Journal of Chemical Ecology, Vol. 11, No. 3, 1985

MALE OLIVE FRUIT FLY ATTRACTION TO SYNTHETIC SEX PHEROMONE COMPONENTS IN LABORATORY AND FIELD TESTS

B.E. MAZOMENOS and G.E. HANIOTAKIS

Biology Department
N.R.C. "Democritos" Aghia Paraskevi
Attikis, Greece

(Received March 22, 1984, accepted July 23, 1984)

Abstract—Male olive fruit fly attraction to the four synthetic components of the female sex attractant pheromone was studied under laboratory and field conditions. In laboratory tests males responded to all four components tested separately. Component I, (1,7-dioxaspiro[5.5]undecane) was more attractive than any of the remaining three components alone, but a combination of all four was more attractive than component I alone. In field tests with polyethylene vials as pheromone dispensers, the complete mixture, although not statistically significant, was constantly more attractive to males than component I alone. A tendency of enhancement of attraction of component I by combining it with component II (α-pinene) or III (*n*-nonanal) was also observed. In field tests with rubber septa as pheromone dispensers only component I was attractive. Mixtures containing component I were also attractive but not more attractive than component I alone. Evaporation rate and ratio of components as they come out of the dispenser appear to be critical for male response.

Key Words—*Dacus oleae*, olive fruit fly, Diptera, Tephritidae, pheromones, sex attractants, multicomponent pheromones, field tests of pheromones.

INTRODUCTION

The sex attractant pheromone produced by female olive fruit flies, *Dacus oleae* Gmelin, was found to be a mixture of four components (Mazomenos and Haniotakis, 1981). Two of these components were isolated from the female rectal glands and the other two from female volatiles trapped by a total condensation cold trap operating with liquid nitrogen (Haniotakis et al., 1977).

Baker et al. (1980) identified the major component as 1,7-dioxaspiro[5.5]undecane (I). Mazomenos et al. (1981) identified the same component

0098-0331/85/0300-0397$04.50/0

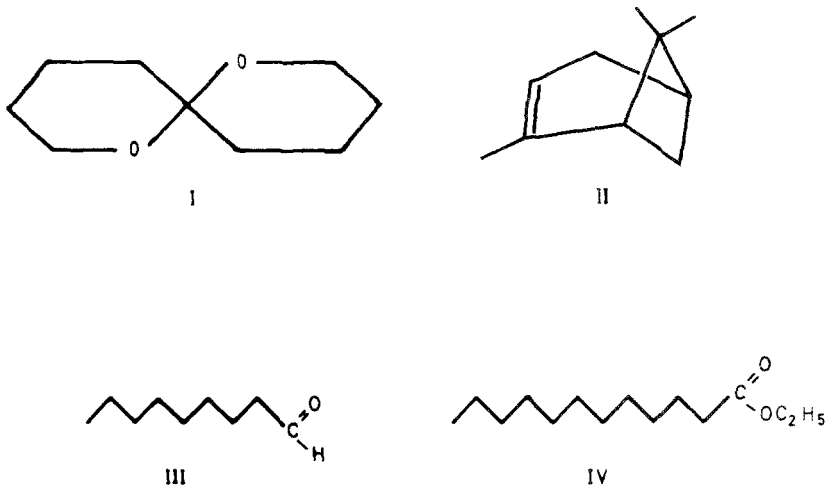

FIG. 1. Chemical structures of the sex pheromone components of female olive fruit fly, *Dacus oleae* Gmel.

plus the three secondary ones as α-pinene (II), *n*-nonanal (III), and ethyl-dodecanoate (IV). The chemical structures of all four components are shown in Fig. 1.

Laboratory and field cage bioassays showed that all components attract males. Component I is more attractive than the other three. The complete mixture is more attractive than component I (Mazomenos and Haniotakis, 1981).

Rossi et al. (1978) and Gariboldi et al. (1982) reported that the components *p*-cymene and (*Z*)- and (*E*)-6-nonen-1-ol, which were isolated from *D. oleae*, showed biological activity. However, our laboratory bioassays with these components revealed no such activity. Jones et al. (1983) tested these same components in the field and observed no fly activity. The same authors tested (*E*)- and (*Z*)-6-nonen-1-ol in combination with component I and found that (*E*)-6-nonen-1-ol depressed male catch while (*Z*)-6-nonen-1-ol had no significant effects.

In the present study we tested the synthetic pheromone components individually and in combinations in laboratory and field tests and compared them with concentrated ether extract of virgin female flies.

METHODS AND MATERIALS

Insects Used. The insects used were obtained from a colony maintained at the Entomology Laboratory, N.R.C. "Democritos," Athens, Greece, on artificial diet for about 15 generations (Tsitsipis, 1977). Flies 24 hr postemergence

were separated according to sex and maintained in screen cages 270 cm^3 in artificial light (3000 lux intensity) and a 12 : 12 hr light–dark regime. Temperature was 25 ± 2°C and relative humidity 65 ± 5%.

Pheromone Collection. Pheromone was collected from 4- to-6-day-old virgin females by extracting the whole female body in ether for 24 hr. The extract was concentrated and stored at − 15°C until use (Mazomenos and Haniotakis, 1981).

Synthetic Chemicals. Component I, 1,7-dioxaspiro[5.5]undecane, was supplied by Vioryl Chemical Co. (Terma Kato Kifissia, Attikis, Greece) and was 99% pure. α-Pinene, *n*-nonanal, and ethyl-dodecanoate, components II, III, and IV, respectively, were purchased from Fluka AG, Chemical Co., Buchs SG, and were 95–98% pure. The chemicals were used without further purification.

Laboratory Bioassays. Bioassays were conducted in a screen cage as described by Mazomenos and Haniotakis (1981). The components to be tested were dissolved in hexane at concentrations of 6, 2, 0.6, and 2 μg/100 μl for I, II, III, and IV, respectively. These concentrations were found in preliminary tests to give optimum male response. Combinations of components were prepared similarly. Aliquots of 100 μl were poured on 7.5 cm^2 sections of Whatman No. 1 filter paper for each test. The solvent was allowed to evaporate before the paper was introduced into the insect cage. The number of males visiting the paper was recorded for 10 min. Each experiment was repeated four times on four different days. Filter paper with the same volume of hexane was used as control. Bioassays were conducted, one per day, during the last 2 hr of the photophase, the period with the highest mating activity (Zervas, 1982).

Field Experiments. During 1980, comparative male attraction studies were conducted between the complete synthetic pheromone mixture and the natural pheromone crude extract, between the major pheromone component and its various combinations with the secondary components, as well as between some combinations of secondary components alone. Yellow sticky posterboard rectangles, 15 × 20 cm, were used as traps and 1 ml polyethylene vials as pheromone dispensers (Mazomenos et al., 1983). Synthetic chemicals or their mixtures were dissolved in hexane. Concentrations of the major component tested ranged from 1 to 15 mg. When secondary components were added, the ratio of 3 : 1 : 0.3 : 1 was observed for components I, II, III, and IV, respectively. This is the ratio of components found in natural pheromone mixture (Mazomenos and Haniotakis, 1981).

The experimental design was either the Latin square, with traps placed in every other tree at distances of about 20 m (experiment 1 of Table 1), or the randomized block (experiment 2 of Table 1 and Figure 2 at Amarousion), or completely randomized with trap distances of about 100 m (experiment at Spata). Traps were inspected and cleaned once per week. Pheromone dispensers were not replaced throughout the experiments because of their long residual activity

TABLE 1. NUMBER OF MALE OLIVE FRUIT FLIES RESPONDING TO SYNTHETIC SEX PHEROMONE COMPONENTS AND THEIR BLEND IN LAB BIOASSAYS[a]

Attractant	Concentration (μg)	Male response[b]
I	6	27.5c
II	2	10.5b
III	0.6	11.3b
IV	2	7.8b
I + II + III + IV	6 + 2 + 0.6 + 2	37.3d
Blank		3.3a

[a]Means of four replicates.
[b]Means followed by same letter are not significantly different, Duncan's multiple-range test, $P = 0.05$.

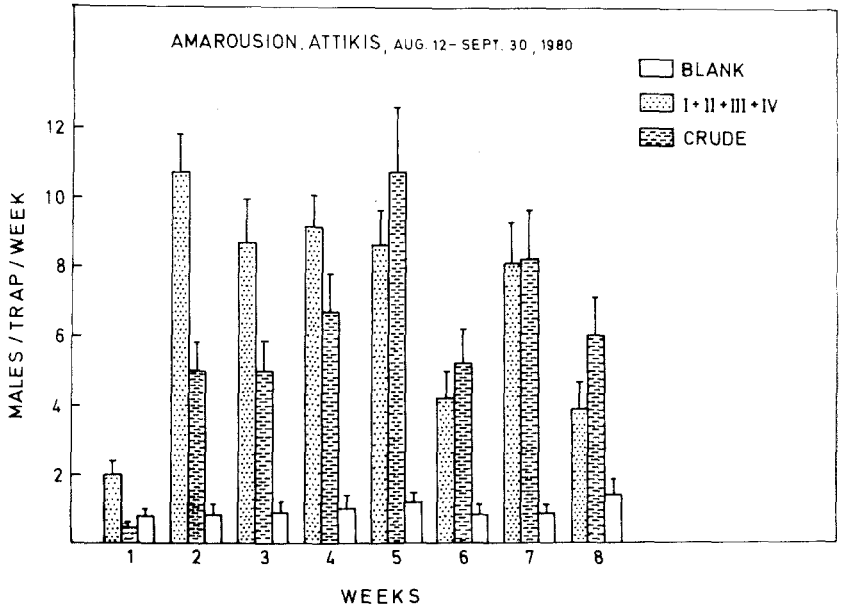

FIG. 2. Number of male olive fruit flies per trap per week captured by yellow sticky traps baited with polyethylene vials containing 400 FE of female whole body crude extract or 1 mg of the major component plus the appropriate concentrations of the other components of the blend. Means of 10 traps/treatment. Vertical lines above bars indicate standard deviations.

(Mazomenos et al., 1983). Tests relied on natural insect populations except during the period with low population densities and no wild male response to pheromones, between mid-May and the end of July (Haniotakis et al., 1982), when releases of artificially reared insects were made at weekly intervals.

During 1981, comparative male attraction studies were conducted between all four synthetic pheromone components individually and in all possible combinations with component (I). In these tests rubber septa were used as dispensers charged with 5 mg for individual component tests. For combinations, the above-mentioned ratio of components found in natural mixtures was observed. The same type of traps were used at distances of about 20 m. Traps were inspected, cleaned, rebaited, and rotated daily. Field experiment data were transformed to $\log(x + 1)$ prior to statistical analysis. Means comparisons were made with Duncan's multiple-range test.

RESULTS AND DISCUSSION

Laboratory Tests. Male attraction to synthetic sex pheromone components and the complete mixture is shown in Table 1. Component I showed the major attraction, while attraction of the other three components was at lower levels. The number of males attracted to the complete pheromone mixture was higher than that to component I. Actually component I attracted 73.2% of the males attracted by the mixture. The above results coincide with those obtained from similar tests with the natural pheromone components (Mazomenos and Haniotakis, 1981), except in the case of component IV in which the natural product appears to be more active than the synthetic one.

Field Tests. Male olive fly attraction to various combinations and different concentrations of synthetic pheromone components and crude virgin female whole body ether extract at different periods of the year is shown in Table 2. In experiment 1, although no significant differences were found between treatments, the results were similar to the case of laboratory bioassays, i.e., combination of the major component I with the secondary components was more attractive than I alone. In addition, synthetic component I and its mixtures were more attractive than crude female extract in the concentration used.

In experiment 2, similar results were found, i.e., combination of component I with the other components, except IV, is more attractive than I alone. In addition, it can be seen that various combinations of the secondary components in the absence of I were not attractive.

Comparative male attraction studies between crude female extracts at a concentration of 400 female equivalents (FE), which contain approx. 1, 0.3, 0.1, 0.3 mg of components I, II, III, and IV, respectively, and a complete mixture of synthetic components at the above concentrations were carried out. Figure 2

TABLE 2. NUMBER OF MALE OLIVE FRUIT FLIES CAPTURED BY YELLOW POSTERBOARD STICKY TRAPS BAITED WITH POLYETHYLENE VIALS CONTAINING VIRGIN FEMALE CRUDE EXTRACT OR DIFFERENT COMBINATIONS OF SYNTHETIC PHEROMONE COMPONENTS; AMAROUSION, ATTIKIS, GREECE[a]

Attractant	Concentration (mg)	Total males captured	Mean/trap/week[b]
Experiment 1			
Crude	400 FE	207	4.6
I	1	353	7.8
I + IV	1 + 0.33	326	7.2
I + II + III	1 + 0.33 + 0.1	375	8.4
I + II + III + IV	1 + 0.33 + 0.1 + 0.33	395	8.8
Experiment 2			
I	15	1903	90.6bc
I + II	15 + 5	2510	119.6c
I + III	15 + 1.5	2046	97.6bc
I + IV	15 + 5	1727	82.3b
II + IV	5 + 5	256	11.7a
III + IV	5 + 5	280	13.3a
Blank		528	25.1a

[a]Means of five traps/treatment (exper. 1, May 5–July 15, 1980) and three traps/treatment (exper. 2, Sept. 2–Nov. 25, 1980).
[b]Means followed by the same letter within the same experiment are not significantly different. Duncan's multiple-range test, $P = 0.05$.

shows the results. Synthetic pheromone mixture was more attractive than crude extract during the first four weeks of the experiment while during the remaining four weeks male catches were in favor of the crude extract. It seems that the evaporation rates of the two kinds of attractants were different.

In another field test with polyethylene vials as dispensers and distances between traps over 100 m the synthetic major pheromone component I at a concentration of 15 mg was compared to the complete synthetic mixture. The concentration of component I in the mixture was also 15 mg. The results are shown in Figure 3. Although not statistically different, the complete mixture attracted more males than component I alone during all 12 weeks of the experiment except of one (week 9). It should be noted that environmental temperatures dropped after the 8th week. This may have had a different effect on the evaporation rate of the different components of the mixture which resulted in a change of the existing ratio. A change of the original ratio due to different evaporation rates of the components should also be taken into consideration.

In one additional field experiment, comparative male attraction studies were conducted between all four components individually and all possible combinations of component I with the other three. In this experiment rubber septa were

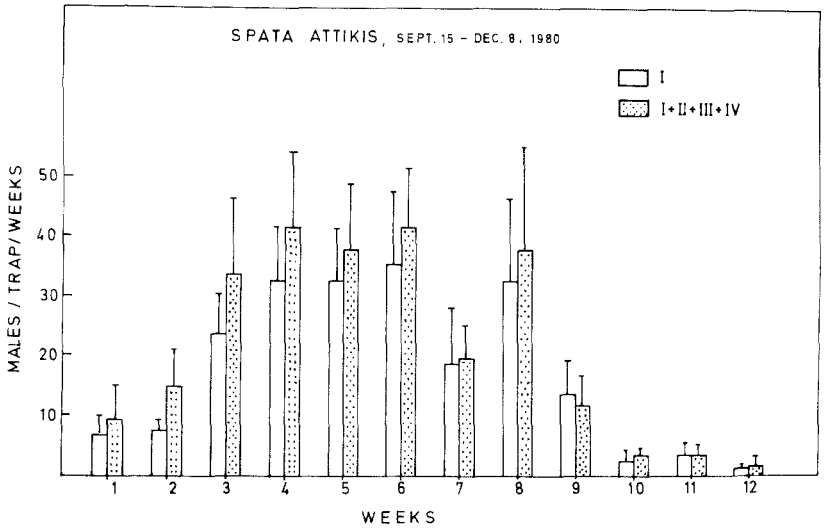

FIG. 3. Number of male olive fruit flies per trap per week captured by yellow sticky traps baited with polyethylene vials containing 15 mg of the major pheromone component or 15 mg of the major pheromone component plus the appropriate concentrations of the other components of the blend. Means of 10 traps/treatment. Vertical lines above bars indicate standard deviations.

used as dispensers. The results are shown in Table 3. As in laboratory bioassays, component I attracted also the highest number of males. In this field experiment, however, components II, III, and IV showed no significant attraction when tested individually. Furthermore, combinations of component I with one or more of the secondary components did not increase male attraction as was the case both in laboratory bioassays and in field experiments with polyethylene dispensers. It seems that the type of dispenser, i.e., the evaporation rate of individual components and subsequently their resulting ratio as they come out of the dispenser, has a decisive effect on male attraction. If this is the case, it is possible that ratio of the pheromone components as they come out of the polyethylene dispenser is suboptimal and that further increase of the biological activity of the complete pheromone mixture may be possible by appropriate formulation.

Electroantennogram (EAG) studies showed that all four pheromone components were detected by both sexes of laboratory-reared and wild insects (van der Pers et al., 1984). Components I and III elicited higher EAG response than components II and IV. These results coincide with our findings in laboratory bioassays (Table 1). Also of interest is the observation that components I and III are detected by independent sensory systems of the insect antennae.

In conclusion, even though component I and the complete mixture are attractive enough to males to be useful for practical applications, further studies

TABLE 3. NUMBER OF MALE OLIVE FRUIT FLIES CAPTURED BY YELLOW STICKY TRAPS BAITED WITH RUBBER SEPTA CONTAINING SYNTHETIC SEX PHEROMONE COMPONENTS SEPARATELY OR COMBINATIONS OF COMPONENT I WITH SECONDARY COMPONENTS[a]

Attractant	Concentration (mg)	Total males captured	Mean/trap/day[b]
I	5	641	23.7c
II	5	71	2.6a
III	5	121	4.5a
IV	5	45	1.7a
I + II	5 + 1.7	636	23.5c
I + III	5 + 0.5	546	20.2c
I + IV	5 + 1.7	513	19.0bc
I + II + III	5 + 1.7 + 0.5	587	21.7c
I + II + IV	5 + 1.7 + 1.7	331	12.3b
I + III + IV	5 + 0.5 + 1.7	487	18.0bc
I + II + III + IV	5 + 1.7 + 0.5 + 1.7	568	21.0c
Blank		31	1.2a

[a] Means of three traps/treatment × 9 repetitions, between Aug. 24 and Sept. 4, 1981; Amarousion, Attikis, Greece.

[b] Means followed by the same letter are not significantly different. Duncan's multiple-range test, $P = 0.05$.

are needed to understand the mode of action and function of this pheromone system and to increase the activity of the synthetic pheromone and consequently its potential for practical use.

Acknowledgments—This work was partially supported by the Greek Dept. of Agriculture. The authors would like to thank Mr. A. Ioannou and N. Liopas for technical assistance, Mrs. Filipopoulou for making the drawings, and A. Kanousi for typing the manuscript.

REFERENCES

BAKER, R., HERBERT, R., HOWSE, P.E., JONES, O.T., FRANCKE, W., and REITH, W. 1980. Identification and synthesis of the major sex pheromone of the olive fly (*Dacus oleaé*). *J. Chem. Soc. Chem. Commun.* 1:52–53.

GARIBOLDI, P., JOMMI, G., ROSSI, R., and VITA, G. 1982. Studies on the chemical constitution and sex pheromone activity of volatile substances emitted by *Dacus oleae*. *Experientia* 38:441–444.

HANIOTAKIS, G.E., MAZOMENOS, B.E., and TUMLINSON, J.H. 1977. A sex attractant of the olive fruit fly *Dacus oleae* and its biological activity under laboratory and field conditions. *Entomol. Exp. Appl.* 21:81–87.

HANIOTAKIS, G.E., MAZOMENOS, B.E., and HARDAKIS, I.M. 1982. Monitoring and control of the olive fruit fly with pheromone traps. Proc. Meeting E.E.C. on L'état d'avancement des travaux

et echanges d'informations sur les problèmes posés par la lutte integrée en oleiculture, Antibes, France. Nov. 4–6, 1981, pp. 46–60.

JONES, O.T., LISK, J.C., LONGHURST, C., HOWSE, P.E., RAMOS, P., and CAMPOS, M. 1983. Development of a monitoring trap for the olive fly, *Dacus oleae* (Gmelin) (Diptera: Tephritidae), using a component of its pheromone as lure. *Bull. Entomol. Res.* 73:97–106.

MAZOMENOS, B.E., and HANIOTAKIS, G.E. 1981. A multicomponent sex pheromone of *Dacus oleae* Gmel. Isolation and bioassay. *J. Chem. Ecol.* 7:437–443.

MAZOMENOS, B.E., HANIOTAKIS, G.E., TUMLINSON, J.H., and RAGOUSIS, N. 1981. Isolation, synthesis and bioassays of the olive fruit fly sex pheromone. 1st Panhellenic Congress of Agricultural Research. Kalithea, Halkidiki. May 5–8, 1981. Abstract.

MAZOMENOS, B.E., HANIOTAKIS, G.E., IOANNOU, A., SPANAKIS, I., and KOZIRAKIS, A. 1983. Field evaluation of olive fruit fly pheromone traps with various dispensers and concentrations, pp. 171–177, *in* R. Cavalloro (ed.). Fruit Flies of Economic Importance. Proc. CEC/IOBC Inter. Symp. Athens, November 1982.

ROSSI, R., CARPITA, A., and VITA, G. 1978. (*Z*)-6-Nonen-1-ol and related compounds as attractants of the olive fruit fly, *Dacus oleae* (Gmelin) (Diptera: Trypetidae). *Gazz. Chim. Ital.* 108:709–712.

TSITSIPIS, J.A. 1977. An improved method for mass rearing of the olive fruit fly, *Dacus oleae* (Gmel.) (Diptera: Tephritidae). *Z. Angew. Entomol.* 4:419–426.

VAN DER PERS, J.N.C., HANIOTAKIS, G.E., and KING, B.M. 1984. Electroantennogram responses from olfactory system in *Dacus oleae*. *Entomol. Hellenica.* In press.

ZERVAS, G.A. 1982. Reproductive physiology of *Dacus oleae* (Gmelin) (Diptera: Tephritidae). *Geoponika* 282:10–14. (in Greek with English summary).