(fig. 1). None of the remaining variables made a significant additional contribution to the regression (table).

Exposure of *H. pomatia* to nitrogen for 24 h always leads to a highly significant ($p < 0.001$) elevation of LDH activity in the foot of summer animals, but has no effect on winter animals in which LDH activity is already at its highest. On the other hand, the activity of the non-equilibrium enzyme, pyruvate kinase, is not affected by exposure to a nitrogen atmosphere.

This ecological approach indicates that the activity of an equilibrium enzyme in 1 particular tissue from a species of poikilothermic animals derived from a single locality is modifiable by environmental factors, mainly by time of year, day temperature, and pO_2. The mean variability inherent in this system is approximately 5-fold, ranging from a minimum of 8 to a maximum of 39 units · g f.wt^{-1} but individual minima and maxima cover more than a whole order of magnitude. The sensitive response of LDH to environmental cues can be rationalized by either of 2 assumptions:

Either, the D-LDH of this tissue does not catalyze an equilibrium reaction in vivo; or, the concentrations even of enzymes catalyzing equilibrium reactions are geared to the *maximum flux rates* expected under a given set of conditions. A change of conditions (be it exo- or endogenously determined) may lead to higher flux rates and thus to the induction of additional enzyme activity. As far as LDH is concerned a number of reports suggest that this enzyme can be activated quite rapidly and thus seems to be a site of metabolic control in certain animals[9,10].

1 I thank U. Platzer for technical assistance and D. Frank for help with the statistics. The investigation was supported by the 'Fonds zur Förderung der wissenschaftlichen Forschung in Österreich', project No. 1257 and 2919.

2 E.A. Newsholme and C. Start, Regulation in Metabolism. Whiley, New York 1973.
3 E.A. Newsholme and B. Crabtree, Trends Biochem. Sci. *6*, 53 (1981).
4 F. Rolleston, Trends Biochem. Sci. *5*, 10 (1980).
5 P.W. Hochachka and G.N. Somero, Strategies of Biochemical Adaptation. W.B. Saunders, Philadelphia, London, Toronto 1973.
6 H. Tsukuda, Comp. Biochem. Physiol. *52B*, 343 (1975).
7 W. Wieser and E. Wright, Hoppe-Seyler's Z. Physiol. Chem. *360*, 533 (1979).
8 W. Wieser, J. comp. Physiol. *141*, 503 (1981).
9 H.O. Hultin, in: Isoenzymes, vol.2, p.39. Ed. C. Markert. 1975.
10 A.F. Finn, Jr, and R.A. Lockshin, Comp. Biochem. Physiol. *68C*, 1 (1980).

Studies on the chemical constitution and sex pheromone activity of volatile substances emitted by *Dacus oleae*[1,2]

P. Gariboldi, G. Jommi, R. Rossi and G. Vita

Laboratorio di Chimica Organica, Università degli Studi di Milano, I-20133 Milano (Italy), Istituto di Chimica Organica Industriale, Università di Pisa, Pisa (Italy), and Laboratorio di Valorizzazione Colture Industriali, CNEN Casaccia, Roma (Italy), 16 April 1980

Summary. Volatile substances from the females of *Dacus oleae* have been submitted to GLC-MS analysis and several components identified. E-6-nonen-1-ol and p-cymene displayed attractive and aphrodisiac effects in both laboratory and field experiments.

Dacus oleae (Gmelin) Diptera, Trypetidae, is a serious pest of olive-trees causing considerable economic damage every year by decreasing olive-oil production, especially in the Mediterranean area.

Up to date only protein degradation products seem to be attractive to this fly, therefore the interest in a more effective and specific attractant for pest preventive control is well justified. Research on a possible pheromone in *D. oleae* started in 1971 with the work of Economopoulos et al.[3]. In recent years Haniotakis et al.[4-6] reported the isolation of a mixture of compounds displaying pheromone activity in both laboratory and field assays; however, no characterization of the components of such mixture has been so far achieved.

In this paper we report on our studies directed towards the identification of some volatile substances emitted by *D. oleae* and the evaluation of their sex-pheromone activity.

The insects were reared in perspex cages ($20 \times 20 \times 20$ cm, nylon net) at 25 ± 5 °C with a 70 ± 10% humidity and a light exposure similar to the natural one. They were fed with a mixture of hydrolyzed soy, sugar and egg-powder. Adults were immediately separated by sex after emergence.

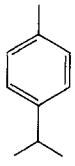
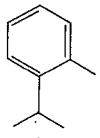
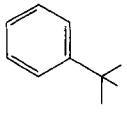
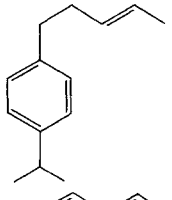
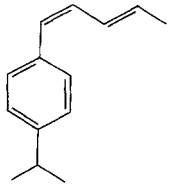
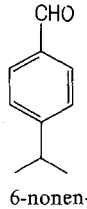
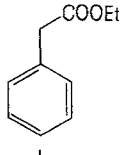
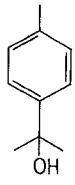
About 3000, 6 days old virgin females were caged in a 100×25 cm perspex cylinder and gently flushed with air over a total period of 9 h (last 3 h of the photoperiod for 3 days). Effluent air and volatile substances were totally condensed in a trapping system cooled by liquid nitrogen. After slow evaporation of the air and salting, the condensate was extracted several times with highly purified ethyl ether. After careful concentration at atmospheric pressure, the extracts were directly used for GLC-MS analysis without any further treatment.

After several attempts, the best gas-chromatographic resolution was obtained by injecting the mixture into a SCOT glass capillary column (CW 20 M; 40 m) working with a programmed temperature (60–200 °C; 5 °C/min). The gas-chromatographic system was connected with an LKB 2091 mass-spectrometer equipped with an LKB 2130 data system.

A typical total ion current plot is represented in the figure and the identified peaks are listed in table 1. All structure attributions were accomplished first by MS-spectra analysis and then by comparison of retention times and fragmentation of each peak with those of pure samples.

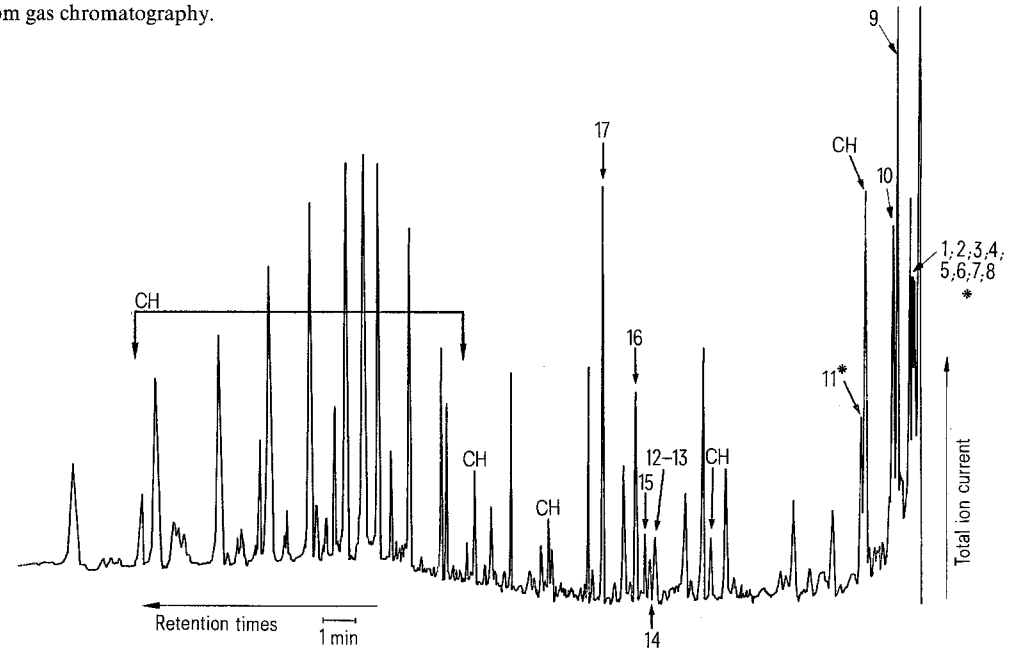
Peaks marked with CH refer to aliphatic hydrocarbons which have not been further investigated. Substances marked with an asterisk are not emitted by the insects as could be demonstrated by a GLC-MS analysis in the same conditions of a 'blank' obtained by the above described procedure but in the absence of insects. All of them, together with compounds 9, 10, 14, 16 and 17 were commercially available or easily synthesized.

Table 1. Substances identified by GLC-MS in the cold trap condensate from female *Dacus oleae*

Peak No.	Formula	Fragmentation (relative abundance %)
1*	CH_3OH	32 M^+ (58); 31 (100); 29 (78)
2*	C_2H_5OH	46 M^+ (10); 31 (100); 29 (39)
3*	CH_3CHO	44 M^+ (77); 29 (100)
4*	n-C_4H_9Br	138-136 M^+ (68); 57 (100); 56 (16.4); 55 (71); 41 (61.9)
5*	$C_6H_5CH_3$	92 M^+ (100); 91 (11.7); 77 (1.5); 51 (10.5)
6*	$C_6H_4(CH_3)_2$	106 M^+ (41.2); 105 (19); 92 (16.5); 91 (100); 77 (14)
7*	$C_6H_5C_2H_5$	106 M^+ (32); 91 (100); 77 (9.5)
8*	n-$C_5H_{11}Br$	152-150 M^+ (2); 123-121 (10); 71 (100); 57 (41); 55 (30)
9**		134 M^+ (21); 119 (100); 91 (61); 77 (28)
10**		134 M^+ (27.7); 119 (100); 91 (37.5)
11*		134 M^+ (3.5); 119 (24.3); 91 (33); 77 (11.8); 41 (100)
12		188 M^+ (4.7); 134 (12.9); 133 (100); 105 (43.5)
13		186 M^+ (23.4); 171 (15.8); 143 (100); 91 (9)
14		148 M^+ (62.9); 147 (15.2); 133 (100); 119 (36.4)
15	6-nonen-1-ol	124 (5.3); 96 (10.6); 95 (25.7); 67 (100)
16		164 M^+ (5.5); 119 (1.3); 91 (100); 65 (28)
17		150 M^+ (7.5); 135 (64); 132 (57); 117 (46); 43 (100)

* Compound not emitted by the insects. ** p- and o-Cymene are also present in the fruits of the host plant (unpublished results from our laboratory).

Recording from gas chromatography.



Alkyl aromatic hydrocarbons 12 and 13 have been prepared as a mixture of diastereoisomers and their synthesis will be the object of a further publication.

MS-spectrum of peak 15 was not easily attributable owing to its low intensity and the overlapping of at least 2 substances. As some fragmentations suggested the presence of an aliphatic alkenol, and some nonen-1-ols have been shown either to be attractants for *D. oleae*[7] or components of the pheromone of the mediterranean fruit fly, *Ceratitis capitata*[8], we tried to compare these substances with the unknown peak 15.

We had available all the 13 regio- and stereoisomers of nonen-1-ol and, by fragmentographic analysis (focusing the spectrometer on ions 124, 96, 95 and 67 m/e), we were able to detect within the peak 15 the presence of a 6-nonen-1-ol. Every attempt to assign the stereochemistry to the double bond of the natural alkenol failed, as both E and Z diasteroisomers displayed very similar fragmentation patterns and identical retention time even on several stationary phases and under different conditions.

Table 2. Attractiveness to males displayed in an olfactometer by the substances emitted by females of *D. oleae*

Treatment	Total number of tests	Medium number of visits	Range
Whole extract from 50 females	10	15	8–25
p-Cymene	10	12	8–17
E-6-Nonen-1-ol	10	14	11–25
Blank	20	3	0– 6

Table 3. Number of *D. oleae* captured during 7 weeks by 4 traps

Treatment	Captured males	Captured females	Total
Blank	42	84	126
p-Cymene	59	151	210
E-6-Nonen-1-ol	76	156	232
p-Cymene/E-6-Nonen-1-ol 1/1	57	118	175

Laboratory bioassays were run in a cubic (60 cm) plexiglass olfactometer similar to the model of Beroza and Baker. 3 samples alternated with 3 blanks were exposed to 100, 3–5 days old males and tested for 1 h. Each population was utilized for 7 days. Counting of the visits of the insects to the samples (paper rolls absorbed with 1 µl of a n-pentane solution (1:1 weight) were positioned on a branch rotating at 1 rpm) was done during the last 3 h of the photoperiod using a television recording system.

The results for p-cymene and E-6-nonen-1-ol in comparison with the whole mixture of substances emitted by females are summarized in table 2.

Field evaluation of the attractiveness of the same 2 compounds was run in an olive-grove with plants of the same size and belonging to the same variety. The population dynamics of *D. oleae* were investigated using 9 blank traps, which were placed at the center of the sectors into which the field was divided.

5 mg of each substance or mixture were supported on 2.3 g of cellulose and placed in a yellow triangular 'delta' shaped trap (wall size: 10 × 15 cm) where 2 inner walls were coated with silicone glue; 4 such traps were placed within each sector. The experiment lasted 7 weeks; every week captured insects were numbered by sex and the chemical attractant replaced by a fresh sample. The results are summarized in table 3.

As shown in table 3, both the tested compounds induce an appreciable increase in the captures, whereas a 1:1 mixture of them displays a reduced activity. In relation to the sex of the captured insects, a higher activity towards the females is observed for p-cymene, whereas E-6-nonen-1-ol does not alter the ratio females: males in comparison with the blank. These results seem rather promising in view of a large-scale field application, where the largest possible number of captured females is generally desirable.

The 2 compounds emitted by the females of *D. oleae* so far tested influence the behavior of the insect, displaying both an attractive and an aphrodisiac effect; the former seems to be exerted on both sexes, whereas the latter is predominantly perceived by the males.

The assertion of some authors[4–6] that there is of a pheromone emitted by the females of *D. oleae* is in contrast to other results[3,9] which demonstrate that chemical signals

having a pheromone effect are emitted by the males of this insect.

For Trypetidae Diptera sex pheromone emission has up to now been attributed to the males and, with the one exception of *Ceratitis capitata* Wied.[8] no chemical characterization of these substances has been accomplished. However for this insect also field experiments[10] did not ascertain the sexual specificity of the substances previously reported as sex pheromones.

For *D. oleae* we can affirm that the females emit chemical signals both attracting and aphrodisiac; however, their direction in sexual terms has not so far been ascertained.

1 Part of this work has been presented at the 4th International Congress of Pesticide Chemistry (IUPAC), Zurich, July 1978 - Comm. III, 230 and at the 11th International Symposium on the Chemistry of Natural Products (IUPAC) Varna, September 1978.

2 This work has been supported by the CNR, special 'ad hoc' program 'Fitofarmaci e Fitoregolatori' subproject 2.

3 A.P. Economopoulos, A. Giannakakis, M.E. Tzanakakis and A.V. Vouyadjoglaou, Ann. ent. Soc. Am. *64*, 2 (1971).

4 G.E. Haniotakis, Envir. Ent. *3*, 82 (1974).

5 G.E. Haniotakis, Ann. Zool. Ecol. Anim. *9*, 273 (1977).

6 G.E. Haniotakis, B.E. Mazomenos and J.H. Tumlinson, Ent. exp. Appl. *21*, 81 (1977).

7 R. Rossi, A. Carpita and G. Vita, Gazz. chim. ital. *108*, 709 (1978).

8 M. Jacobson, K. Ohinata, D.L. Chambers, W.A. Jones and M.S. Fujimoto, J. med. Chem. *16*, 248 (1973).

9 L. De Marzo, G. Nuzzacci and M. Solinas, Entomologica *14*, 203 (1978).

10 K. Ohinata, M. Jacobson, S. Nakagawa, M.S. Fujimoto and H. Higa, J. Envir. Sci. Health *A12*, 67 (1977).

Inhibitory effect of fasting on the glucagon-induced increase of liver phosphorylase A activity in rats[1]

Š. Németh, J. Kolena and I. Klimeš

Institute of Experimental Endocrinology, Centre of Physiological Sciences, Slovak Academy of Sciences, 833 06 Bratislava (Czechoslovakia), 1 June 1981

Summary. The increase of liver phosphorylase A activity observed 1, 2 and 3 min after i.v. administration of 0.1 μg kg^{-1} glucagon to fed rats was found to be completely absent in 24 h fasted animals, although there is even an exaggerated liver cAMP response to glucagon after fasting.

In a previous paper from our laboratory[2], maximal increases in the activity of the active (A) form of liver glycogen phosphorylase (EC 2.4.1.1) in rats subjected to low 'stress-producing' doses (400 revolutions during 6 min and 40 sec) of the Noble-Collip[3] drum procedure were described. An important stimulator of phosphorylase A activity is glucagon, which increases the level of cAMP and activates the whole glycogenolytic cascade (for review see Van de Werve[4]). Under the conditions of the above-mentioned procedure, we have also demonstrated the release of glucagon[5] and an increased level of hepatic cAMP[2]. However, fasting for 24 h substantially reduced both glucagon release (unpublished results) and enzyme response[6] during the stress. Yet the possibility that decreased tissue sensitivity to glucagon might also be involved after fasting cannot be ruled out on the basis of the results available.

In this paper, the phosphorylase response of fed and 24 h fasted rats to physiological doses of exogenous glucagon is compared. As glucagon is able to increase enzyme activity within seconds[7], and the response to the drum procedure also appears very rapidly (after 2 min), phosphorylase activity was studied during the 1st 6 min after i.v. glucagon. Our results have shown the absence of phosphorylase response to 0.1 μg kg^{-1} of glucagon after fasting.

Materials and methods. Adult, male SPF rats of the Wistar strain with an average weight of 330 g supplied by VELAZ (Prague) were used. They were kept at a constant light (from 06.00 h to 18.00 h) - dark schedule and on a standard laboratory diet (caloric percent protein 25, carbohydrate 53, lipid 22, minerals and vitamins added) and tap water. The animals were allowed to adapt to the conditions of our animal house for 3 weeks. Before glucagon administration, which took place during the morning hours, 50% of the animals were fasted for 24 h. Both fed and fasted rats were anaesthetized with Pentobarbital (SPOFA, Prague, 50 mg kg^{-1} i.p.) and injected via a tail vein with 1.0 ml kg^{-1} of saline (controls) or 1.0 ml kg^{-1} of a glucagon solution (Eli Lilly and Comp., USA) in saline. In a pilot study in which only phosphorylase activity was determined the hormone concentration was 0.2 μg ml^{-1} and the animals were studied

Effect of 24 h fasting in rats on the response to an i.v. bolus of 0.1 μg kg^{-1} of glucagon. Means of 6 values per group ± SEM

	Fasting	Control	Min after glucagon 1	2	3	6
cAMP nmole g^{-1}	+	5.10±0.95	19.06±3.40[c,e]	7.94±1.68	6.20±0.37	-
(protein)	−	5.72±0.59	9.08±1.39[a]	5.74±1.31	5.45±0.65	3.07±0.22[c]
Phosphorylase	+	15.3±1.6	18.8±2.2[f]	15.8±1.7[h]	13.5±1.6[h]	-
Form A	−	18.9±2.4	26.1±1.2[a]	34.8±2.4[d]	34.2±2.4[c]	13.2±1.1
Total	+	48.3±1.9	45.1±2.6	43.4±6.4	42.2±2.5	-
	−	56.2±3.9	50.7±2.3	50.9±2.2	46.2±3.1	44.4±1.5[b]
Percent A	+	31.9±3.9	43.8±8.0	37.8±3.8[g]	32.7±4.3[h]	-
	−	33.2±2.9	51.5±1.9[d]	69.0±5.8[d]	75.0±4.9[d]	29.9±3.1

[a] Against control significant at $p<0.05$; [b] at $p<0.02$; [c] at $p<0.01$; [d] at $p<0.001$; [e] Against fed significant at $p<0.05$; [f] at $p<0.02$; [g] at $p<0.01$; [h] at $p<0.001$.