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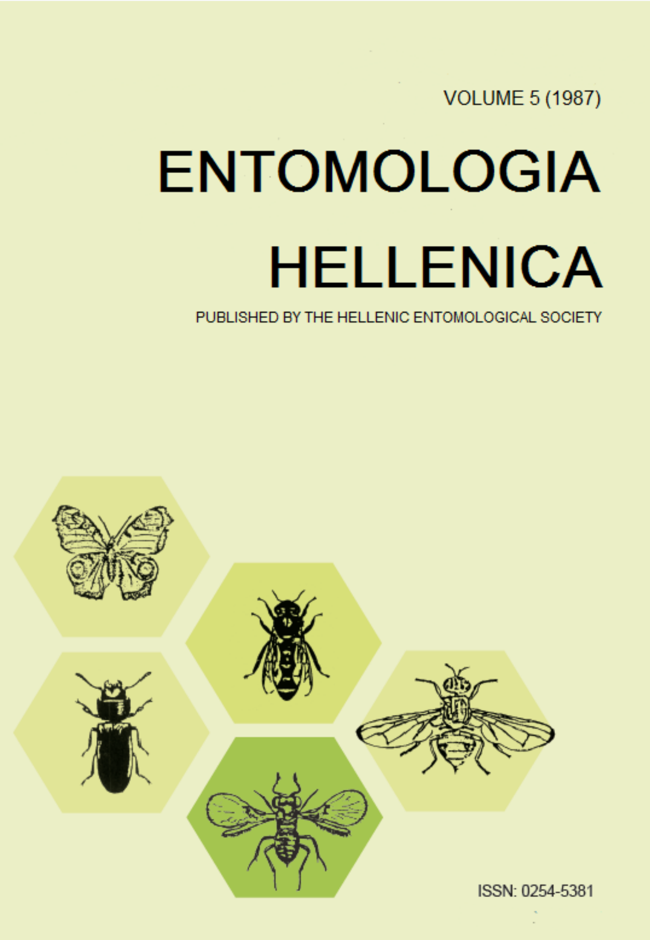

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Effect of Combining Food and Sex Attractants on the Capture of *Dacus oleae* Flies[1]

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ABSTRACT

Sex pheromonal compounds i.e. the most potent male attractants, and ammonia, one of the most potent female attractants available today for *Dacus oleae* (Gmelin), were combined on the same trap. This combination resulted in an overall increase of female captures as compared to those of ammonia alone, but a decrease of male captures as compared to those of pheromone alone. Deviations from this overall effect were observed during certain periods of the year and are caused by varying environmental and biological factors which influence insect response to pheromones. Explanations for these results based on recent findings concerning the pheromone system of *D. oleae* are presented here.

Introduction

Attractants available today for *Dacus oleae*, effective under field conditions, include:

I. Chemical attractants. a) Food attractants. These include various ammonia - releasing compounds, such as aqueous ammonia solutions, ammonium salts (Bua 1933), protein or yeast hydrolysates (Orphanidis et al. 1958), heterocyclic amines probably responsible for protein attraction (Stavrakis 1970) and the fruit volatiles, hexanol, octanal, nonanol and nonanal (Guerin et al. 1983). b) Sex attractants. Female *D. oleae* flies release a sex pheromone which functions as a potent long-range male attractant (Haniotakis 1981, Delrio et al. 1983). This pheromone was found to be a mixture of the following substances: 1,7-dioxaspiro (5.5) undecane, a-pinene, n-nonanal and ethyl dodecanoate at a ratio of 3:1:0.3:1 (Mazomenos and Haniotakis 1981, 1985). The first substance, which is the major component of the mixture (Baker et al. 1980), has also been isolated from male *D. oleae* flies (Mazomenos and Pomonis 1983). Recent studies have shown that male olive fruit flies respond only to the R-(-)- enantiomer of the major pheromone component to which they are attracted from long distances, while females respond only to the S-(+)- enantiomer which functions as an arrestant and aphrodisiac (Haniotakis et al. 1986 c). The same studies have shown that females and probably males release the major pheromone in its racemic form.

II. Visual attractants. The colour yellow with maximum reflectance between 500-520 nm (Girolami and Cavalloro 1973, Prokopy et al. 1975, Delrio et al. 1979) has been found to be most attractive for *D. oleae*.

A number of substances which have attracted *D. oleae* flies in laboratory tests but not in the field have also been reported (Delrio 1984). Much effort has been made during the last decade towards the development of an effective trap for *D. oleae* which could be used for monitoring, control, or both. For this purpose all types of attractants showing field activity, namely food, visual and sex, were tested individually or in various combinations. A recent review article covers adequately these subjects (Delrio 1984).

For monitoring, satisfactory trapping systems are not available. Efficiency of traps used for this purpose depends on a number of factors, environmental and biological, the effects of which are not well known. Reliable correlations therefore between trap catches and wild population densities or fruit infestation, applicable to different olive ecosystems, cannot be established for any type of

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trap. However, some papers on such correlations have been published (Kapatos and Fletcher 1983 and references cited therein). For direct control, there have been several attempts to control the olive fruit fly by means of mass trapping. Brief reviews of the subject are included in Haniotakis et al. (1986a, 1986b). In these attempts various trap types based on one or more attractants have been used. It seems that no definite decision has been reached yet which is acceptable to all scientists working in this field as to the best trap type and attractant or combination of attractants for this purpose. It was the aim of the present work to field test the sex and food attractants available today individually and in combination under the same conditions and evaluate the results for the purpose of selecting the best attractant or combination for use in mass trapping systems. Such an evaluation has not been reported previously. The visual attractant was intentionally not included because, for the type of traps used here, it increases manufacturing and operational costs with only limited contribution to the total trap catch (Haniotakis 1986a) and at trap densities required for mass trapping it may be detrimental to the beneficial fauna of the olive ecosystem (Neuenschwander 1982).

Materials and Methods

Studies of the relative attractiveness of sex attractant pheromones, ammonia - releasing sources and combination of the two were made during 1982 and 1983 in an olive orchard with medium size trees of mixed varieties at Paradissos, Athens, Greece. Before initiation of the main experiment, the effects of the combination of the two attactants on the captures of *D. oleae* were examined in a preliminary test at the same orchard.

In 1982 the traps were sticky poster-board rectangles (15×20cm) of a dark yellow colour (Imperial, Vivechrom, Athens, Greece) with maximum light reflectance outside of the preference range of *D. oleae* (Prokopy et al. 1975) which nevertheless still attracts a small number of flies (Haniotakis 1986a). In 1983 traps were sticky plywood rectangles of the same dimensions but of natural wood colour, neutral as far as *D. oleae* attraction is concerned (Haniotakis unpublished data). These traps, when unbaited, do not catch any flies. The complete blend of the sex pheromone of *D. oleae* (Mazomenos and Haniotakis 1985), at the ratios mentioned above, was used at a concentration of 50 mg/trap, dispensed from 1 ml polyethylene vials (Mazomenos et al. 1983). The major component of the pheromone blend, 1,7-dioxaspiro (5.5) undecane, kindly supplied by Vioryl, Greece, was in racemic form. Pure enantiomers of the major pheromone were not available in amounts required for this type of test.

Ammonium bicarbonate salt (20 mg/trap) in polystyrene cups with perforated polyethylene lids was used as the food attractant during 1983. Ammonium bicarbonate was selected because it is inexpensive, easy to handle, and readily releases ammonia under field conditions for a long period, characteristics highly desirable for practical uses. An aqueous ammonia solution (25%) in polyethylene vials with perforated lids (25 ml/trap), which was used during 1982, was replaced by bicarbonate salt because the residual activity of the ammonia solution is short and this necessitates refilling of the vials, an operation which raises the cost in practical applications.

In preliminary experiments, a small number of traps was placed at spacings of 40 m or more. In the remaining experiments, there were arrangements of one trap in every other tree, i.e. approx. 16 m apart. The latter trap density was chosen because previous experiments (Haniotakis 1981) had indicated that it is the most suitable for mass trapping purposes. More specifically, the proportional recovery of released flies was not affected when trap densities were between one trap per two trees and two traps per tree, but was significantly lower when lower trap densities were used. Competition between traps, however, is expected to increase as the trap distance decreases (Delrio et al. 1983, Haniotakis 1986b).

Traps were checked once a week and replaced when necessary so that an adequate clean sticky surface would be available for effective capture of landing insects at all times. Dispensers were not replaced in 1982; in 1983 they were replaced once on June 30th. The active life of the dispensers used in these tests is expected to be at least 4 months (Mazomenos et al. 1983). The following experimental designs were used: preliminary experiment 1982 (Table 1) block design, experiments 1982 (Table 2) block design, experiments 1983 (Table 3) completely randomized design. The traps in the completely randomized design were rotated at every check. Numbers of flies captured on individual traps were subjected to analysis of variance after logarithmic transformation ($x=\log(x+1)$).

Results and Discussion

Table 1 shows the numbers of *D. oleae* male and female flies captured on yellow sticky traps baited with pheromone, aqueous ammonia solution, and a combination of the two in the 1982 experiments. In this experimental design in which competition between traps was limited, the combination of ammonia and pheromone drastically decreased captures of males (compared to pheromone alone) and increased captures of females (compared to ammonia alone). In other words, traps combining pheromone and ammonia caught fewer males than pheromone alone, but more females than ammonia alone. The decrease of male captures in the combination traps could be due to either interference of ammonia with pheromone action, or to an increase of female numbers in the vicinity of the trap in response to the S-(+)- enantiomer of the major pheromone (arrestant). Such an increase has actually been observed (Haniotakis et al. 1983, Zervas 1987). The naturally - produced pheromone of these females would compete with the pheromone dispenser on the trap, thus reducing its effectiveness. A negative correlation of pheromone

TABLE 1. Numbers of *Dacus oleae* flies captured per trap per week on yellow sticky traps baited with pheromone (Ph), aqueous ammonia solution 25% (A), and Ph+A dispensers. Means of three replicates. Preliminary experiment, Paradissos, Athens, 1982.

	Mean no. of flies captured by*					
	Ph		A		Ph+A	
Date	males	females	males	females	males	females
Oct. 7	111.0 a	4.3 b	32.0 c	38.7 d	72.0 e	94.7 f
Oct. 18	272.7 a	6.1 b	44.3 c	63.0 d	67.2 e	82.1 f

Analysis of Variance

Source of variation	d.f.	S.S.	M.S.	F-calc.	F-rec. (p=0.05)
Blocks	2	0.0051	0.0027	0.50	3.44
Sex	1	1.2113	1.2113	228.48	4.30
Treatments	2	0.6962	0.3481	65.64	3.44
Dates	1	0.0708	0.0708	13.36	4.30
Treat. x Sex	2	4.1567	2.0784	392.02	3.41
Sex x Dates	1	0.0674	0.0674	12.71	4.30
Treat. x Dates	2	0.0861	0.0431	8.12	3.44
Treat. x Dates x Sex	2	0.1378	0.0689	13.00	3.44
Residual	22	0.1166	0.0053		
Total	35	6.5483			

*Means followed by same letters are not significantly different, Duncan's Multiple Range Test, p=0.05.

TABLE 2. Numbers of *Dacus oleae* flies captured per trap per week on yellow sticky traps baited with pheromone (Ph), aqueous ammonia solution 25% (A), and Ph+A dispensers, Paradissos, Athens, 1982.

	Mean no. of flies captured by*					
	Ph		A		Ph+A	
Date	males	females	males	females	males	females
Oct. 26	115.8 a	27.7 b	23.2 c	52.7 d	43.8 e	80.0 f
Nov. 2	73.7 a	12.7 b	8.7 c	27.0 d	47.3 e	24.8 f
Nov. 10	77.9 a	6.0 b	22.8 c	27.4 d	34.7 e	26.0 f
Nov. 17	49.0 a	10.8 b	18.5 c	29.3 d	26.2 d	37.2 de
Nov. 24	6.5 a	2.3 b	5.0 c	10.5 ad	8.5 ad	16.3 d
Dec. 8	4.1 a	2.5 bc	1.5 c	3.7 ac	5.0 a	3.9 ab
Dec. 21	2.5 a	1.8 b	1.2 c	1.8 b	4.2 d	3.6 d
Dec. 30	1.1 a	0.3 b	0.5 bc	0.5 bc	0.7 ac	0.7 ac

Analysis of Variance

Source of variation	d.f.	S.S.	M.S.	F-calc.	F-req. (p=0.05)
Blocks	5	1.2456	0.2491	11.51	2.71
Sex	1	0.2097	0.2097	9.69	3.84
Treatments	2	0.3650	0.1825	8.43	3.00
Dates	7	17.9371	2.5624	118.35	2.01
Treat. x Sex	2	2.2802	1.1401	52.66	3.00
Sex x Dates	7	0.3937	0.0562	2.60	2.01
Treat. x Dates	14	0.3564	0.2550	1.18	1.69
Treat. x Dates x Sex	14	1.6195	0.1157	5.34	1.69
Residual	235	5.0879	0.0217		
Total	287	29.4951			

*For each date, mean catches of male and female flies followed by the same letter are not significantly different, Duncan's Multiple Range Test, p=0.05.

trap efficiency with insect population density has also been observed on certain occasions in *D. o-0ae* (Haniotakis unpublished data). The increase of female captures in the combination traps could also be the result of female population increase around the pheromone sources. Traps with pheromones alone do not catch females because the female arrestant does not function as a female attractant as well. The small number of females caught by these traps are attracted by their yellow colour.

Insect population densities and therefore trap catches vary significantly with the date of observation. Table 2 shows the results for 1982 and Table 3 for 1983. In general the results of Tables 2 and 3 agree with those of Table 1. However, if we examine more closely Table 3 in particular, which covers the entire period of the year during which the in-

TABLE 3. Numbers of *Dacus oleae* flies captured per trap per week on natural color plywood sticky traps baited with pheromone (Ph), ammonium bicarbonate salt (A), and Ph+A dispensers. Means of 6 replicates, Paradissos, Athens, 1983.

	Mean no. of flies captured by*					
	Ph		A		Ph+A	
Date	males	females	males	females	males	females
Mar. 29	1.2 a	0.5 b	0.8 ab	0.5 b	7.7 c	7.5 c
Apr. 5	1.8 a	0.0 b	3.8 c	2.8 a	10.7 d	14.5 e
Apr. 14	17.2 a	1.1 b	9.9 c	5.3 d	25.3 e	16.1 a
Apr. 19	2.8 a	0.0 b	0.9 c	0.5 d	4.2 e	5.1 a
Apr. 26	20.2 a	0.8 b	4.5 c	1.7 b	16.7 a	6.2 c
May 3	17.2 a	0.2 b	3.2 c	1.2 d	8.5 e	1.0 d
May 11	2.9 a	0.0 b	2.1 c	1.2 c	1.9 c	0.3 b
May 16	3.5 a	0.0 b	0.3 c	0.0 b	1.2 d	0.4 c
May 31	0.4 a	0.0 b	0.2 c	0.0 b	0.9 d	0.0 b
June 15	0.1 a	0.0 b	0.2 c	0.0 b	0.7 d	0.2 c
June 30	0.4 a	0.0 b	0.4 a	0.0 b	0.3 c	0.0 b
July 7	6.3 a	0.3 b	1.3 c	1.8 c	8.3 a	2.5 d
July 14	9.8 a	0.2 b	2.3 c	2.0 c	2.3 c	0.7 d
July 21	5.2 a	0.0 b	1.3 c	0.5 d	6.0 e	0.5 d
July 28	7.7 a	0.0 b	0.7 c	1.2 d	3.3 e	1.2 d
Aug. 4	2.5 a	0.2 b	0.5 c	0.2 b	1.2 d	0.7 c
Aug. 11	4.0 a	0.0 bc	0.3 b	0.2 b	1.3 d	1.5 d
Aug. 18	3.2 a	0.2 b	1.0 c	0.5 d	1.0 c	2.3 a
Aug. 24	3.1 a	0.0 b	0.0 b	0.8 c	1.6 d	2.0 d
Aug. 31	4.3 a	0.0 b	0.2 b	0.2 b	1.0 c	1.7 d
Sep. 8	13.7 a	0.2 b	2.8 c	3.4 c	3.4 c	3.8 c
Sep. 15	30.5 a	0.0 b	2.2 c	0.7 d	13.7 e	1.3 f
Sep. 22	22.0 a	0.5 b	2.8 c	6.6 d	9.8 e	6.3 d
Sep. 29	27.0 a	1.8 b	4.8 c	6.2 d	5.2 cd	4.8 c
Oct. 6	19.0 a	1.0 b	3.0 c	2.3 cd	8.8 e	3.5 c
Oct. 13	21.7 a	1.2 b	4.2 c	6.2 d	18.3 e	12.8 f
Oct. 20	6.5 a	1.2 b	5.8 a	3.7 ac	9.3 a	8.5 a
Oct. 27	2.5 a	0.7 b	2.5 a	2.3 a	5.0 c	3.3 c
Nov. 3	4.0 a	0.2 b	5.3 c	3.7 a	5.3 c	4.8 ac
Nov. 10	1.7 a	0.8 b	1.8 a	3.3 c	4.8 d	6.7 e
Nov. 16	0.3 a	0.2 a	2.2 b	2.2 b	2.5 b	2.7 c

Analysis of Variance

Source of variation	d.f.	S.S.	M.S.	F-rec.	F-calc. (p=0.05)
Replicates	5	1.9699	0.3940	5.99	2.21
Sex	*1*	*22.5104*	*22.5104*	*342.29*	*3.84*
Treatments	2	8.8008	4.4004	66.91	3.00
Dates	30	60.4106	2.0137	30.62	1.46
Treat. x Sex	2	18.2802	9.1401	138.98	3.00
Sex x Dates	30	10.5990	0.3533	5.37	1.46
Treat. x Dates	60	14.0300	0.2338	3.56	1.32
Treat. x Dates x Sex	60	9.4970	0.1583	2.41	1.32
Residual	925	60.8313	0.0658		
Total	1115	206.929			

*For each date, mean catches of male and female flies followed by the same letter are not significantly different, Duncan's Multiple Range Test, p=0.05.

TABLE 4. Mean numbers of *Dacus oleae* flies captured per trap per week on sticky traps baited with pheromone (Ph), ammonia-releasing dispensers (A), and Ph+A for periods during which insect response to traps varies due to differences in environmental or biological factors (critical factors).

	No. of flies captured by*						
	Ph		A		Ph+A		
Period	males	females	males	females	males	females	Critical factor**
Oct. 26–Nov. 17, 1982	77.6 d	14.0 a	17.7 c	33.1 b	35.1 b	40.8 b	2
Nov. 18–Dec. 30, 1982	3.4 a	1.7 b	2.1 b	4.1 a	4.5 a	6.1 a	1
Mar. 29–Apr. 19, 1983	5.8 b	0.4 d	3.9 bc	2.2 c	12.0 a	10.0 d	1
Apr. 20–May 16, 1983	10.8 a	0.3 e	2.5 c	1.0 d	7.0 b	1.9 cd	2
May 17–June 30, 1983	0.3 b	0.0 c	0.2 b	0.0 c	0.6 a	0.0 bc	3
July 1–July 21, 1983	7.1 a	0.2 c	1.6 b	1.4 b	5.5 a	1.2 b	4
July 22–Oct. 13, 1983	13.9 a	0.4 e	1.9 d	2.5 d	5.7 b	3.5 c	2
Oct. 14–Nov. 17, 1983	3.0 c	0.6 d	3.5 bc	3.1 c	5.4 ab	5.2 a	1

*For any particular time period, means followed by same letter are not significantly different, Duncan's Multiple Range Test, p=0.05.
**Critical factor: 1 = low temperatures, 2 = full insect activity, 3 = sexual inactivity, 4 = reduced sex activity.

sect is active, we can make the following observations (summarized in Table 4).

With regard to capture of males by ammonia, pheromone and the combination, the following periods can be distinguished: a) The cold periods (Nov. 18 - Dec. 30, 1982, Mar. 29 - Apr. 19 and Oct. 14 - Nov. 17, 1983), during which mating activity and therefore insect response to pheromone was restricted by low afternoon temperatures. Ammonia as food attractant is attractive throughout the day. During these periods an additive or slight synergistic effect of sex and food attractants was observed in the combination traps. The limited interference between sex (long-range activity) and food (short-range activity) attractants due to restricted pheromone effectiveness could be the reason for these results. b) The period of no sexual activity of the insect (May 17 - June 30, 1983), which coincided with the lack of mature fruit in the orchard. During this period males do not respond to pheromones. As was expected, the results agreed with those of the previous case and could be attributed to the same reason. c) The period of partial sexual activity (July 1 - July 21, 1983), during which the results of the combination of the two attractants appeared but were not yet significant. d) The period of insect reproductive activity (Oct. 26 - Nov. 17, 1982, Apr. 20 - May 16 and July 22 - Oct. 13, 1983), when results agreed with those of Table 1. Differences in trap catches between the various traps were not as pronounced as in Table 1, probably due to the increased trap competition allowed in this experimental design.

With regard to females, the combination of pheromone and ammonia resulted in an increase of capture of females as compared to the capture by ammonia traps throughout the year, except in the periods of insect reproductive inactivity or reduced activity. Probably females, like males, do not respond to pheromones during such periods. The effect of cool afternoons which was observed in the case of capture of males did not occur in the case of females. The fact that females responded to arrestant pheromone throughout the day as opposed to male response to the sex pheromone during afternoon hours only can very well explain this difference (Haniotakis et al. 1986a).

Simulation models indicated that combination of sex and food attractants on the same trap is the most efficient system of those tested here for mass trapping (Karandinos and Haniotakis unpublished data). This is understandable since in addition to males, a large number of females, i.e. the damaging sex, is also removed. On the basis of the above findings, it is likely that traps using only the pheromonal enantiomer which functions as a male attractant will attract more males than those baited with the racemic mixture, since in the former case competing females will not aggregate in the area of the trap. Such an attractant may be more suitable for monitoring purposes.

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KEY WORDS: *Dacus oleae*, Olive fruit fly, Sex pheromones, Sex attractants, Food attractants, Traps

Επίδραση Συνδυασμού Προσελκυστικών Φύλου και Τροφής στην Ίδια Παγίδα στις Συλλήψεις Εντόμων Δάκου

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ΠΕΡΙΛΗΨΗ

Προσελκυστικά φύλου, τα πιο ισχυρά προσελκυστικά για τα αρσενικά έντομα του δάκου της ελιάς, και αμμωνία, ένα από τα πιο ισχυρά προσελκυστικά για τα θηλυκά του ίδιου εντόμου που έχομε στη διάθεσή μας σήμερα, συνδυάστηκαν στην ίδια παγίδα. Ο συνδυασμός αυτός είχε σαν αποτέλεσμα την κατά μέσο όρο αύξηση των συλλήψεων θηλυκών εντόμων, σε σύγκριση με τις συλλήψεις των παγίδων αμμωνίας, αλλά τη μείωση των συλλήψεων αρσενικών, σε σύγκριση με τις συλλήψεις των παγίδων προσελκυστικών φύλου. Αποκλίσεις από το αποτέλεσμα αυτό παρατηρήθηκαν κατά τη διάρκεια ορισμένων περιόδων του έτους οι οποίες οφείλονται στην επικράτηση διαφορετικών φυσικών και βιολογικών παραγόντων που επηρεάζουν τη δράση των προσελκυστικών φύλου. Με βάση πρόσφατες πληροφορίες που αφορούν τη λειτουργία του συστήματος χημικής επικοινωνίας των δύο φύλων του δάκου δίδονται εξηγήσεις για τα αποτελέσματα αυτά.