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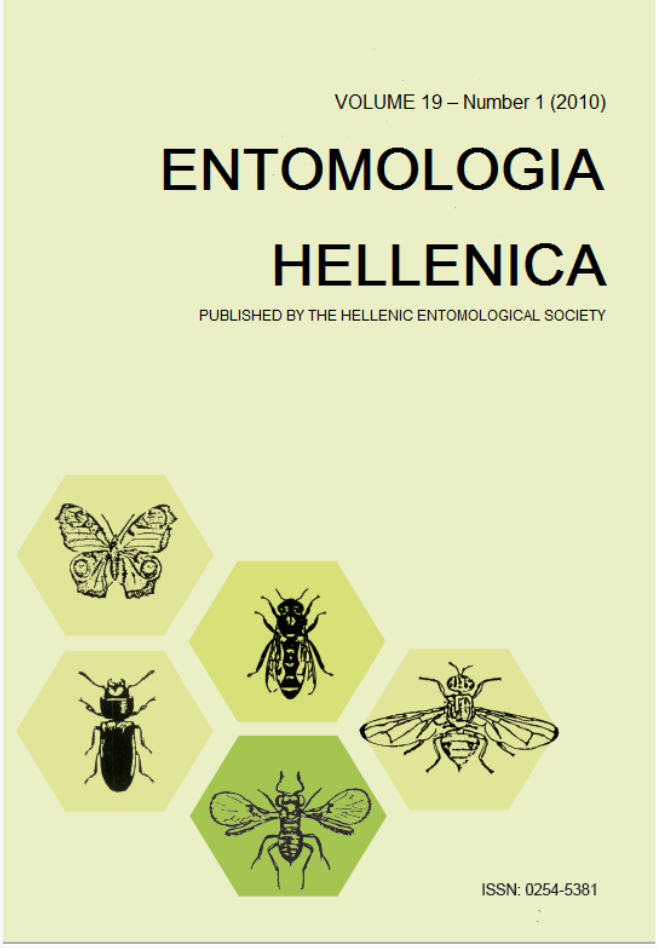


# Extract of olive fruit fly males (Diptera: Tephritidae) attract virgin females

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# Extract of olive fruit fly males (Diptera: Tephritidae) attract virgin females

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## ABSTRACT

Research conducted during the past three decades suggests that in contrast to most other tephritid fruit flies, in which sexual pheromones are produced by males, the olive fruit fly *Bactrocera oleae* (Rossi) relies for its sexual communication on a pheromone that is produced by females. However, our present study suggests that virgin, mature females are attracted to male odors. In olfactometer assays extracts of male bodies obtained with a two-solvent system of methanol and dichloromethane were highly attractive to virgin females. This was observed during the last two hours of the photophase, when males are sexually active, but not during the first hours of the photophase, or when mated females were tested. Extracts of male bodies obtained with diethyl ether were also attractive to virgin females, albeit not as strongly as the two-solvent extracts. These results strongly indicate that males of the olive fruit fly elicit attraction to virgin females based on olfactory stimuli. The importance of these findings for understanding the sexual behavior of the olive fruit fly is discussed.

KEYWORDS: *Bactrocera oleae,* bioassays, male pheromone.

## Introduction

The olive fruit fly, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is the most important pest of cultivated olive, *Olea europaea* L., in the Mediterranean and Middle East regions (Tzanakakis 2006), and during the last few years also in California (Rice et al. 2003, Gutierrez et al. 2009). Although most tephritid species rely on male pheromones for sexual communication (Wicker-Thomas 2007), it is unclear if this is also true for the olive fruit fly. Economopoulos et al. (1971, 1975) observed that in places where large numbers of laboratory cultured males or females were released there was a characteristic odor in response to which wild males and females tended to aggregate. This mainly happened during the evening hours, when mating occurs (Haniotakis 1974), which suggests that the odors emitted by either of the sexes are possible related to sexual behavior.

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Some further evidence showing that male olive fruit flies may employ a sexual pheromone that attracts virgin females come from a study by De Marzo et al. (1978) who run olfactometer trials to investigate whether living males of the olive fruit fly, or rectal glands from males, attract virgin females. Though some response was observed the work was not received as a persuasive demonstration of this phenomenon by the scientific community. For example, as acknowledged by the authors, the experimental setup did not allow a clear separation between chemical, visual and auditory stimuli. Furthermore, females were influenced in their response by natural light entering the experimental room from a window.

Other works have shown that a four-component pheromone identified from the female olive fruit fly acts as a strong male attractant (see references in Mazomenos 1989, Haniotakis 2000). Though the major component of this pheromone is also produced by males, apparently from secretory cells of the posterior rectum in adult males, it does not seem to attract females (Haniotakis et al. 1986). In other species of the genus *Bactrocera* cells of this type produce the male sex pheromone(s) that elicits strong attraction to females searching for mates (Fletcher 1969, Schultz and Boush 1971). Given that none of all the above studies has proved the existence of a male sex pheromone, the established notion among fruit fly workers is that males of the olive fruit fly do not employ a sexual pheromone to attract females (Haniotakis 1974, 1977, 2000, Haniotakis et al. 1977, 1986, Baker et al. 1980, Mazomenos and Haniotakis 1981, 1985), and are therefore, different than other tephritid species that rely on male pheromones for sexual communication (Wicker-Thomas 2007).

So far only one study attempted to prove the existence of such a pheromone by implementing an extraction technique (Mazomenos and Pomonis 1983). In this study diethyl ether extracts from the rectal glands of sexually active males were tested in laboratory bioassays for their attraction to virgin females, but the response observed was rather negligible. The purpose of the present work was to follow up on the above study using whole body extracts of males instead of rectal glands. We used diethyl ether but also other solvents and methods of extraction not used by the above workers. Our aim was to demonstrate that a chemical component in the body of olive fruit fly males does in fact elicit high attraction by virgin females.

## Materials and Methods

Flies were retrieved from naturally infested olives collected in September 2008 in the area of East Attica (N 38° 09′, E 24° 00′), Greece. Before being used the flies were reared on green unripe olive fruits in the laboratory for 1-4 generations. Upon emergence adults were placed in Plexiglas cages (25 × 25 × 25 cm) provided with a mixture of yeast hydrolyzed (ICN Biomedicals Inc.) and sugar in a ratio of 1:3 respectively and water. When virgin females were required for an experiment the two sexes were kept separately. Rearing and the experiments were conducted under 25 ± 2°C, 65% RH, and a 12:12 h (light: dark) photoperiod. All the flies used, either for extraction (males) or to test their response (females) were 12-15 day of age and therefore sexually mature.

The extractions were performed during the last two hours of the photophase. Batches of ca. 400 intact males were extracted three times with 20 ml of diethyl ether and were left for 24 hours in 20 ml of two-solvent system consisting of methanol and dichloromethane (1:9) and then extracted another two times with 20 ml by the same solvent system. The diethyl ether and two-solvent extracts from five such batches (i.e.,

from approximately 2,000 males) were separately combined and concentrated by evaporation to 4,000 μl using a slow stream of He gas.

The bioassay used was a modified version of that described in Mazomenos and Haniotakis (1981). Seventy-two hours before a test 100 virgin or mated females were introduced into a plexiglas test cage (100 × 50 × 50 cm) with wire screen on the two opposite small sides and on the top. The cage was situated in a large (6 × 3 m), well-ventilated room with uniform light conditions (*ca.* 3,000 lux). The bioassays were conducted during last two hours of the photophase, when most matings occur (Causse et al. 1966), or during the first two hours of the photophase. Two hundreds μl of extract (or 200 μl of the respective solvent in the case of the control), corresponding to 100 male equivalents, were pipetted onto a 25 $cm^2$ of Whatman no. 1 filter paper and left to evaporate in a nearby room. To start a bioassay a weak air stream current (about 0.3 m/s) generated by a fan (40 cm diameter, located two meters away from the experimental cage) was directed through the cage. The filter paper was suspended upstream from the top of the cage near the side towards the fan by an 8 cm wire and for a 5 min period the number of females visiting the paper was recorded. Following a bioassay the cage was cleaned before subsequent tests. Control bioassay was run using new insects. The position of both cage and fun was rearranged within experimental room before running a new test. Four to seven such replicates were run for each test. Bioassays were conducted one at a time testing the different extracts in random sequence.

**Statistical analysis**

The Mann – Whitney U test was used to compare the response of females to adult male extracts against control (solvents only). The response of the various categories of females to male extracts (excluding controls from the analyses) was analyzed using the non parametric ANOVA Kruskal-Wallis.

## Results

The results showed that the diethyl ether extract of males had a statistically significant, albeit weak effect in attracting virgin females (Table 1; Mann-Whitney U test, $P = 0.021$). On the other hand, the extract obtained with the two-solvent system of methanol and dichloromethane was highly attractive to virgin females when tested during the last two hours of the photophase. No significant response was observed by mated females tested during the last two hours of the photophase, or by virgin females tested during the first two hours of the photophase. The response of the various categories of females to male extracts (excluding controls from the analyses) differed significantly (Kruskal – Wallis, chi-square test = 14.9; $df = 3$; $P < 0.01$). Pairwise comparison among the four treatments revealed significant differences between the response of virgin females, during the last two hours of the photophase to the methanol – dichloromethane extract and the other three treatments (Mann – Whitney U test, $P < 0.01$), which did not differ among each other ($P > 0.05$). Similar results were obtained when only the response of females to methanol – dichloromethane extract was included in our analysis.

## Discussion

Previous observations and experiments have not unequivocally proved that a pheromone is present in male olive fruit flies (Economopoulos et al. 1971, De Marzo et al. 1978). The vast amount of research data

TABLE 1. Response of virgin or mated *Bactrocera oleae* females to whole-body extracts of males with diethyl ether or a two-solvent system of methanol and dichloromethane (1:9). In each treatment or control assay (a replicate) one hundred females were tested. Five to 8 replicates ran for each test.

| Female category | Period of day | Solvent | % females responding (±SE) | | Mann - Whitney U test | |
|---|---|---|---|---|---|---|
| | | | Treatment | Control | Z - value | *P** |
| Virgin | Last two hours of photophase | Diethyl ether | 3.26 ± 0.58 | 0.95 ± 0.29 | 2.3 | 0.021 |
| Virgin | Last two hours of photophase | Methanol & dichloromethane | 18.98 ± 1.14 | 0.71 ± 0.19 | 3.2 | 0.001 |
| Virgin | Morning hours | Methanol & dichloromethane | 1.57 ± 0.54 | 1.65 ± 0.39 | 0.0 | 1.000 |
| Mated | Last two hours of photophase | Methanol & dichloromethane | 3.18 ± 0.91 | 0.79 ± 0.28 | 0.9 | 0.344 |

*$P < 0.05$

indicates that females produce a male-attracting pheromone (Mazomenos 1989, Haniotakis 2000). Nevertheless, our above results strongly suggest that olive fruit fly males possess odor(s) that attracts females. Since this odor(s) was highly attractive to virgin females when tested during the last two hours of the photophase, when olive flies are known to be sexually active (Causse et al. 1966), it is probably a pheromone related to the sexual behavior of the species.

Our results indicate that besides the well documented female pheromone, this species employ a second mechanism of sexual communication that is similar to other members of the family Tephritidae. Our findings are not in accordance with laboratory bioassays by Mazomenos and Pomonis (1983) who found response of males to diethyl ether extracts of male-rectal glands but not any response of virgin females. Mazomenos and Pomonis used only anal glands for their extracts that may contain lower concentration of the respective pheromone than whole body extracts that were used in our study (Levinson et al. 1987, Mavraganis et al. 2008). Our findings contribute to a greater understanding of the mating system of the olive fly and may help construct improved trapping systems to better monitor and control the olive fruit fly population. However, the role of this pheromone in the sexual behavior of the olive fruit fly needs to be studied more profoundly. Extracts from male rectal glands are attractive to other males (Mazomenos and Pomonis 1983) and may therefore act as an aggregation signal for males foraging for food resources. These aggregations may be of importance in the sexual behavior of this fly. The role of female attraction to male odor should also be determined more profoundly.

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Wicker-Thomas, C. 2007. Pheromonal communication involved in courtship behavior in Diptera. J. Insect Physiol. 53: 1089-1100.

# Εκχυλίσματα ενηλίκων αρσενικών του δάκου της ελιάς (Diptera: Tephritidae) προσελκύουν παρθένα θηλυκά άτομα

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## ΠΕΡΙΛΗΨΗ

Τα αποτελέσματα ερευνών που διεξάγονται τις τελευταίες δεκαετίες δείχνουν ότι, σε αντίθεση με άλλα έντομα της οικογένειας Tephritidae, η σεξουαλική επικοινωνία του δάκου της ελιάς, *Bactrocera oleae* (Rossi), βασίζεται κυρίως στη φερομόνη που απελευθερώνεται από τα ενήλικα θηλυκά. Η παρούσα μελέτη ωστόσο δείχνει ότι και τα ενήλικα αρσενικά του δάκου ελκύουν παρθένα θηλυκά. Σε πειράματα εργαστηρίου, με ολφακτόμετρο, μελετήθηκε η ανταπόκριση θηλυκών του δάκου της ελιάς σε εκχυλίσματα αναπαραγωγικά ώριμων αρσενικών. Τα αποτελέσματα έδειξαν ότι εκχυλίσματα των αρσενικών με διχλωρομεθάνιο/μεθανόλη και λιγότερο με διαιθυλαιθέρα, ήταν ιδιαιτέρα ελκυστικά για τα παρθένα θηλυκά κατά τις τελευταίες ώρες της φωτοπεριόδου, οπότε αυτά είναι σεξουαλικά δραστήρια. Η ανταπόκριση των παρθένων θηλυκών στα παραπάνω εκχυλίσματα ήταν αμελητέα όταν αυτά ήταν συζευγμένα. Επιπλέον, παρθένα θηλυκά δεν ανταποκρίνονταν στα παραπάνω εκχυλίσματα τις πρώτες ώρες της φωτοπεριόδου. Τα αποτελέσματα αυτά δείχνουν ότι, εκτός από την ύπαρξη της σεξουαλικής φερομόνης των θηλυκών στο δάκο της ελιάς, υπάρχουν οσμηρές ουσίες στα αρσενικά που προσελκύουν παρθένα θηλυκά. Περισσότερη έρευνα απαιτείται για να διευκρινιστεί σε βάθος ο ρόλος των οσμηρών ουσιών των αρσενικών. Τα παραπάνω ευρήματα συμβάλλουν στην πληρέστερη κατανόηση της σεξουαλικής συμπεριφοράς του δάκου της ελιάς και μπορούν να αξιοποιηθούν για την αποτελεσματικότερη αντιμετώπισή του με φιλικά προς το περιβάλλον μέσα.