

Results and discussion. The concentration of F-actin-bound ADP in muscle was 2.44 ± 0.46 (11) $\mu\text{mole g}^{-1}$ protein, a value in good agreement with published data^{3,8}. The concentration of ATP in muscle was 4.48 ± 0.32 (11) $\mu\text{mole g}^{-1}$ muscle.

Preliminary experiments indicated that the specific-radioactivity of ATP increased linearly with time. This is characteristic of a labelling procedure in which the tracer is administered at a single localized site from which absorption may be slow and where the administered tracer has to be converted to the metabolite being measured⁹. Both conditions were present in the experiments described in this communication. Thus the sp. act. of ATP (S_{ATP}) is proportional to time and the ratio of ATP sp. act. to F-actin-bound ADP sp. act. (S_{ADP}) is described by the equation

$$\left(\frac{S_{\text{ADP}}}{S_{\text{ATP}}}\right)_t = \frac{1}{kt} (kt - 1 - e^{-kt})$$

(for derivation see Zilversmit⁹)

where k is the fractional turnover rate of F-actin-bound ADP (h^{-1}) and t is time (h). The fractional turnover rate, calculated according to this formula, was 0.88 ± 0.13 (11) h^{-1} (table). However, the validity of the method relies upon ADP being bound only to F-actin. This would seem probable since other components of muscle fibres which bind ADP exhibit considerably weaker binding^{3,10,11}. Furthermore, the muscle homogenate is subjected to prolonged washing in the procedure reported here.

As discussed above the fractional turnover rate of F-actin-bound ADP should be directly proportional to the turnover

rate of F-actin. This assumes that the incorporation of bound ^3H -ADP reflects the addition of monomers to F-actin rather than the exchange of the nucleotide on pre-existing polymers. Evidence both for and against exchange occurring in vitro and in vivo has been presented¹² and reviewed elsewhere^{12,13} with the conclusion that exchange does not occur in vivo. The procedure described in the present report is currently being used to investigate further whether exchange of F-actin-bound ADP may indeed occur in vivo¹⁴.

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- 2 F. Oosawa and M. Kasai, *Biological Macromolecules*, vol. 5, p. 261. Marcel Dekker, New York 1971.
- 3 J. Uchino and K. K. Tsuboi, *Am. J. Physiol.* 219, 154 (1970).
- 4 W. E. Cohn and C. E. Carter, *J. Am. chem. Soc.* 72, 4273 (1950).
- 5 H. Adam, in: *Methods of Enzymatic Analysis*, 2nd ed., p. 539. Ed. H. V. Bergmeyer. Academic Press, New York and London 1965.
- 6 P. D. Swanson and W. L. Stahl, *Biochem. J.* 99, 396 (1966).
- 7 O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. Randall, *J. biol. Chem.* 193, 265 (1951).
- 8 G. E. Griffin and G. Goldspink, *Differentiation I*, 355 (1973).
- 9 D. B. Zilversmit, *Am. J. Med.* 29, 832 (1960).
- 10 H. O. Hultin and S. H. Richardson, *Archs Biochem. Biophys.* 105, 288 (1964).
- 11 M. Young, *J. biol. Chem.* 242, 2790 (1967).
- 12 H. Strzelecka-Golaszewska, M. Jakubiak and W. Drabikowski, *Eur. J. Biochem.* 55, 221 (1975).
- 13 M. Appenheimer, D. von Chak and H. H. Weber, *Biochim. biophys. Acta* 256, 681 (1972).
- 14 L. C. Ward, *IRCS Med. Sci.* 7, 231 (1979).

Laboratory and field studies of the female sex pheromone of the olive moth, *Prays oleae*¹

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Summary. Analysis by GC-EAG indicates that abdominal tip extracts of female *Prays oleae* contain a tetradecenal. Synthetic (Z)-7-tetradecenal elicits a strong EAG response from male *P. oleae* and field tests show it to be comparable in attractancy with the virgin female moth. (Z)-9-Tetradecenal also produces a strong EAG response but it is not an attractant and, when added to (Z)-7-tetradecenal, markedly reduces trap catches.

The olive moth, *Prays oleae* (Bern.) (Lepidoptera, Yponomeutidae) is an important pest of olives in the Mediterranean region, successive generations of larvae causing excessive flower drop in the spring and further damage in the summer by boring into the kernels of the developing fruits⁵. Following the identification of the female sex pheromone of *P. citri*⁶, an investigation of the pheromone of *P. oleae* was undertaken to provide a tool for monitoring and control of this pest. Attraction of the male moths to traps baited with virgin female *P. oleae* had already been demonstrated⁷, and the use of these traps to detect successive generations had been reported⁸.

Materials and methods. Larvae attacking olive flowers were collected in the field in the Evia region of Greece and allowed to pupate in the laboratory before being flown to London. Preparation of female moth abdominal tip extracts and examination of the extracts by gas chromatography (GC) linked to electroantennographic (EAG) recording from the male moth were carried out as for *P. citri*⁶, as were the preparation of synthetic chemicals and the record-

ing of the EAG response profile from the male moth to these compounds.

Field trials were carried out in the Chania region of Crete. Traps were of the delta type, having triangular cross-section (8 cm sides) and length 18 cm, with the inner basal surface coated with an adhesive. Potentially attractant synthetic chemicals were mixed with an equal amount of 2,6-di-tert-butyl-p-cresol (BHT) as antioxidant, and dispensed from sealed polyethylene vials (32 × 16 mm, 1.5 mm thick)⁹. Traps were hung from olive trees, normally 2–3 m above ground level and at least 50 m apart in a circular array. The traps comprising one replicate were rotated one position clockwise every 2–3 days, and the polyethylene vials were renewed every 30 days. Virgin female moths used to bait traps were obtained from field-collected larvae. Special care was taken to distinguish *P. oleae* and *P. citri* in all trap catches. Where appropriate, catch data was transformed to $\log(10x+1)$ and subjected to analysis of variance, differences between treatment means being graded for significance at $p=0.05$.

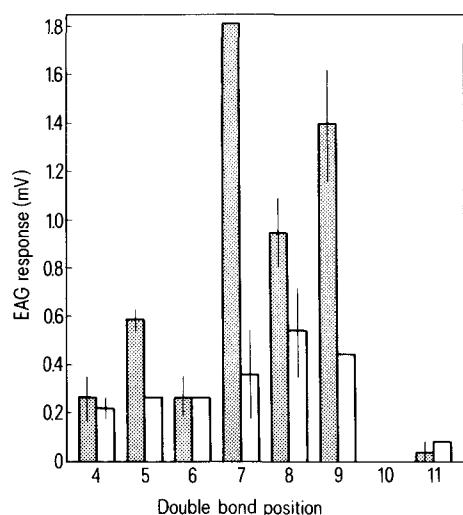
Results. Analysis of female abdominal tip extract by GC-EAG using packed GC columns with temperature programming showed a single EAG response from the male moth at the retention temperatures corresponding to a mono-unsaturated C_{14} aldehyde. (Equivalent chain lengths based on retention temperatures for n-alkyl acetates: 12.9 on Carbowax 20 M; 11.9 on Apiezon L.) Yields of pheromone were so low that no significant peak was seen on the GC trace, and insufficient material was available for high-resolution GC-EAG analysis or microchemical reactions. The EAG response profile of the male moth to synthetic tetradecenals (figure) showed a maximum for (Z)-7-tetradecenal as for *P. citri*⁶, but the response of *P. oleae* to (Z)-9-tetradecenal was relatively much greater than that of *P. citri*. In further tests, 'puffing' a mixture of 2 ng (Z)-7-tetradecenal and 2 ng (Z)-9-tetradecenal consistently produced a larger EAG response from male *P. oleae* than 4 ng of (Z)-7-tetradecenal (means of 7 replicates 1.83 mV and 1.32 mV respectively, difference significant at 1% level by 't'-test).

An initial field experiment showed that the attractiveness to male *P. oleae* moths of a polyethylene vial containing 1 mg of (Z)-7-tetradecenal was comparable to that of 1, 4 or 8 virgin female moths (total catches over 10 nights: 30, 11, 3 and 23 respectively).

Numbers of male *P. oleae* caught in Delta traps (DT) baited with 0.1, 0.5 and 1.0 mg (Z)-7-tetradecenal and in McPhail traps (MT) baited with 1.0 mg (Z)-7-tetradecenal

Replicate	Duration (days)	Times sampled	1.0 mg, DT	0.5 mg, DT	0.1 mg, DT	1.0 mg, MT
1	42	28	341 a	323 a	180 b	105 c
2	73	30	351 a	343 a	322 b	-
3	94	28	242 a	348 a	282 a	60 b
4	50	20	104 a	101 a	57 a	24 b

Catches followed by the same letter were not significantly different at $p=0.05$ after analysis of variance of $\log(10x+1)$ transformed data. Numbers of *P. citri* males caught during replicates 1-4 respectively: 51, 90, 129, 0 (1.0 mg, DT); 20, 70, 71, 2 (0.5 mg, DT); 8, 45, 32, 5 (0.1 mg, DT); 32, -, 26, 3 (1.0 mg, MT).



Male *P. oleae* EAG responses to tetradecenal isomers, standardized against the mean response to (Z)-7-tetradecenal and corrected for the mean blank value; the bars indicate the spread of duplicate tests. (Z)-isomers, dark columns; (E)-isomers, light columns. Responses to (Z)- and (E)-10-tetradecenal were not recorded.

During 1978, catches in sticky traps baited with vials containing 0.1, 0.5 and 1.0 mg (Z)-7-tetradecenal were compared with each other and with catches by vials containing 1 mg (Z)-7-tetradecenal in glass McPhail traps of the type used for olive fly, *Dacus oleae*; the experiment was replicated 4 times (table). Catches by the McPhail traps were always significantly lower than those by any of the sticky traps. In the latter, catches by the 0.1 mg loading were significantly lower than those by the 0.5 and 1.0 mg loadings in 2 of the 4 replicates. Addition of (Z)-9-tetradecenal to (Z)-7-tetradecenal greatly reduced the attractiveness of the latter. In 2 experiments run over 57 and 55 nights, total catches of male *P. oleae* moths by 1 mg of (Z)-7-tetradecenal and by a combination of 0.8 mg (Z)-7-tetradecenal and 0.2 mg (Z)-9-tetradecenal were 255 and 2 respectively. Total catches by the 2 aldehydes combined at loadings of 0.6+0.4 mg, 0.4+0.6 mg, 0.2+0.8 mg and by 1 mg (Z)-9-tetradecenal alone in these experiments were 1, 1, 0 and 1 respectively. Small numbers of *P. citri* male moths were caught in most of these experiments, although very rarely in traps baited with virgin female *P. oleae*.

Discussion. The studies described here suggest that (Z)-7-tetradecenal, the aldehyde previously identified as the female sex pheromone of *P. citri*⁶, is also a sex pheromone component in *P. oleae*: - GC-EAG analyses indicated that a tetradecenal was present in female tip extract and (Z)-7-tetradecenal was the most potent of the isomers tested for EAG activity. When dispensed from polyethylene vials in the field, 1 mg of this aldehyde was comparable in attractiveness with virgin female moths, and it has been used in place of virgin females for monitoring *P. oleae* populations^{7,8}. The relatively strong EAG responses of male *P. oleae* to (Z)-9-tetradecenal, alone and combined with an equal amount of (Z)-7-tetradecenal, suggested that this might also be a pheromone component, which acted on different antennal receptors from the (Z)-7 isomer. Field tests showed that it was not itself an attractant and that it masked the attractiveness of (Z)-7-tetradecenal when the 2 compounds were combined. The fact that in many of the field experiments with (Z)-7-tetradecenal as attractant source both *P. oleae* and *P. citri* males were caught, whilst *P. citri* males were very rarely caught in traps baited with female *P. oleae*, suggests that there may be some additional chemical basis for species isolation. The ability of (Z)-9-tetradecenal to markedly reduce the attractancy of (Z)-7-tetradecenal to male *P. oleae* but not to male *P. citri*⁹ could be significant in this respect and further studies are in progress. For control purposes, (Z)-9-tetradecenal could be of potential use as a communication and mating disruptant.

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- 5 C.E.D. Pelekassis, Annls Inst. phytopath. Benaki 4, 185 (1962).
- 6 B.F. Nesbitt, P.S. Beever, D.R. Hall, R. Lester, M. Sternlicht and S. Goldenberg, Insect Biochem. 7, 355 (1977).
- 7 R. Pralavorio, Y. Arambourg and D. Codou, Ann. Zool. Ecol. Anim. 7, 269 (1975).
- 8 R. Pralavorio and Y. Arambourg, Revue Zool. Agric. Path. Veget. 76, 63 (1977).
- 9 M. Sternlicht, S. Goldenberg, D.R. Hall, R. Lester and B.F. Nesbitt, Phytoparasitica 6, 101 (1978).