

## Female calling behaviour and male response to the synthetic sex pheromone components of *Palpita unionalis* (Lepidoptera: Pyralidae)

**Basilios E. Mazomenos, Maria Konstantopoulou, Dimitra Stefanou, Spiros Skareas and Leonidas C. Tzeiranakis<sup>1</sup>**

*Chemical Ecology and Natural Products Laboratory, Institute of Biology, NCSR "Demokritos" P.O. Box 60228, 153 10 Aghia Paraskevy, Attikis Greece.*

<sup>1</sup> *Regional Centre for Plant protection and quality control, Ministry of Agriculture Iraklio Crete*

**Abstract:** The pheromone biology of the jasmine moth *Palpita unionalis* was studied under Laboratory conditions. Female began calling during the second day following emergence. Calling activity and pheromone production is periodic and synchronous. Maximal calling and pheromone production was obtained the fourth day. The peak of the female calling occurred during the dark phase, six hours after lights off, of a 14:10 (Light: Dark) regime. Male response to each one of the two synthetic pheromone components [(E)11-16:Ac], [(E)11-16:Ald] and their blend was tested in a wind tunnel at different dosages. The (E)11-16:Ald stimulates more males to take flight, but the vast majority of them did not approach the pheromone source. With the (E)11-16:Ac fewer males took flight, but most of them flew close to the pheromone source and some landed on the source expanding their hair pencils. A two component blend at the ratio of 7:3 [(E)-11-16:Ac:(E)11-16:Ald] was the one that evoked the full behavioural repertoire by males. The proportion of males responding was lower at the dose of 2 µg and remained relatively unchanged for the other doses tested with a trend toward decreased responses at the higher dose of 32 µg. Field tests revealed that funnel type traps baited with 1 mg of the two compounds blend captured significant number of males.

**Key words:** *Palpita unionalis*, calling behaviour, pheromone titter male response, wind tunnel, pheromones (E)-11-hedecenal, (E)-11-hexadecenyl acetate.

### Introduction

The jasmine moth *Palpita unionalis* Huebner (Lepidoptera: Pyralidae) is a serious pest of *Jasminum* sp., *Ligustrum* sp., *Olea europea* and *Phiirea media*, causing severe damages on the foliage of these plants. *P. unionalis* occurs throughout the Mediterranean region (Balachowsky, 1972). In olive trees, larvae usually attack young leaves and shoots, while in years of high population densities; they attack

also olive fruits, making them unsuitable for marketing.

The sex pheromone of *P. unionalis* was reported to consist (E)11-hexadecenyl acetate [(E)11-16:Ac] and (E)11-hexadecenal [(E)11-16:Ald] (Mazomenos *et al.*, 1994). The blend of the two components at the ratio of (7:3) [(E)11-16:Ac:(E)11-16:Ald] attracted males in field test. Further field tests indicated that the efficacy of the traps baited with the blend was limited and male catches were low. In order to improve the pheromone efficacy in monitoring and control studies it is essential to know the pheromone biology of *P. unionalis*

In many moth species, female calling and pheromone production is synchronous and usually depended on moth age as well as on other endogenous and exogenous factors. (Howlader and Gerber, 1986; Raina *et al.*, 1986; Snir *et al.*, 1986; Dunkelblum *et al.*, 1987;; Noldus and Potting, 1990; Babilis and Mazomenos, 1992; Kakimura and Tatsuki, 1993).

Wind tunnel and field studies as well, have shown, that specific pheromone components or their blends can be responsible for several quantitative and qualitative aspects of male mating behaviour for many moth species (Baker and Carde, 1979; Linn and Gaston, 1981a, 1981b; Quartey and Coaker, 1993). Therefore knowledge of the role of each component is essential for understudying the behavioural mechanisms associated with male mating behaviour.

In this paper we report results on the effect of age on the female calling behaviour, the pheromone production and the role of the synthetic pheromone components on male attraction in wind tunnel and field tests.

## Material and Methods

*Insects.* The insects used were from a laboratory colony established from larvae collected from infested olive trees. The colony was maintained on *Ligustrum ovalifolium* (L) leaves at  $25 \pm 2^\circ\text{C}$ ,  $60 \pm 5\%$  relative humidity (rh), and 14:10 h light-dark (L:D) regime (Vasilaina-Alexopoulou and Santorini, 1973). Pupae were segregated by sex and were kept at  $25^\circ\text{C}$  on a 14:10 Light:Dark (L:D) cycle. Emerged females/males were transferred daily to  $20\text{ cm}^3$  Plexiglas cages in separate chambers and kept under the conditions mentioned above. Moths were provided with a 10% sucrose solution.

*Female calling behaviour.* Thirty newly emerged females were caged individually in 10x10x10cm screen cages provided with 10% sucrose solution. The calling behaviour of the females was observed during the scotophase at 15 min intervals, observations were facilitated with red light.

Data on the percentage of female calling, daily, the age at which females initiated calling, as well as the mean onset time (time after lights off), and the mean time spent calling were collected on successive nights from the first night of emergence until the seventh day.

*Pheromone collection and analysis.* To quantify the pheromone produced, ovipositors of individual females 1 to 7-old were excised and extracted in 10  $\mu$ l dichloromethane for 20 min in the sixth hour of the scotophase. Ten samples were prepared and analysed for each day. The extract was transferred to 0.3 ml conical screw caps vials. Five  $\mu$ l 25 ng of dodecyl acetate was added as internal standard. The samples were concentrated to about 2  $\mu$ l and were analysed on Varian Model 3400 chromatograph, with flame ionization detector (FID) and a splitless injector system. The column was a DB-5 30 m x 0.32 mm (id0) (J&W, Scientific, Folsom, Ca, U.S.A.). The column temperature program was 80°C (hold 2 min) at 10°C/min to 250°C. Helium was used as carrier gas, at a flow rate of 2 ml/min

*Chemicals.* Synthetic (E)11-16:Ald and (E)11-16:Ac, used for the wind tunnel tests, were provided from (Vioryl Co. Kato Kifissia, Athens, Greece). The components were found to be 97% pure, when analysed on DB-5 30 m x 0.32 mm-ID capillary GC column (J&W, Scientific, Folsom, Ca, U.S.A.).

*Male behaviour.* The male response to the pheromone source was studied in a 150 x 35 x 35 cm glass wind tunnel similar to that described by Carde and Hagaman (1979) maintained at  $25 \pm 2^\circ\text{C}$ ,  $60 \pm 5\%$  rh. Male response was tested during the fourth to seventh hour of a ten-hour scotophase. The males 2 to 5-d-old were placed individually in 15 x 10 cm cylindrical screen cages. The cages were transferred into the testing room, operated under the same conditions described, before the onset of the scotophase. Each cage was placed close to the downwind end of the tunnel five minutes prior to testing for male acclimation to the light intensity and airflow. The chemicals and the baits (5x5 cm filter paper Whatman No 1) were kept and prepared outside the testing room. The piece of the filter paper impregnated with the pheromone component was hung 15 cm downwind of the fan. Filter papers impregnated with the same volume of hexane were also introduced into the tunnel. Twenty-five males were tested to the following dosages: 2, 4, 8, 16 and 32  $\mu$ g, of each of the component and their blend at the ratio of 7:3 [(E)11-16:Ald:(E)11-16:Ac].

*Field trapping.* The two compounds blend in hexane solution was formulated in white rubber septa. Three funnel type traps baited with 1 mg of the pheromone blend were hung on olive trees in an olive grove located in Island Crete from 23 April to 15 November. Traps were suspended at least 50 meter apart the number of males caught was recorded weekly and the pheromone dispensers were replaced every 30 days.

*Data recording and analysis.* The responses of the male to either the pheromone component alone or the blend was recorded for 5 min. The behavioural steps recorded included: wing fanning and taking flight; flight close to the pheromone source; landing on the pheromone source and hail pencil display. Data recorded from the wind tunnel were analysed using a chi-square 2 x 2 test of independence (Zar, 1984). Responses of males of individual components and their blend were compared with respect to each dose and to the specific behavioural steps. The  $P=0.05$  level was set for the rejection of the null hypothesis.

## Results

*Calling behaviour.* Moths emerged during the scotophase, 2 to 3 h before lights on. The following scotophase (scotophase 1) none of the 50 observed females initiated calling. Calling was initiated during the second scotophase. Maximum calling occurred during the fourth scotophase (68.5%). The percentage of the females calling for scotophase 2 to 5 ranged from 42.5 to 61.5% and then decreased so that by the seventh scotophase only 12.4% of the females were observed calling. Calling activity was initiated during the 5 to 6 h after lights off and terminated during the 7 to 8 h of the scotophase. The mean onset of calling advanced from 6.4 h on scotophase 2 to 5.1 h on scotophase 7. The mean time spent calling was similar from scotophases 2 to 5 and was decreased for the next two scotophases (6 and 7) (Table 1).

Table 1. Effect of age on calling, the mean time of onset calling and the mean time spent calling of virgin females *P. unionalis*. The females emerged during the last two hours of the scotophase and were kept at 14:10 (L:D) regime (N=30).

Female Age Scotophase	% Calling	Mean onset	Calling Time (min)
1	-	-	-
2	42.5 ± 1.3	384.0 ± 11.7	55.4 ± 3.6
3	65.2 ± 2.6	387.6 ± 7.8	62.8 ± 1.4
4	68.4 ± 1.4	360.1 ± 11.3	58.8 ± 2.7
5	61.5 ± 3.4	351.9 ± 20.2	61.2 ± 4.3
6	17.3 ± 3.0	331.9 ± 18.6	38.4 ± 2.0
7	12.4 ± 3.1	304.2 ± 19.3	31.2 ± 3.1

*Pheromone gland content.* The pheromone content in female gland was quantified from scotophase 1 until the scotophase seven. Age affected the pheromone production of *P. unionalis*, not detectable quantities of both components were found in scotophase 1. The pheromone production begins from scotophase 2, increased progressively and reached at maximal level on scotophase 4 (Table 2). The pheromone production coincides with the female calling activity. The quantity of (E)-11-16:Ac produced was approximately 7 times more than the quantity of (E)-11-16:Ald.

*Male response to pheromone blend and the two components.* The role of each component on male behaviour and the blend was studied at the 8 µg dosage (Fig. 1). At a dosage of 8 µg, the males exhibited all the stages of the behavioural sequence. The proportion of males responding was maximal when blend of (E)11-16:Ac, and (E)11-16:Ald was tested. Wing fanning and taking flight was 100%, significantly

higher than the level observed for the (E)11-16:Ac alone ( $Z=13.3$ ,  $P=0.05$ ), but was not different from that observed for males responding to the (E)-11-16:Ald.

Table 2. Titre of (E)-11-16:Ald and (E)-11-16:Ac female *P. unionalis* sex pheromone components (ng) obtained from individual pheromone gland extracts of 2 to 7-d-old virgin females (N=10).

Female Age Scotophases	(E)-11-16:Ac	(E)-11-16:Ald	Ratio Ac/Ald
1	-	-	-
2	173 ± 11.3	23 ± 1.2	7.5
3	262 ± 48.7	36 ± 6.6	7.2
4	296 ± 8.2	45 ± 8.2	6.6
5	292 ± 33.3	40 ± 8.9	7.3
6	241 ± 24.5	36 ± 3.1	6.7
7	171 ± 18.9	24 ± 3.2	7.2
Mean	239.4 ± 22.7	34 ± 3.5	7.1

Comparing males exhibiting wing fanning and taking flight, when the two components were tested alone, significantly more males were found to be responding to the (E)11-16:Ald than to the (E)11-16:Ac ( $Z=7.62$ ,  $P=0.05$ ).

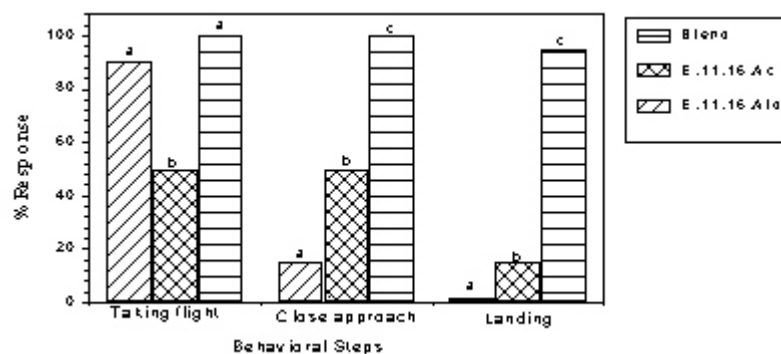


Figure 1. Proportional effect of (E)-11-16:Ald, (E)-11-16:Ac and their blend, on males *P. unionalis* response of various behavioral stages in a wind tunnel. Bars with different letters for each chemical are significant different (chi-square 2X2 test of independence  $P<0.05$ ) (N=25).

The majority of males that made upwind progress in response to the blend flew close to the pheromone source and many of them landed on the filter paper, exposing their

hair pencils. The number of males that flew close to the pheromone source with the (E)11-16:Ac was relatively high, however it was significantly lower than that of the blend ( $Z=13.33$ ,  $P=0.05$ ). Significantly more males landed on the filter paper with the blend, compared with the (E)11-16:Ac ( $Z=23.1$ ,  $P=0.05$ ). Few males landed on the filter paper with the (E)11-16:Ald alone.

*Effect of pheromone dose on male behavioural steps.* The proportion of males that performed wing fanning and taking flight was not significantly different to all the doses tested with the (E)11-16:Ald and the blend (Fig. 2A). Male response and taking flight, at the dose of 2  $\mu\text{g}$  was significantly lower than that of 4  $\mu\text{g}$  ( $Z=3.95$ ,  $P=0.05$ ) for (E)-11-16:Ac. Above this dose, the proportion of males that performed wing fanning and taking flight were not significantly different and seemed to reached a plateau at the doses of 8 to 16  $\mu\text{g}$ .

Few males flew close to the pheromone source, when (E)11-16:Ald was tested. The percentage of males that flew close to the pheromone source with the (E)11-16:Ac increased as the pheromone dosage increased from 2 to 4  $\mu\text{g}$  and reached a plateau for higher dosages tested (Fig 2B). With the blend the proportion of males flying close to the source was high to all dosages and reached the 100% at the dose of 8  $\mu\text{g}$  (Fig. 2B).

For all the doses of (E)11-16:Ald, very few males landed on the filter paper source and everted their hair pencils. The percentage of males landing and everting their hair pencils for (E)11-16:Ac increased as the dose increased and reached 35% at the concentration of 32  $\mu\text{g}$  dose (Fig. 2C). The proportion of males that landed and everted their hair pencils when the blend was tested increased as the pheromone dose increased, 38% and 44% of males were landed on the filter paper at the 2  $\mu\text{g}$  and 4  $\mu\text{g}$  doses respectively, whilst at the higher doses (8, 16, 32  $\mu\text{g}$ ) the percentages of males landing reached 90 to 95% (Fig. 2C).

*Field trapping.* Data collected from field experiments during 1998 and 1999 in Island Crete indicated that pheromone traps baited with the two-component blend attracted males. The number of males caught in pheromone traps compares well with the infestation levels recorded during the testing period on olive trees. During 1998 and 1999 the moth develops low population in Crete. Field results revealed that funnel traps baited with the pheromone blend are effective tools to monitor the moth population and time the application of control measures. Trap catches also indicated that in Island of Crete *P. unionalis* develops at least three generations per year (Fig. 3).

## Discussion

Calling behaviour and pheromone production of *P unionalis* females is synchronous. Maximal pheromone content in the gland and maximal calling activity occurred during the scotophase the 6<sup>th</sup> h after lights off. It appears that *P. unionalis* follows a

calling and pheromone biosynthesis pattern that is common for many moth species (e.g. *Heliothis zea*, Raina *et al.*, 1986; *Heliothis subflexa*, Heath *et al.* 1991; *Sesamia nonagrioides*, Babilis and Mazomenos, 1992; *Helicoverpa assulta*, Kakimura and Tatsuki, 1993; *Cydia pomonella* Bäckman *et al.*, 1997), In these species pheromone production occurs during the period where females are calling and releasing pheromone.

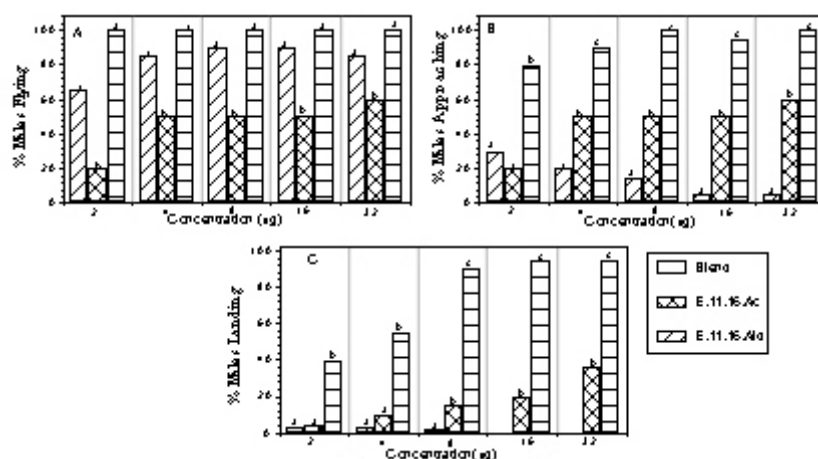


Figure 2. Percentage of males *P. unionalis* taking flight (A), flying close (B), and landing (C) on the pheromone source at various doses of the two-sex pheromone components (E)11-16:Ald, and (E)11-16:Ac and their blend. Bars with different letters for each dose are significant different (chi-square 2X2 test of independence  $P < 0.05$ ) (N=25).

Aside from the quantity of pheromone produced by females every day, the ratio of the two components was the same. The amount of (E)11-16:Ac produced was approximately seven times more compared to the amount of (E)11-16:Ald.

Evaluating the results obtained from the wind tunnel, a significant difference was observed between the two-pheromone components on the effects of the male *P. unionalis* mating behaviour. The (E)11-16:Ald stimulates more males to take flight, but the vast majority of them did not approach the pheromone source. On the contrary, for (E)11-16:Ac fewer males took flight, but most of them flew upwind, approaching the source. Therefore, the males oriented themselves better towards the (E)-11-16:Ac source than when the (E)11-16:Ald was used. As a result, more males landed on the pheromone source, exposing their hair pencils in the presence of (E)11-16:Ac.

The two components blended at a ratio of 7:3 (E)11-16:Ald:(E)11-16:Ac evoked the whole sequence of behavioural steps. From these results it is clarified that the two-pheromone components act synergistically and the blend caused maximal response. Moreover, the blend elicited satisfactory male response, even at low concentrations. Synergism of the different components of sex pheromone blends is widely known amongst several species of Lepidoptera. Much work has been carried out with many moth species that use different multicomponent blends in their sexual be-

behaviour such as: *Trichoplusia ni* (Linn and Gaston, 1981 a,b) *Diaphania nitidalis* (Klun *et al.*, 1986), *Cochylis hospes* (Underhill *et al.*, 1986). *Ephestia cautella* (Quartey and Coaker, 1993). In all of these species, it has been shown that the single presence of some of their pheromone components is not sufficient for successful male attraction and copulation. On the other hand, both in laboratory bioassays and field tests, most of these species use specific blends of appropriate components that act as a unit to evoke maximal stimulation of the males.

Age affected pheromone production the greatest amount of pheromone in the gland was measured, when the females were four days old. After the fourth day pheromone gland content and calling activity were decreased progressively. Similar results have been reported for other moths (Howlander and Gerber, 1986; Raina and Klun, 1986).

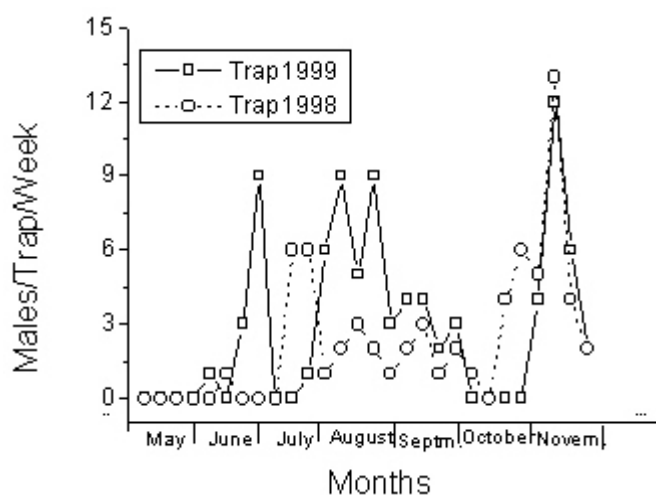


Figure 3. Males *P. unionalis* caught in funnel traps baited with rubber septa, loaded with 1 mg of the pheromone blend (E11-16: Ac: E11-16: Ald). Field tests were conducted in an olive grove in Island Crete during 1998 and 1999.

The different dosages of the two components and their blend tested quantitatively affected male *P. unionalis* behaviour. When the two components were tested alone, with the (E)11-16:Ald no dosage dependence was observed with respect to wing fanning, and taking flight. With the (E)11-16:Ac all the behavioural stages are dosage dependent. Dosage dependence to various behavioural steps has been reported for other moth species. The male response was influenced as the dosage of the pheromone increases for the species *T. ni* (Linn and Gaston, 1981) and *Lymanthia dispar* (Carde and Haganan, 1979).

The data presented shows that calling behaviour and pheromone production of *P. unionalis* is a synchronous process. The two pheromone components individually tested affected male behaviour, however the blend of the two components at the ratio found in the pheromone grand elicited to males maximal response in wind tunnel

experiments. Field data revealed that pheromone traps are effective tools to monitor *P. unionalis* population.

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