ac	Z7-12:OH
C 1	1000					200	
C 2	1000	50					
C 3	1000	50				200	
C 4	1000	200					
C 5	1000		200				
C 6	1000	200				200	
C 7					1000	200	
C 8	1000				200		
C 9	1000			200			
C 10	1000	100	100	100		100	
C 11	1000	100				100	
C 12	1000	100	100				
C 13	1000		100			100	
C 14	1000	20	35	40	15	100	
C 15	1000	20	35	40	15	100	15

Fifteen blends (Table 2) were tested in the field and the results from the field tests are summarized in Table 3. The first test in the present study compared binary and ternary blends containing *Z*7-12:Ac, *Z*9-12:Ac and *Z*9-14:Ac in different ratios (C 1, C 2, C 3, C 4 and C 6) (Tests 1 & 2, Table 3). It was found that both binary blends *Z*7-12:Ac + *Z*9-12:Ac and *Z*7-12:Ac + *Z*9-14:Ac gave the same trap catches. The ternary blends C 3 and C 6 caught generally more males than any of the binary blends C 1, C 2 and C 4, but the differences were statistically nonsignificant. These results indicate that *Z*9-12:Ac and *Z*9-14:Ac can be used interchangeably without affecting the efficiency of the baits (C 1 versus C 2 or versus C 4). A similar phenomenon was observed in field tests in New Zealand with the closely related species *Chrysodeixis eriosoma* (Benn *et al.*, 1982).

TABLE 3. FIELD TRAPPING OF *CHRYSODEIXIS CHALCITES*

Test	*Bait*	*Start of test*	*No. of traps*	*No. of nights*	*Total No. of males*[1]
1.	C 1 C 2 C 3	August 17, 1984	5 5 5	21 21 21	470a 459a 608a
2.	C 1 C 4 C 5 C 6	July 7, 1985	4 4 4 4	46 46 46 46	231a 276a 24b 333a
3.	C 1 C 7 C 8 C 9	August 10, 1986	4 4 4 4	8 8 8 8	437a 0b 1b 0b
4.	C 10 C 11 C 12 C 13	August 27, 1986	4 4 4 4	18 18 18 18	174a 135ab 117ab 85b
5.	C 6 C 14 C 15	August 18, 1986	5 5 5	8 8 8	307a 247a 87b

[1]Numbers followed by same letter do not differ at 5% level of significance (ANOVA and Duncan's Multiple Range Test).

All other binary blends of *Z*7-12:Ac with 11-12:Ac, *Z*8-13:Ac and 12:Ac (C 5, C 8 and C 9) were tried in Tests 2 and 3 (Table 3). The results showed that to trap *C. chalcites* males, none of these acetates is useful as a synergist for *Z*7-12:Ac. The odd compound in these tests is *Z*8-13:Ac, which was found in the pheromone gland volatiles (Dunkelblum *et al.*, 1987) but is very rare as a pheromone component (Nesbitt *et al.*, 1986). This compound might arise either through a shortening by one carbon or the lenghtening by one carbon of a *Z*9-14 precursor or a *Z*7-12 precursor.

Replacing Z7-12:Ac (C 7) or Z9-14:Ac (C 8) in the binary blend C 1 by Z8-13:Ac resulted in complete loss of attractiveness (Test 3, Table 3).

Tests with ternary blends of Z7-12:Ac with Z9-12:Ac, 11-12:Ac and Z9-14:Ac (C 11, C 12 and C 13) (Test 4, Table 3) indicated that Z9-14:Ac and 11-12:Ac can be interchanged without affecting the performance of the baits, whereas substitution of Z9-12:Ac for 11-12:Ac resulted in diminished catches. The use of a five component blend (C 10), which produced the highest response in the wind tunnel (Dunkelblum *et al.*, 1987), and a six component blend (C 14) of all acetates, identified in the gland volatiles (Dunkelblum *et al.*, 1987), did not result in higher trap catches than those obtained with the ternary blends C 6 and C 11 (Tests 4 & 5, Table 3). The ratio of the components in blends C 14 and C 15 is approximately as found in the pheromone gland volatiles.

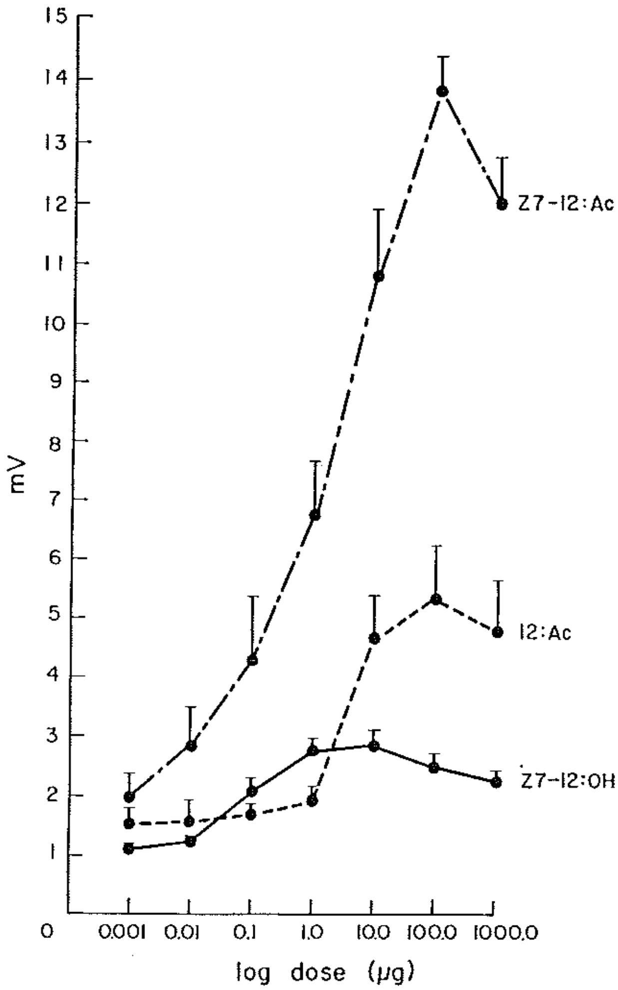

Fig. 1. Representative EAG response curves of *Chrysodeixis chalcites* with two acetates and one alcohol detected in the pheromone gland volatiles of the female. Means ± SE (vertical bars); for graphic clarity only half bars are presented.

The alcohol Z7-12:OH (1.5% relative to Z7-12:Ac), which was found in both gland extracts and volatiles, was incorporated into blend C 15. A significant reduction in trap catch was observed (Test 5, Table 3) as compared with C 14 and C 6. This result confirmed previous field tests which showed that Z7-12:OH reduces the catch of *C. chalcites* males (Dunkelblum *et al.*, 1982). This alcohol is a pheromone component in other Plusiinae species and apparently has an important role in the species separation within the Plusiinae subfamily (Dunkelblum and Gothilf, 1982).

EAG measurements

The EAG response dose curves of several acetates and alcohols were measured by the biological amplification technique (Moore, 1981). These compounds included all components identified in the pheromone gland volatiles of *C. chalcites* (Dunkelblum *et al.*, 1987). Three representative curves are illustrated in Figure 1. The maximum response of the major component Z7-12:Ac, comprising 84% of the volatiles (Dunkelblum *et al.*, 1987), was 13.8 mV at a loading of 100 μg, as compared with only 4.1-8.3 mV of the other acetates (Fig. 2). The EAG response of 14:Ac and 16:Ac, which were found in the gland extracts but not in the volatiles (Dunkelblum *et al.*, 1987), were very low, 1-2 mV (Fig. 2).

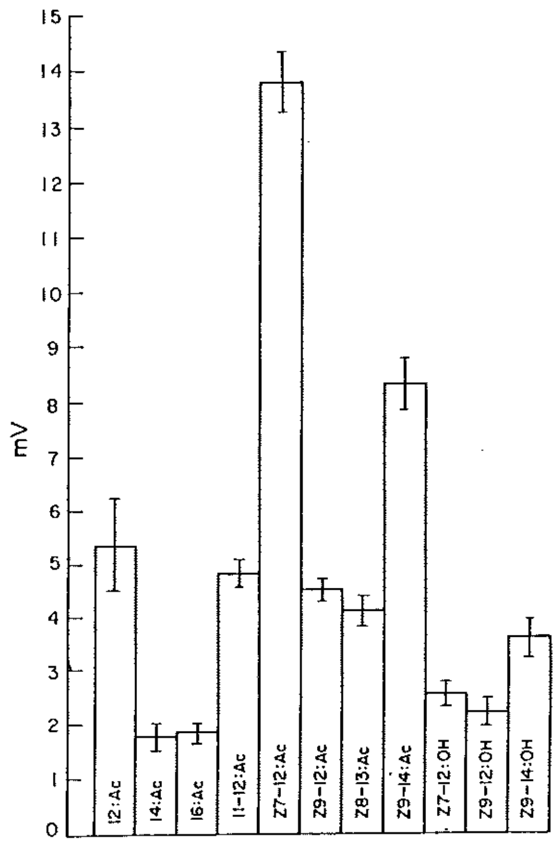

Fig. 2. Relative EAG responses, at a 100 μg loading, to *Chrysodeixis chalcites* pheromone components and related compounds. Means ±SE (vertical bars).

The dose response curve of Z7-12:OH was different from the curves of the acetates (Fig. 1): it ws flat and exhibited a maximum of 2.8 mV only. For comparison, we measured the EAG responses of Z9-12:OH and Z9-14:OH; again flat curves with low maxima, as for Z7-12:OH, were obtained. The Z7-12:OH alcohol, although detected in the volatiles, is probably a biological artifact (Dunkelblum *et al.*, 1987). It was found previously (Dunkelblum *et al.*, 1982) that Z7-12:OH is an inhibitor for trap catches of *C. chalcites* males, as in the present study. The same phenomenon was observed for *Trichoplusia ni* (Tumlinson *et al.*, 1972; Bjostad *et al.*, 1984). However, in the case of *T. ni*, similar EAG amplitudes were obtained from the major pheromone component, Z7-12:Ac, and the inhibitor Z7-12:OH (Mayer, 1973).

According to Benn *et al.* (1982), the antennae of male *C. chalcites* (French specimen), as well as those of the closely related species *C. eriosoma*, have five types of receptor cells responding to Z5-12:Ac, Z7-12:Ac, Z9-12:Ac, Z9-14:Ac and Z7-12:OH. The present EAG measurements suggest that some of the chemoreceptors of the local *C. chalcites* respond to more than one compound.

CONCLUSIONS

The main purpose of this study was to obtain the best bait for trapping of *C. chalcites*, superior to the two component blend used before (Dunkelblum *et al.*, 1981). The present field tests showed that several blends, containing 2-6 components, gave statistically similar trap catches. However, two components, Z7-12:Ac with either Z9-12:Ac or Z9-14:Ac, were essential in any efficient bait. The ternary mixtures of Z7-12:Ac + Z9-12:Ac + Z9-14:Ac gave generally higher trap catches than any two component mixtures and are therefore recommended for trapping of *C. chalcites*. The five and six component blends had the same attractancy as this mixture.

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