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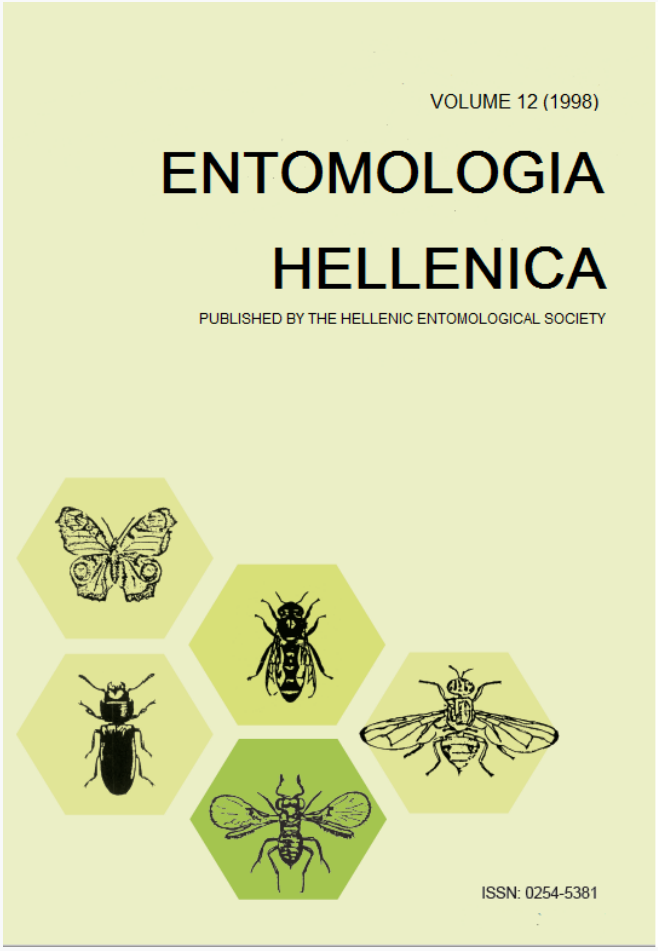

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Elecroantennogram Responses of the Cherry Fruit Fly *Rhagoletis cerasi* (Diptera: Tephritidae) to Naturally Occurring Volatiles[1]

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ABSTRACT

EAG responses of the cherry fruit fly, *Rhagoletis cerasi* (Linne) (Diptera: Tephritidae) were recorded in response to seventy-eight generally occurring plant volatiles and male cherry fruit fly volatiles. The test compounds are representatives of three major classes of organic compounds, aldehydes, ketones, and monoterpenes. No differences were observed in the degree of response between males and females. Carbon-chain length, unsaturation, and position of functional groups all have significant effect on the magnitude of EAG response.

Introduction

In phytophagous insects, host location and acceptance is the result of a sequence of behavioural responses to a complex array of visual, olfactory, gustatory and mechanical stimuli (Averill et al., 1988). Among these, host kairomones in combination with sex pheromones, play the major role for oligophagus insects such as the *Rhagoletis*, where the host plant is used as a rendezvous site for courtship, mating, oviposition site selection and egg-laying. Discrimination among host- and non-host-plants depends on the insect's ability to detect and process essential chemical information. Several species of phytophagous insects show specific behavioral responses to typical odours of their host plants (Visser, 1986). Experiments with sibling species such as the apple maggot (*Rhagoletis pomonella*) and the blueberry maggot (*R. mendax*), indicate that antennal sensitivity is selectively adapted to species-specific host fruit odours (Frey and Bush, 1990).

The fragrance of a plant and, in particular, a ripening fruit, is a complex blend of tens to nearly hundreds of volatile compounds, many relatively unique and others extremely common; having various functional groups and ranging in structure from simple, short, straight carbon-chains to complex multi-ring sesquiterpenes (Van Straten and Maarse, 1983).

In the last few years research has been increasing on the olfactory system of insects, with the aim of devising new strategies using semiochemicals for the development of alternative pest management methods (Topazzini et al., 1990).

Oviposition deterring pheromones have been identified in *R. cerasi* and their efficiency for control has been tested with promising results (Boller et al. 1987). Sex attractant pheromones have also been found (Katsoyannos, 1982) and some active compounds have been identified (Raptopoulos et al., 1995).

However, as Baker (1993) points out, successful pest management with semiochemicals re-

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quires adequate knowledge of the "insect language" (i.e. communication system) involved.

The purpose of this initial study was to investigate by means of EAGs the olfactory selectivity of adult *R. cerasi* to commonly occurring classes of insect, host-plant, and fruit volatiles. This research is further intended to provide a basis for single-cell responses by surveying and assessing the degree that the antennal olfactory system of the cherry fly is receptive to classes and/or particular plant/fruit volatiles.

Materials and methods

Insects

The insects used in this study, were collected as larvae from infested cherries, from the Pelion and Crete regions (Greece). After emergence, adults were sexed and kept on adult *Bactrocera oleae* diet (Tsitsipis 1974), at 25±2oC, and 60±5% R.H., under a 16:8 light : darkness regime.

EAG Studies

Preliminary EAG recordings were obtained from antennae on excised male and female *R. cerasi* heads. All compounds were tested on three individuals of each sex. Since statistical analysis (Student *t* test, p=0.05) revealed no difference in the degree of response between males and females, additional five replications for subsequent evaluation were limited to females only.

For EAG recordings, the indifferent glass capillary microelectrode was positioned into the hemocoel of the cranial cavity, while the recording electrode made contact to the distal end of the funiculus. The microelectrodes used (GC150F-10, Clark Electromedical Instruments, U.K.) contained Ag electrodes, and 0.1N aqueous solution of KCl as electrolyte. (Van der Pers et al. 1984). The electrodes were connected to a high impedance amplifier (Un-03 Syntech General Research Instruments, Borneolaan 4, NL-1217 HA Hilversum, Netherlands).

All compounds tested were dissolved in hexane. For each compound, aliquots (10 μl) containing 10 μg of the compounds tested were pipetted onto a filter paper (1 cm x 2 cm) which, after solvent evaporation, was placed inside a Pasteur pipette. Hexane was also used as control. In each experiment, a new pipette was used. Air was purified by means of an activated charcoal trap, then "puffed" through the pipette and onto the preparation by an air pump (CS-27, Syntech). Stimulus duration was adjusted to 6 ltr/min for 0.7 sec, while the constant air current that bathed the antenna was 5 ltr/min. A valve, when activated, diverted the air current through the test compound pipette. The antennal EAG signals were recorded, digitised, stored, and analysed on a PC, using the EAG v.3 program (Syntech). The various stimuli were randomly presented to the insect. An interval of 60 sec between two consecutive stimuli was found to be necessary for full recovery of the antenna. Every compound was tested on at least five individuals.

The examined series of compounds ranged between 6 to 14 carbon atoms in chain-length, and belonged to three general structural/functional classes (aldehydes, ketones and terpenes). These chemicals have either been identified in the behaviourally active fractions of the headspace volatiles of male insects (Raptopoulos, et al., 1995), or are known to be components of the "General Green Leaf Volatile Complex" (Guerrin et al. 1982, Light and Jang 1987, Visser 1986, Light et al. 1988) or have been identified in cherry volatiles (Schmid and Grosch 1986, Mattheis et al. 1992) (Table 1).

The insect's EAG response to a tested compound was evaluated by the maximum negative deflection (–mV) produced by that compound after subtracting the control. The –mV values to test compounds obtained in this way were transformed to percentages relative to the response elicited by 6-methyl-5-hepten-2-one, which was used as standard (EAG Response=100%). This compound was selected because it was identified both in cherry (Mattheis et al. 1992), and insect volatiles (Raptopoulos et al., 1995). The transformation of absolute mV values into percentages provides for a better evaluation of the relative response activities of the various compounds obtained from the same insect, as well as from different insects (Payne 1975). Furthermore, this procedure minimises the variability due to both the presentation order of the various compounds to the insects, and to the degree of the antennal response which is a time dependent variable (Light and Jang 1987).

Results and discussion

No difference was observed in the degree of response between males and females. According to Light et al. (1988), this similarity in antennal responses between males and females suggests the common ecological need for discrimination and assessment of host plant and/or habitat recognition. Sexual dimorphism in EAGs to plant or insect volatiles has also not been found in other tephritids, as in *Rhagoletis pomonella* (Fein et al., 1982), *Bactrocera oleae* (Van der Pers et al., 1984), *Anastrepha ludens* (Robacker et al., 1986), *A. ludens* (Robacker and Hart 1987) and *Dacus dorsalis* (Light and Jang 1987). However compound-dependent sexual dimorphism in EAGs has been observed for *Ceratitis capitata* to plant volatiles (Light et al. 1988, 1992) and male emissions (Jang et al. 1989).

Table 1 shows the results obtained by electrophysiological studies of female cherry fruit flies to a wide spectrum of naturally occurring volatiles.

Saturated aldehydes: Among straight chain aldehydes, nonanal exhibits the highest EAG res-

TABLE 1: Source and purity of chemicals used in electrophysiological studies, their presence in cherry and male *Rhagoletis cerasi* volaties, the mean eag response (X% of standard) recorded from female antennae and their respective EAG ranking.

Chemicals Tested	Source[1]	Purity[2]	Cherries[3]	Male Volat.[4]	EAG ±SEM[5]	EAG Ranking[6]
Saturated aldehydes						
Isobutyraldehyde	A	99%			40.3 ± 8.2	47
Isovaleraldehyde	A	97%			97.6 ± 5.4	11
2-Methylbutyraldehyde	A	95%			50.7 ± 5.5	39
Benzaldehyde	A	99%	+	I	100.4 ± 9.9	8
Hexanal	A	98%	+	I	29.4 ± 6.0	64
Heptanal	A	95%	+	Tr	48.2 ± 5.1	41
Octanal	A	99%	+	I	62.6 ± 3.7	27
Nonanal	A	95%	+	M	69.8 ± 5.1	20
Dodecanal	A	95%		I	63 ± 5.2	26
Tridecanal	A	90%		I	57.4 ± 5.3	31
Unsaturated aldehydes						
E-2-hexenal	A	99%	+		44.8 ± 5.1	44
E-2-heptenal	A	97%			59.4 ± 5.3	29
E-2-nonenal	A	97%		I	81.7 ± 2.6	16
2-undecenal	A	90%		Tr	73.1 ± 4.6	19
Ketones						
3-Methyl-2-butanone	A	99%			10.1 ± 2.2	78
3-Hydroxy-2-butanone	A	95%			26.1 ± 3.1	69
4-Methyl-3-penten-2-one	A	90%		M	50.4 ± 6.1	40
2-Methyl-3-pentanone	A	97%			57 ± 8.0	32
4-Methyl-2-pentanone	A	99%			37.3 ± 5.9	54
2-Methylcyclopentanone	A	98%			107.3 ± 8.1	4
3-Methylcyclopentanone	A	97%			138.5 ± 10.1	2
1,3-Cyclopentanedione	A	97%			13.6 ± 4.5	77
6-Methyl-5-hepten-2-one	A	99%	+	I	100 ± 5.7	10
2-Hexanone	A	98%		M	100.5 ± 12.8	7
3-Hexanone	A	98%		I	101.2 ± 9.1	6
2-Heptanone	A	98%			55.7 ± 8.1	33
3-Heptanone	A	99%		M	145.3 ± 8.9	1
4-Heptanone	A	98%			79.1 ± 10.1	18
2-Octanone	A	96%			112.8 ± 10.3	3
3-Octanone	A	98%			104.8 ± 11.2	5
2-Nonanone	A	99%		Tr	100.4 ± 11.0	9
2-Decanone	A	98%			44.2 ± 5.6	45
3-Decanone	A	98%			54.8 ± 6.4	34
4-Decanone	A	98%		Tr	92.5 ± 5.2	13
2-Undecanone	A	99%			54 ± 2.8	36
2-Tridecanone	A	99%			33.2 ± 3.9	60
Monoterpenes						
2-Carene	U	90%			27 ± 5.3	67
3-Carene	A	95%		Tr	37.4 ± 3.0	52
α-Pinene	A	97%		I	24.6 ± 6.0	70
β-Pinene	A	95%		I	27.9 ± 2.7	65
α-Terpinene	A	85%			36.3 ± 4.2	56
β-Terpinene	U	90%			24.1 ± 4.6	72
β-Citronellene	U	90%			51.2 ± 4.7	38
β-Phellandrene	U	85%		M	79.7 ± 12.4	17
p-Cymene	A	95%		Tr	23.5 ± 6.5	73
R-Limonene	A	97%		M	44 ± 6.5	46
Alloocimene	U	70%			19.3 ± 1.9	74

TABLE 1 (continued)

Chemicals Tested	Source[1]	Purity[2]	Cherries[3]	Male Volat.[4]	EAG ±SEM[5]	EAG Ranking[6]
Myrcene	U	88%			54.2 ± 7.1	35
Sabinene	A	98%			65.5 ± 5.6	22
Oxygenated Monoterpenoids						
Eugenol	A	99%			31 ± 7.1	63
Cineol (Eucalyptol)	U	85%			27.8 ± 6.9	66
Myrtanol	U	90%			36.7 ± 4.6	55
Geraniol	A	98%	+		59.4 ± 2.5	30
Nerol	A	97%			24.5 ± 4.3	71
Linalool	A	92%	+	Tr	37.4 ± 3.8	53
Veratrol	U	94%			85.9 ± 8.7	15
(1R)-(+)-Fenchol	A	97%			51.6 ± 5.6	37
Pinocarvol	U	90%			68.8 ± 7.9	21
D-Carveol	V	90%			14.2 ± 2.1	76
L-Carveol	V	90%			33.6 ± 4.3	59
R-Borneol	V	96%		Tr	37.8 ± 6.0	51
S-Borneol	V	96%			14.8 ± 2.5	75
β-Citronellol	A	95%			93.3 ± 5.6	12
Citral	A	95%			47.8 ± 3.7	42
R-Camphor	A	98%		I	65.4 ± 3.4	23
S-Camphor	A	96%			61.2 ± 3.3	28
(1R)-(-)-Fenchone	A	98%			47.1 ± 2.9	43
D-Carvone	V	90%			32.2 ± 3.9	61
L-Carvone	V	90%			26.9 ± 3.5	68
Thujone	A	98%			91.2 ± 1.3	14
Verbenone	U	96%			34.1 ± 3.7	58
(D,L)-Piperitone	U	93%			38.1 ± 6.7	50
Pinocarvone	U	90%			64.4 ± 8.8	25
Camphorquinone	U	88%			38.2 ± 4.4	49
Geranyl acetate	A	98%	+	M	64.9 ± 4.4	24
Neryl acetate	A	98%			31.7 ± 4.5	62
Bornyl acetate	A	97%			38.5 ± 3.8	48
Lactone						
γ-Nonalactone	A	98%	+	M	36 ± 8.0	57

[1] Source of the chemicals used; Aldrich Chemical Co (A), Vioryl (V), compounds provided by the laboratory of Chemistry, University of Wales, origin presently unknown (U).
[2] Purity (%) of the compounds used for EAGs, checked by GC.
[3] Compounds identified in cherry volatiles and extracts (Schmid and Grosch 1986, Mattheis et al. 1991).
[4] Compounds identified in the male Rhagoletis cerasi volatiles (Raptopoulos et al., 1995). M=Major, I=Intermediate, Tr=Trace.
[5] EAG response of the female antenna, based on the response to 6-methyl-5-hepten-2-one (100%).
[6] EAG ranking was based on the mean EAG response from highest (1) to lowest (78).

ponse. There was a stepwise progressive decrease in the antennal responsiveness as chain length increased or decreased. Benzaldehyde, a common constituent of the cherry volatiles, showed high EAG response.

Unsaturated aldehydes: Insects were generally more responsive to monoenic-unsaturated straight-chain aldehydes than their saturated analogues. Again, the 9-carbon aldehyde, (E)-2-nonenal showed higher EAG response than unsaturated aldehydes having more or less carbon atoms.

Ketones: EAG responses to ketones were greater than those to complementary aldehydes in all but one case. The position of the carbonyl group for certain of these ketones is more important than carbon-chain length. Furthermore, ketones that have been identified in the volatile emissions of *Rhagoletis* cerasi males (Raptopoulos et al., 1995), exhibit significantly higher EAG response than analog compounds, e.g. 3-heptanone vs. 2-heptanone or 4-heptanone, and 4-decanone vs. 2-decanone or 3-decanone.

Monoterpenes and Oxygenated Monoterpenoids: Terpenoid compounds with hydroxyl or carbonyl groups, generally, show higher EAG response than those of related aliphatic terpenes. Among the aliphatic terpenes tested, β-phellandrene showed the highest EAG response. Geraniol and geranyl acetate showed higher response than their respective stereoisomers (nerol and neryl acetate respectively). Both compounds have been identified as cherry volatiles and the acetate was found also in the volatile emissions of the cherry fruit flies.

Compounds that elicit the greatest EAG response, stimulate the greatest number of chemoreceptors. The antennal responses to the compounds tested ranged between 0.3 and 4.5 mV. Generally, carbon-chain length, unsaturation, and position of functional groups all have significant effect on the magnitude of EAG response. Volatility also plays an important role in the EAG response (eg. 1,3-cyclopentadione, a compound with low volatility, gives practically no response. A key factor that influences the magnitude of the antennal response to the various compounds tested, was the presence or absence of these compounds in either the volatiles of host fruit or insect emissions.

Green-leaf volatiles along with plant species-specific blends of important discriminatory compounds (eg. terpenes and their analogues), have been shown to dominate the EAG responsiveness of oligophagous insects (Visser, 1986). Many constituents of essential oils of plants or fruits are attractive to tephritids; e.g. *Dacus dorsalis* males to methyl eugenol (Steiner, 1952) and *Bactrocera latifrons* to α-ionol (Flath, et al., 1994). The results of this work are but a partial contribution to the elucidation of chemical-odor relationship. A far greater amount of information is needed in order to identify clear structure-activity relationships for *R. cerasi* and Tephritids in general.

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KEY WORDS: *Rhagoletis* cerasi, cherry fruit fly, EAG, plant volatiles.

Ηλεκτροφυσιολογικές αποκρίσεις της μύγας του κερασιού *Rhagoletis cerasi* (Diptera: Tephritidae), σε φυσικές πτητικές ουσίες

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ΠΕΡΙΛΗΨΗ

Πραγματοποιήθηκαν ηλεκτροφυσιολογικές παρατηρήσεις της μύγας των κερασιών *Rhagoletis cerasi* (Linne) (Diptera: Tephritidae) για εβδομήντα-οκτώ ουσίες από τρεις κύριες κλάσεις οργανικών ενώσεων (αλδεΰδες, κετόνες και μονοτερπένια) σε ηλεκτροαντενογράφο (ΗΑΓ). Δεν παρατηρήθηκε καμία διαφορά μεταξύ αρσενικών και θηλυκών εντόμων στον βαθμό απόκρισης για τις ουσίες που δοκιμάστηκαν. Πιστοποιήθηκε ότι το μέγεθος απόκρισης των εντόμων στις διάφορες ουσίες εξαρτάται από το μέγεθος της αλυσίδας των μορίων, την ύπαρξη διπλών δεσμών και την ύπαρξη και θέση χαρακτηριστικών ομάδων.