HGCA

PROJECT REPORT No. 294

CONTROL OF SLUG AND SNAIL DAMAGE USING LOW TOXICITY, PLANT-DERIVED REPELLENTS AND ANTIFEEDANTS

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CONTROL OF SLUG AND SNAIL DAMAGE USING LOW TOXICITY, PLANT-DERIVED REPELLENTS AND ANTIFEEDANTS

by

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P.S. Publication of this report was significantly delayed pending application by Rothamsted Research for Intellectual Property protection to cover some of the project findings.

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ABSTRACT

This study has shown that slug feeding can be manipulated using a range of secondary metabolites found in plant tissues. Chemicals have been identified which reduce or prevent slug feeding when incorporated into wheat flour pellets or when sprayed onto plant leaves.

In a systematic study of the family Apiaceae (*Umbelliferae*) species representing thirty-two of the forty-two genera found in the UK yielded extracts which influenced slug feeding behaviour. Eleven antifeedant chemicals were identified including alcohols, aldehydes and alkaloids suggesting that slug feeing may be controlled by chemicals which have different physiological and toxicological characteristics, some of which may be suitable for practical application as plant protectants.

When the most active slug feeding deterrent, the alkaloid identified from hemlock, was sprayed onto young Chinese cabbage plants exposed to large numbers of starved slugs the plants remained undamaged over the 24-hour test period.

The most serious damage to cereals caused by slugs is done to the newly-sown seed when the embryo is eaten and germination prevented. A seed treatment which deterred slug feeding until the plant started active growth would largely solve the problem. In this initial investigation eleven chemicals with slug antifeedant activity were identified in a survey of only thirty-three plant species. Any one of these may have the correct combination of antifeedant activity, phytotoxicity and persistence for successful deployment as a seed dressing and there must be very many more waiting to be found.

Development of a successful antifeedant cereal seed dressing would largely obviate the need to use conventional molluscicide pellets, with consequent savings in cost and a reduction in pesticide usage on the crop.

1. SUMMARY

Two main techniques were developed to look for naturally-occurring plant secondary metabolites which could be used to deter slug feeding on crop plants. An electrophysiological method was used to record the electrical activity induced in the tentacular nerve of the field slug, *Deroceras reticulatum* (Müller) when the tentacle receptors were exposed to volatile extracts of selected plant species. A complementary feeding bioassay was used to measure the reduction in slug feeding when these extracts were added to a standard food.

A systematic examination was made of the plant family Apiaceae (Umbelliferae) which contains crop plants (e.g. carrot), herbs (e.g. coriander) and weeds (e.g. hemlock). Thirty-three species representing thirty-two genera were tested. Extracts of twenty-one species elicited nervous activity in the tentacular nerve and reduced slug feeding on the standard diet. The most active extracts were from hemlock (*Conium maculatum*), coriander (*Coriandrum sativum*) and parsley (*Peteroselenum crispum*). Eleven active chemicals were identified, three of which significantly reduced slug consumption of the standard diet. When the most active compound, from hemlock, was sprayed onto Chinese cabbage in glasshouse tests, no slug feeding damage ocurred. These chemicals may have commercial potential for protecting seeds or foliage from slug damage.

2. OBJECTIVES

The overall objective was to explore alternative methods of reducing slug damage which did not rely on molluscicidal baits, with the intention to improve crop protection and to reduce pesticide inputs.

The specific objectives were:

(i) Development of an electrophysiological technique for detecting behaviourally-active chemicals.

(ii) Systematic examination of a group of plants for activity.

(iii) Identification of chemicals conferring activity.

(iv) Assessment of their potential for protecting slug damage-susceptible crop plants.

3. INTRODUCTION

3.1 SLUGS: PEST STATUS AND CURRENT CONTROL METHODS

Slugs are perceived as being one of the most serious agricultural pests in temperate countries. The field slug *D. reticulatum*, is important because it is widespread, attacking the seed, foliage or storage organs of many economically important crops. The most important damage, in economic terms, is that done to potato tubers and winter wheat seeds and seedlings (Port & Port, 1986). Other plants susceptible to attack from *D. reticulatum* include oilseed rape and sugar beet just after germination. Much economic loss is a result of slugs grazing the surface causing cosmetic damage resulting in loss of value of the crop, as is the case with vegetables for human consumption such as brussels sprouts, celery stalks and carrots. Contamination of produce is also a problem in salad crops such as lettuce and in peas for freezing. Slugs have also been implicated in transmission of plant pathogens, for example the fungus *Mycocentrospora acerina* affecting carrots and the bacterium *Erwinia carotovora* affecting potatoes.

There are few reliable estimates of direct losses to crops caused by slugs. One indirect measure of size of the problem nationally is the cost of control products purchased. Poison baits delivering the chemicals metaldehyde, methiocarb or thiodicarb are currently the most extensively used method of control. Metaldehyde has been in use since the 1940's

(Gimmingham, 1940). On ingestion of bait pellets containing metaldehyde, slugs exhibit uncoordinated locomotion leading to paralysis. There is a dramatic increase in mucus secretion and death is caused at least in part by dehydration (Mills *et al*, 1993). The carbamates methiocarb and thiodicarb act as a stomach poisons causing hyperactivity leading to immobilization and death.

Currently, over 800,000 hectares of agricultural and horticultural crops in Great Britain are treated with molluscicides each year, metaldehyde accounting for 55%, methiocarb 40% and thiodicarb 5% of the total area treated. The remainder is made up by compounds such as aluminium sulphate or sodium tetraborate (Garthwaite & Thomas, 1996). Due to factors such as the switch to autumn sowing, sowing wheat after oilseed rape and the ban on stubble burning, there has been a dramatic increase in slug pellet usage. The area treated with molluscicides has increased 67-fold since the early 1970's, while the total amount of active ingredient has increased from less than 7 to more than 250 tonnes (Table 1).

Four main commodity groups are treated with molluscicides; arable crops, fodder crops, vegetables and soft fruit (Table 2). Usage on arable crops, in particular on oilseed rape, winter wheat and potatoes, accounts for 99% of the total area treated by molluscicides. The estimated annual cost of mollusc control to the agricultural and horticultural industry using metaldehyde and methiocarb alone is estimated to be around £9 million (Garthwaite & Thomas, 1996).

Table 1 Estimated annual usage of molluscicides in Great Britain (tonnes a.i. applied)

Molluscicide	1970-74	1975-79	1980-83	1984-89	1990-93	1994-95
Metaldehyde	5.2	12.9	81.8	87.3	85.8	190.5
Methiocarb	1.4	9.8	130	84.4	25.5	54.7
Thiodicarb	-	-	-	-	<0.1	6.7
Total	6.6	22.7	211.8	171.7	111.3	251.9

From Garthwaite and Thomas (1996)

Table 2 Estimated annual usage of molluscicides on the four main commodity groups in Great Britain (hectares treated)

	1970-74	1975-79	1980-83	1984-89	1990-93	1994-95
Arable crops	6,862	41,890	666,655	518,698	291,948	800,366
Fodder crops	2,672	12,804	9,839	3,345	22,121	*
Vegetables	894	1,344	3,678	5,781	5,289	9,055
Soft fruit	780	1,334	2,021	3,067	2,008	2,393
Total	11,208	57,372	682,193	530,891	321,366	811,814

* not surveyed during this period From Garthwaite & Thomas (1996)

These data illustrate the level of damage sustained by crops each year and emphasise how current control measures rely almost entirely on chemical control with metaldehyde or methiocarb. Chemical control with toxic baits is becoming less acceptable as it is both unreliable and poses risks to non-target organisms. One major drawback of using pelleted baits is that sufficient bait to provide a lethal dose is not always ingested and under wet conditions, slugs immobilised by metaldehyde can rehydrate and recover (South, 1992). The risks posed by slug baits to other animals are well documented (Booze & Oehme, 1985; Studdert, 1985), and as all but one of the bait poisons currently used were originally developed as insecticides or acaricides, the hazard for non-target animals is implicit. Cases of poisoning of non-target organisms have been noted with hedgehogs, birds, small mammals (Reece *et al*, 1985; Keymer *et al*, 1991), farm and domestic animals (Sutherland, 1983; Giles *et al*, 1984; Booze, 1985; Baum, 1986) and predatory beetles (Purvis, 1996).

3.2 ALTERNATIVE CROP PROTECTION METHODS: ANTIFEEDANTS

The shortcomings of chemical control have encouraged research into alternative means of reducing crop damage without recourse to the use of toxic materials. Current projects include biological control by exploiting the slug-parasitic nematode, *Phasmarhabditis hermaphrodita,* (Glen *et al*, 1996), cultural control methods such as the manipulation of common weed populations as an alternative food source in winter wheat crops (Cook *et al*, 1996) or increasing control through predation by carabid beetles (Symondson *et al*, 1996).

An alternative 'chemical' control strategy is the identification of naturally-occurring, plant-derived chemicals which actively deter feeding (Dodds *et al*, 1996; Dodds, 1996; Dawson *et al*, 1996; Powell & Bowen, 1996), as described in this report.

3.3 THE ROLE OF CHEMORECEPTION IN FEEDING BEHAVIOUR

A primary function of chemoreception is food location and selection and the whole process may be regarded as "a complex series of events leading to ingestion of a potential food source," (Kpikpi & Thomas, 1992).

However, many plants contain secondary metabolites which have no metabolic function except to alter the palatability (Cates & Orians, 1975). By serving as attractants, repellents or toxins, these semiochemicals can lead to extremely specialised associations between animals and plants (Cates & Orians, 1975; Rice *et al*, 1978; Lincoln & Langenheim, 1979; Gouyon *et al*, 1983, Garraway, 1992). A strong selective advantage must therefore exist for a mechanism capable of locating food via these chemical cues (Suzuki, 1967; Preston & Lee, 1973; Townsend, 1973a; Audesirk, 1975; Croll & Chase, 1977, 1980; Croll, 1983).

Terrestrial molluscs have a wide range of foods in their diet, although they do show some preferences. Foods range from living tissue to decaying organic matter and occasionally animal matter (Port & Port, 1986). Shah (1992), reported that the snail *A fulica* fed on vegetable crops, fruit trees, plantation crops, ornamentals and even transplanted rice. Taylor (1902-1907), described how the slug *Milax gagates* rejected only 22 out of 195 different plant foods offered and Frōmming (1954) described the slug *A. ater* as a "polyphagous species". When *Arion hortensis, Arion circumscriptus* and *Arion intermedius* were offered leaves and flowers from 40 different wild plants, each species fed on at least 90% and *A. hortensis* fed on every part of every plant offered (South, 1992).

However, subsequent investigations have shown that molluscs are also capable of a high degree of selection in their feeding behaviour. Getz (1959) offered leaves of 45 different species of plant separately to *D. reticulatum, A. circumscriptus* and *Deroceras laeve*, and found that 28% were very acceptable, 25% relatively acceptable and 47%

rejected. Other evidence for selection comes from Bailey (1989) who showed that *D. reticulatum* actively selected maize flour over pea, and Cook *et al* (1996) who demonstrated preferences within a range of 12 weed species.

Only limited information is available on the specific nature of semiochemicals that elicit a phagostimulatory response to foods. Carr *et al* (1974) gave evidence that many biological fluids and extracts, proteins, peptides and other high molecular weight materials were important stimulants for feeding. Pickett & Stephenson (1980) isolated and identified volatiles from extracts of lettuce, carrot and dandelion. At a concentration of 100ng/µl, (E)-2-hexen-1-ol and 1-hexen-3-ol were shown to cause a response similar to that from the whole plant.

Several examples of plants which are avoided by slugs and snails are known and in some cases the secondary metabolite involved has been identified (Table 3). It was felt that with the development of appropriate techniques it would be possible to identify many other antifeedants. The methods used and the results obtained are described below.

Table 3. Some plants known to exhibit molluscan antifeedant properties

Plant Species	Active Component	Reference
cashew (*Anacordium occidentale*)	anacordic acid	Kubo *et al* (1986)
olive (*Olea europaea*)	oleuropeine	Kubo & Matsuroto (1984)
	ligstodide	
black pepper (*Piper nigrum*)	piperine	Henderson & Parker (1987)
eau-de-cologne mint	unknown	
love-in-a-mist (*Nigella damascena*)	unknown	Bonar (1989)
		"
feverfew (*Chrysanthemum parthenium*)	unknown	
		"
lovage (*Ligusticum officinale*)	unknown	
		"
beard tongues (*Penstmon*)	unknown	
mossy saxifrages (*Saxifraga hypnoides*)	unknown	"
		"
Anemone blanda	unknown	
fennel (*Feoniculum vulgare*)	(+)fenchone	"
		Garraway *et al* (1991)

4. MATERIALS AND METHODS

4.1 TEST ANIMALS

Adult *Deroceras reticulatum* were collected from beneath laminated cork/polystyrene roof insulation boards, on an irrigated plot of mixed herbage. They were maintained in plastic bowls lined with moist cotton wool covered with absorbent, unbleached paper in a controlled environment room (12hr light 15^0C/12 hr dark 5^0C). The slugs were fed on Chinese cabbage (*Brassica chinensis*) grown from seed.

4.2 TEST PLANTS

Foliage from 35 species of Apiaceae (Umbelliferae) was tested. Most species were grown from seed (obtained from Chiltern Seeds or Mr M Southam) and maintained in glasshouses. The more common species, including cow parsley (*Anthriscus sylvestris*), hogweed (*Heracleum sphondylium*), greater-burnet saxifrage (*Pimpinella major*) and ground elder (*Chaerophyllum temulentum*), were collected on Rothamsted Farm. Collected species were extracted the same day and extracts stored in sealed airtight vials and held at -20^0C to avoid evaporation and degradation of material.

4.3 PLANT EXTRACTION

Plant material was extracted by microwave-assisted distillation based on the method of Craveiro *et al* (1989). 30-40g fresh weight of plant material was heated in a 500ml florentine flask for approximately 1 minute in an 800W microwave oven until the plant cells burst. The volatile materials were picked up in an stream of nitrogen, at a flow rate of 800ml/min, carried through PTFE tubing (3mm internal diameter), and trapped in a flask containing cooled solvent (hexane). All connections between tubing and flasks were made airtight by sealing with PTFE tape. Magnesium sulphate was added to remove water and

the extract filtered. All extracts were then concentrated down to 25g equivalent fresh weight of plant foliage per ml of hexane (or appropriate solvent) using a rotary evaporator.

4.4 ELECTROPHYSIOLOGICAL ASSAY

A slug, anaesthetized with gaseous CO_2 was pinned out on to a dish containing a thin layer of Sylgard gel (Dow Corning 184 Silicone Elastomer). The resin and curing agent were mixed in a ratio of 10:1, de-gassed in a vacuum desiccator and poured into the bottom of a 6.0cm glass petri dish. The dish was placed into an oven (30-40^{0}C) for 24 hours to cure the resin.

An incision into the slug was made dorsally from the mantle to the everted pharynx and the epithelium pinned back to reveal the posterior and anterior tentacles, the buccal mass and cerebral ganglion. One of the posterior tentacles was removed by cutting at the tip of the tentacle sheath, at the base of the tentacle retractor muscle and the centre of the cerebral connective. The dissected tentacle was then placed into a new Sylgard dish containing a specially formulated Ringer solution (Garraway *et al,* 1992) (Table 4): preparations survived for up to 24 hours with this buffered saline solution. The tentacle was dissected to expose further both the main olfactory nerve and the sensory pad by cutting away the retractor muscle and sheath. The preparation was then positioned so that the nerve remained in the Ringer solution for recording but the sensory pad was exposed to air so that airborne volatile stimuli could be applied to it.

Two suction electrodes containing silver/silver chloride wire were made. The indifferent or 'reference' electrode was identical to the recording electrode and both were connected to a differential amplifier. The cut end of the olfactory nerve was drawn into the recording electrode (which had been trimmed to be a tight fit over the nerve) by suction generated by a 10ml glass syringe, while the indifferent electrode was placed in the surrounding

Ringer solution.

An airstream, purified by passage through activated charcoal and molecular sieves (0.5μm), was passed through a gas jar containing water to prevent the preparation from drying out. Air from the gas jar was then passed through a flowmeter where the flow was adjusted to 60ml/min, the maximum flow which did not stimulate nerve firing in the olfactory nerve preparation (Garraway, 1992). The air flow then passed continuously through a metal tube to an ejection port (1mm dia.). 2μl aliquots of test extract were introduced into this airstream via an injection port and so passed over the sensory pad. Any electrical activity elicited in the tentacle nerve preparation by the test stimulus was relayed through the electrode before being amplified (x1000) by a DAM 50 differential amplifier (World Precision Instruments). Amplified electrical responses were then simultaneously displayed onto a digital storage oscilloscope ('Wavetek' 20MHz) and recorded on magnetic tape for subsequent computer analysis using appropriate software (Spike 2 (Cambridge Electronic Design) and Autospike (Syntech, Hilversum)).

4.5 COUPLED GAS CHROMATOGRAPHY/MASS SPECTROMETRY

GC was performed on an HP1 50m fused SiO_2 capillary column, starting at 30^0C for 5 minutes then programmed at a 5^0C rise per minute until a maximum temperature of 250^0C was reached. MS was by electron impact at 70eV, 200^0C (VG Autospec system).

1μl aliquots of plant extracts were injected into the GC column where they were chromatographically separated. The GC capillary column was then fed through a heated sheath into the ion source of the MS, where components were ionized and their masses calculated. The resulting mass spectrum indicated the possible identities of individual components within the plant extract. These were subsequently confirmed by a series of GC peak enhancements.

4.6 FEEDING ASSAY (WHEAT FLOUR)

Adult *D. reticulatum* were maintained in a controlled environment room (12hr day 15^{0}C/12hr night 5^{0}C). The slugs were fed *ad lib.* on *B. chinensis* cv. "tip-top", for at least 24 hours and then starved for a further 24 hours before being used in the feeding tests.

Untreated wheat seeds (cv. *Avalon* or *Beaufort*) were milled in a Retsch mill to a fine flour which was then oven-dried at 50^{0}C. Approximately 5g of flour was mixed with c. 2ml water to produce a dough which was pressed flat and cut into 30 pieces of roughly equal size. These flour pellets were placed in numbered positions on a glass plate, oven-dried at 50^{0}C to a constant weight, and then accurately weighed. Each pellet was then placed in a numbered 9.0cm plastic Petri dish containing two 7.0cm filter papers (Whatman No. 1). Thirty replicates were prepared for each treatment and each control.

Individual pellets were then dosed with 30µl test extract, (it was found by experiment that 30µl volatile solvent would saturate a dry pellet without running off).

The Petri dishes were then placed in the controlled environment room (15^{0}C/5^{0}C) and the filter papers moistened with distilled water. One slug was placed in each dish and a stainless steel gauze lid (0.5mm mesh) was used to contain the slug whilst allowing free air circulation to prevent vapour build up from the volatile test materials. After 24 hours the slugs were removed and each pellet was replaced in its numbered position on the glass plate and re-dried in the oven at 50^{0}C to a constant weight. The dry weight of each pellet consumed by the slug during the test was then calculated by difference.

The weights consumed were analysed by ANOVA and the change in feeding (C), i.e. the difference in mean weights consumed by the test ($\bar{T}$) and control ($\bar{U}$) slugs, expressed as a percentage with a standard error.

4.7 FEEDING ASSAY (CHINESE CABBAGE)

The most potent slug antifeedant compound (RES-CO1), isolated from hemlock (*C. maculatum*), was also tested as a topically-applied spray to foliage of pot-grown *B. chinensis*, under simulated field conditions to assess its potential as a crop protectant.

As RES-CO1 had demonstrated signs of phytotoxicity to *B. chinensis* at high levels (100µg/cm^2 of plant foliage), a dose-response test was devised in which 2-week old *B. chinensis* leaves were electrostatically sprayed with varying concentrations to assess at what level it was antifeedant but not phytotoxic. Solutions of RES-CO1 were prepared at six concentrations and dissolved in a mixture of ethanol and water in a ratio of 1:3:1. Ethanol solvent was used as it readily mixes with water, which enables spray droplets to hold an electrostatic charge, preventing coalescence and improving coverage on the leaf surfaces. Plants were treated with RES-CO1 at 2,6,18,54,and 100ug/cm^2. Each treated plant was tested against an untreated control.

Tests were conducted in Darvic trays (20cm x 40cm). At either end a small slot (1cm wide) was cut to enable the bases of the *B. chinensis* petioles to be slid in, so that the tray rested on top of the plant pots. The slots were then closed with fitted covers so that only the leaf foliage was exposed above the tray. A 1-2cm layer of moistened soil covered the base of the tray and crystalline salt was applied to the rim to prevent slugs from escaping. Thirty slugs were placed in the centre of the tray between the plants and the experiment left to run for 24 hours in a controlled environment room (12 hours light 15^{0}C:12 hours dark 5^{0}C). The damage done to the leaves by the feeding slugs was assessed visually and given a score between 1 and 5, relating to the area of leaf consumed where:

4 = entire leaf consumed

3 = over 50% of leaf consumed

2 = approximately 50% of leaf consumed

1 = less than 50% of leaf consumed

0 = leaf undamaged

P = signs of phytotoxity present on the leaf surface

5. RESULTS

5.1 ELECTROPHYSIOLOGICAL RESPONSES TO EXTRACTS OF APIACEAE

Of the 33 different Apiaceae species screened for activity, 22 triggered some degree of nervous activity in the slug tentacle nerve preparation. All 33 species were screened on an individual nerve preparation, with a resting period of at least ten minutes between dosing.

The intensity of responses differed with plant species (Fig 1). Extracts such as *A. sylvestris* (Fig 1b) induced only a few AP's, while species such as *C. sativum* and *C. maculatum* (Fig 1f,g) triggered an intense nerve response lasting for over 60 seconds and others were intermediate between these extremes (Fig 1a,c,d). Within intense traces there was a rapid firing of nerve cells at the onset of stimulation with activity then falling back to baseline levels only after several minutes. Three parameters were taken into consideration when evaluating the intensity of individual traces.

5.1.1 DURATION OF RESPONSE

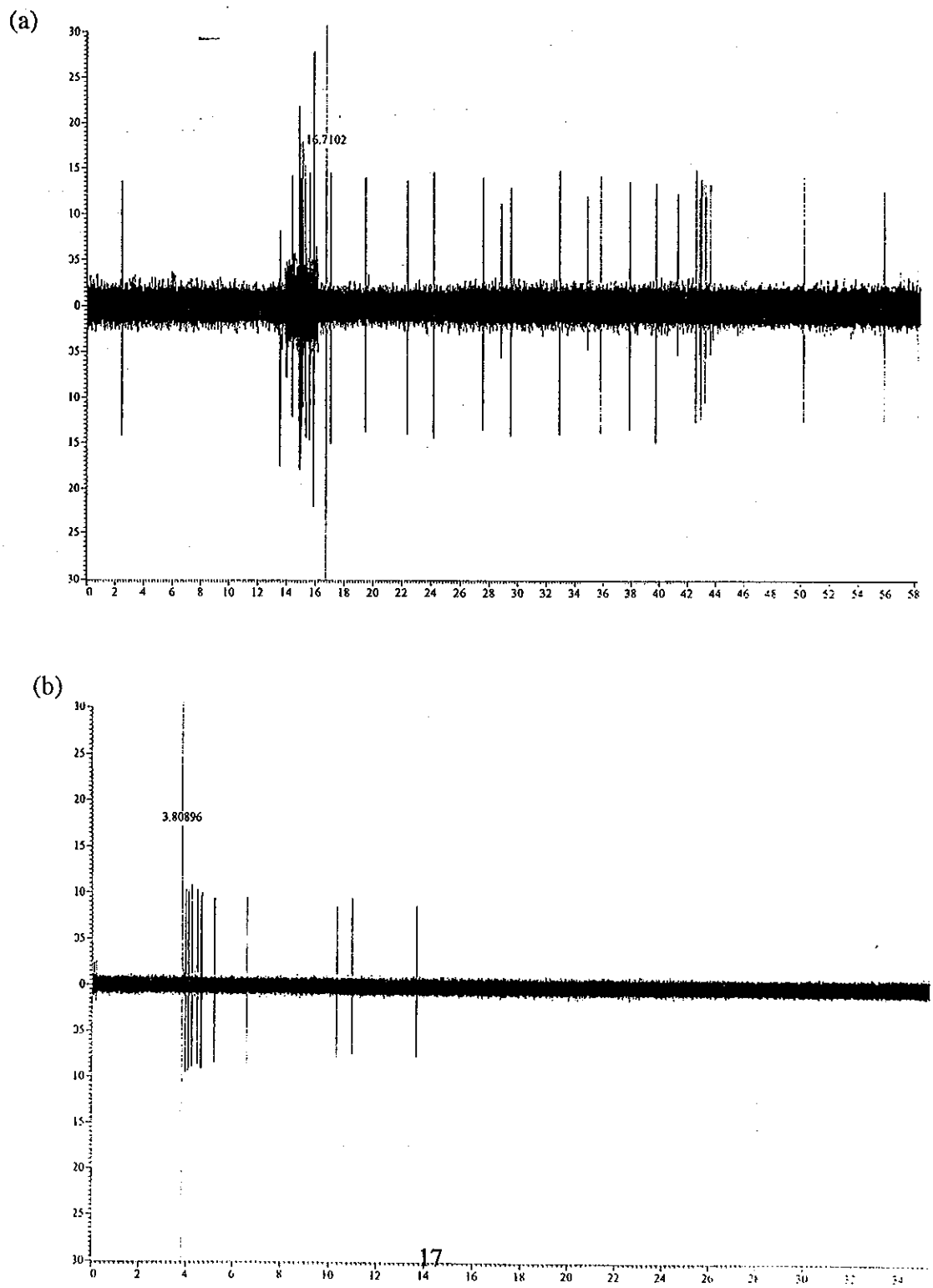
The duration of the response was defined as the time in seconds that activity remained above the spontaneous activity level (Figure 2).

5.1.2 TOTAL NUMBER OF ACTION POTENTIALS (AP's)

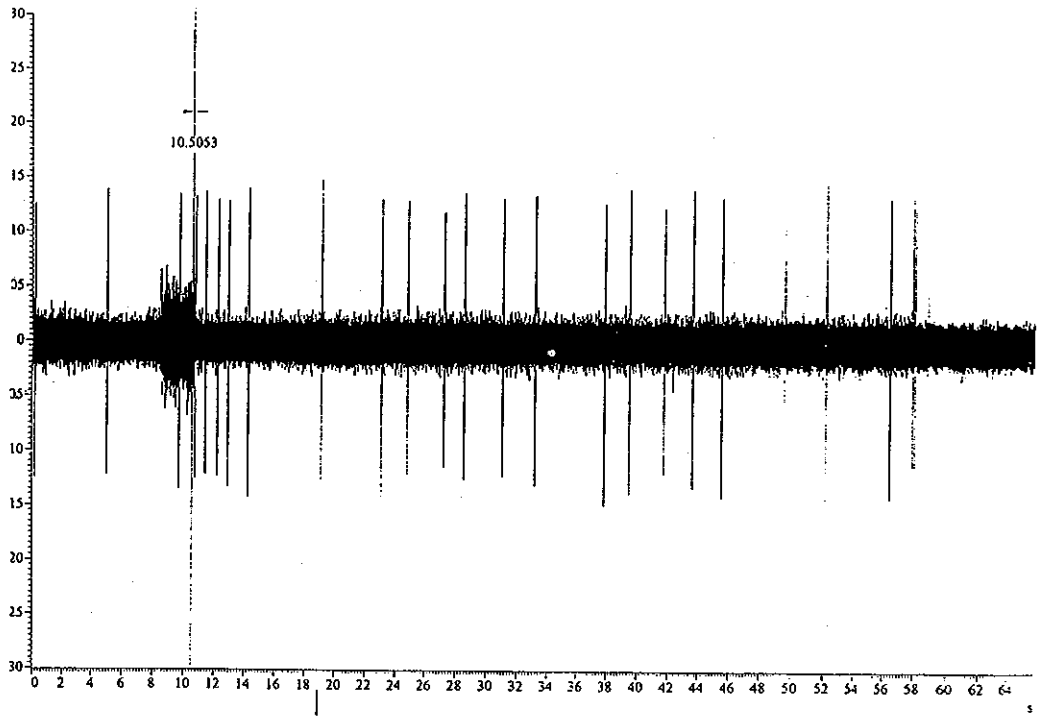
Where spontaneous activity was constant or very low before stimuli were applied, a good measure of the intensity of a response was the total number of AP's produced. In an intense response, e.g. when extract of *C. maculatum* was applied to the olfactory nerve

Fig. 1 Electrophysiological traces from the olfactory nerve preparation of *D. reticulatum*, in response to various Apiaceae extracts

Cursors indicate where extracts were applied to the tentacle preparation. Extracts tested were: (a) ground elder (*A. podagraria*), (b) cow parsley (*A. sylvestris*), (c) sweet cicely (*M. odorata*), (d) stone parsley (*S. amomum*), (e) curled chervil (*A. cerefolium*), (f) coriander (*C. sativum*), (g) hemlock (*C. maculatum*).



(c)



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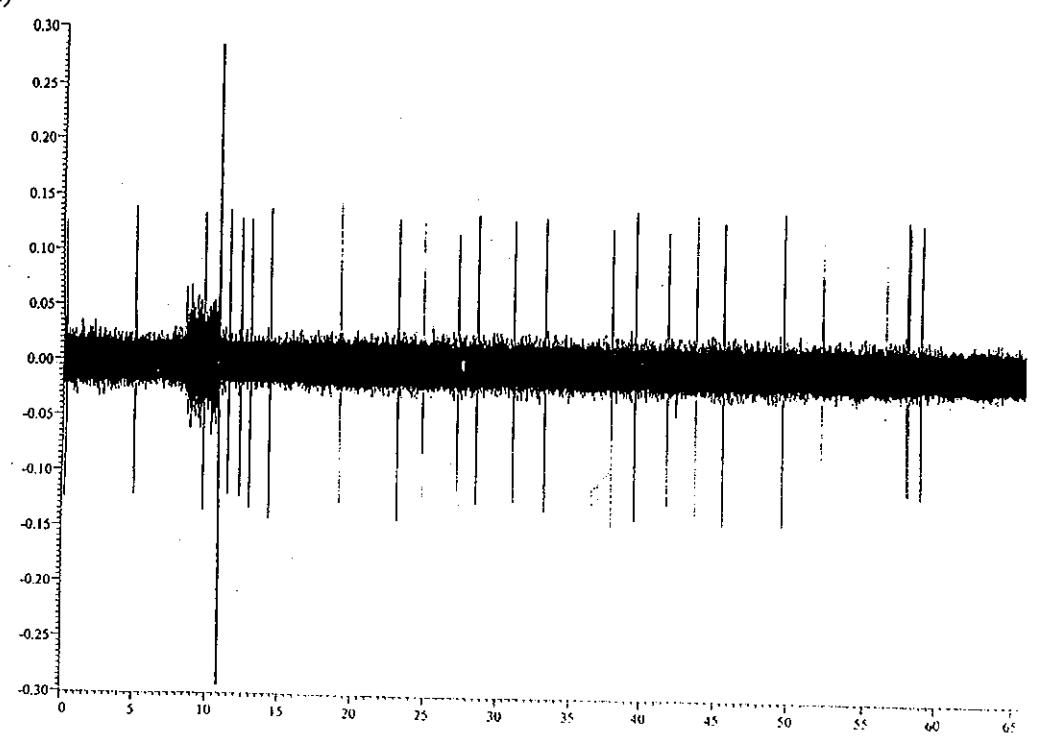

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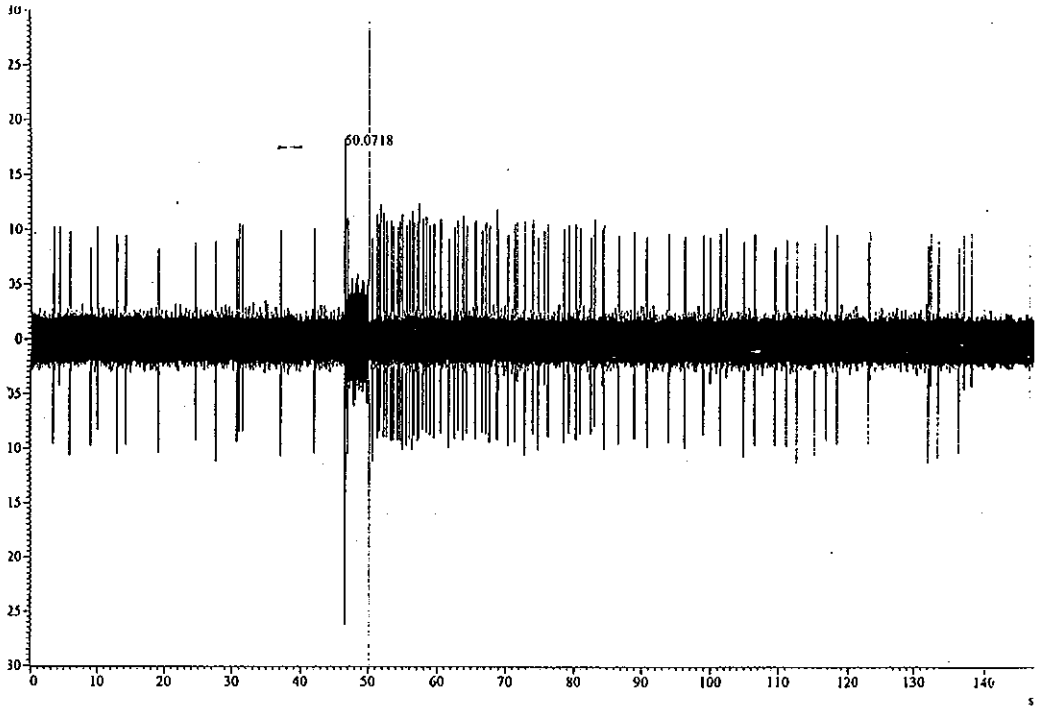

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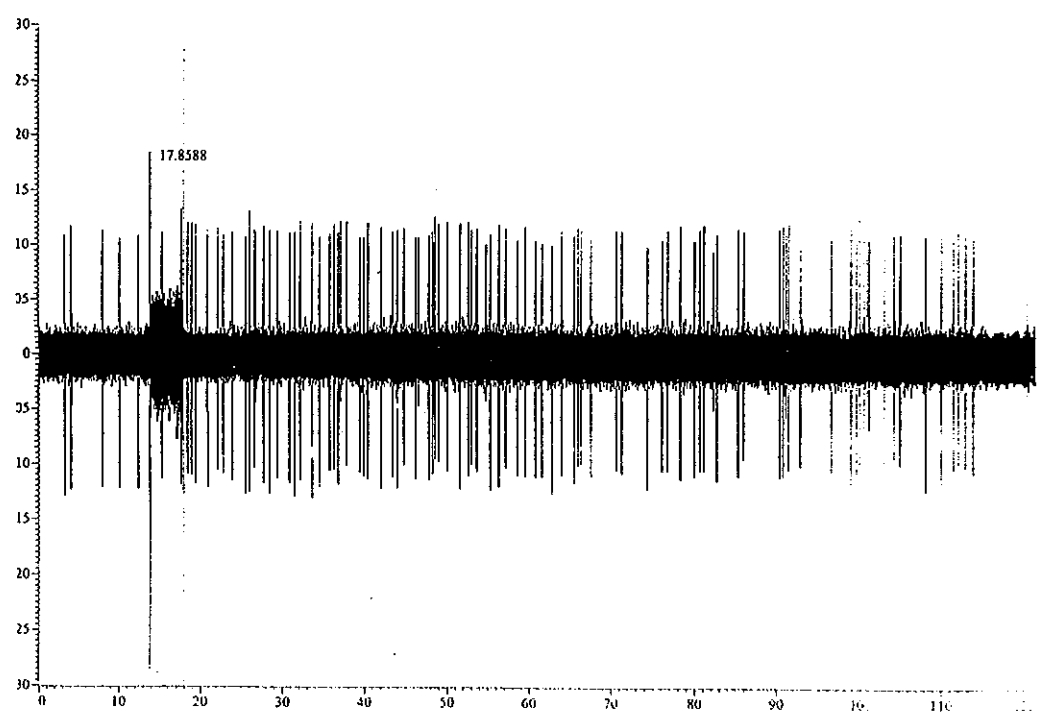

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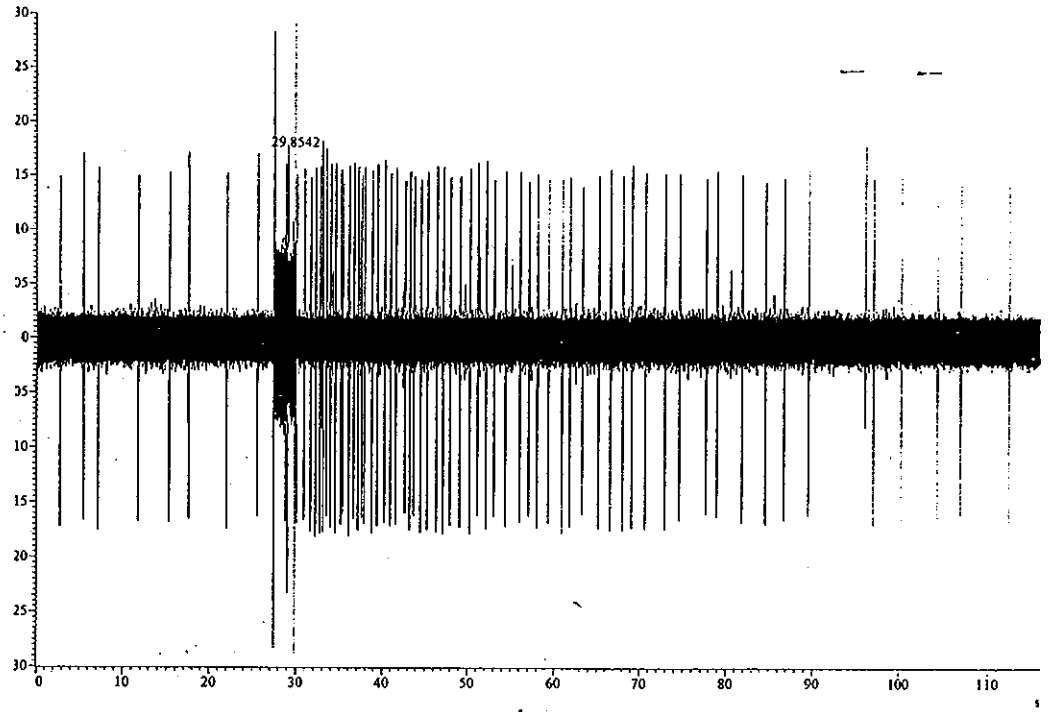
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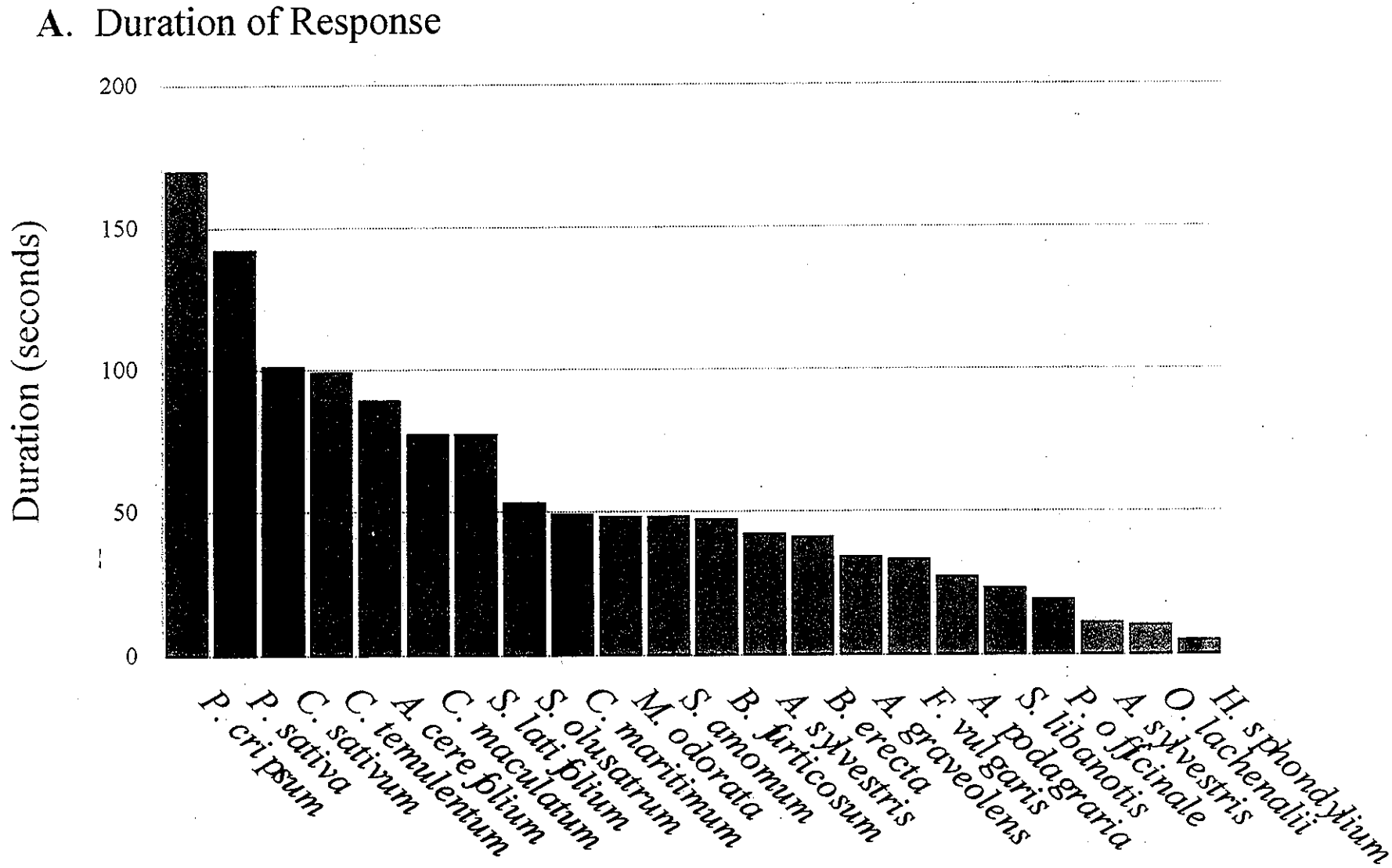
Fig. **2** Effect of volatile components from extracts of Apiaceae species on the electrophysiological activity of the olfactory nerve of *D. reticulatum*



preparation, initial firing was rapid, producing up to 10 spikes per second. Computer software (Spike 2, Cambridge Electronic Design) was used to calculate the number of AP's produced during the response (Figure 3).--

5.1.3 INCREASE IN FREQUENCY FOR THE FIRST TEN SECONDS AFTER STIMULATION

Where spontaneous activity levels were high before the test extract was applied, it was difficult to separate the frequency of the spikes produced in response to the extract alone from that of the cells firing spontaneously. To overcome this, spike frequency was measured for approximately 50secs prior to stimulation and then over a fixed period of ten seconds after the extract was applied. The increase in frequency of nerve firing was then calculated for all 22 active extracts (Fig 4).

5.2 FEEDING BEHAVIOUR RESPONSES TO EXTRACTS

Tests were made to check whether the hexane solvent itself had any effect on the feeding behaviour of *D. reticulatum*. When pellets dosed with hexane solvent was compared with untreated pellets there was no effect on feeding over a 24 hour period (Table 5). Subsequently pellets dosed with hexane extract were tested against untreated control pellets.

Fig. **3** Effect of volatile components from extracts of Apiaceae species on the electrophysiological activity of the olfactory nerve of *D. reticulatum*

B. Total Number of Action Potentials Recorded Following Exposure

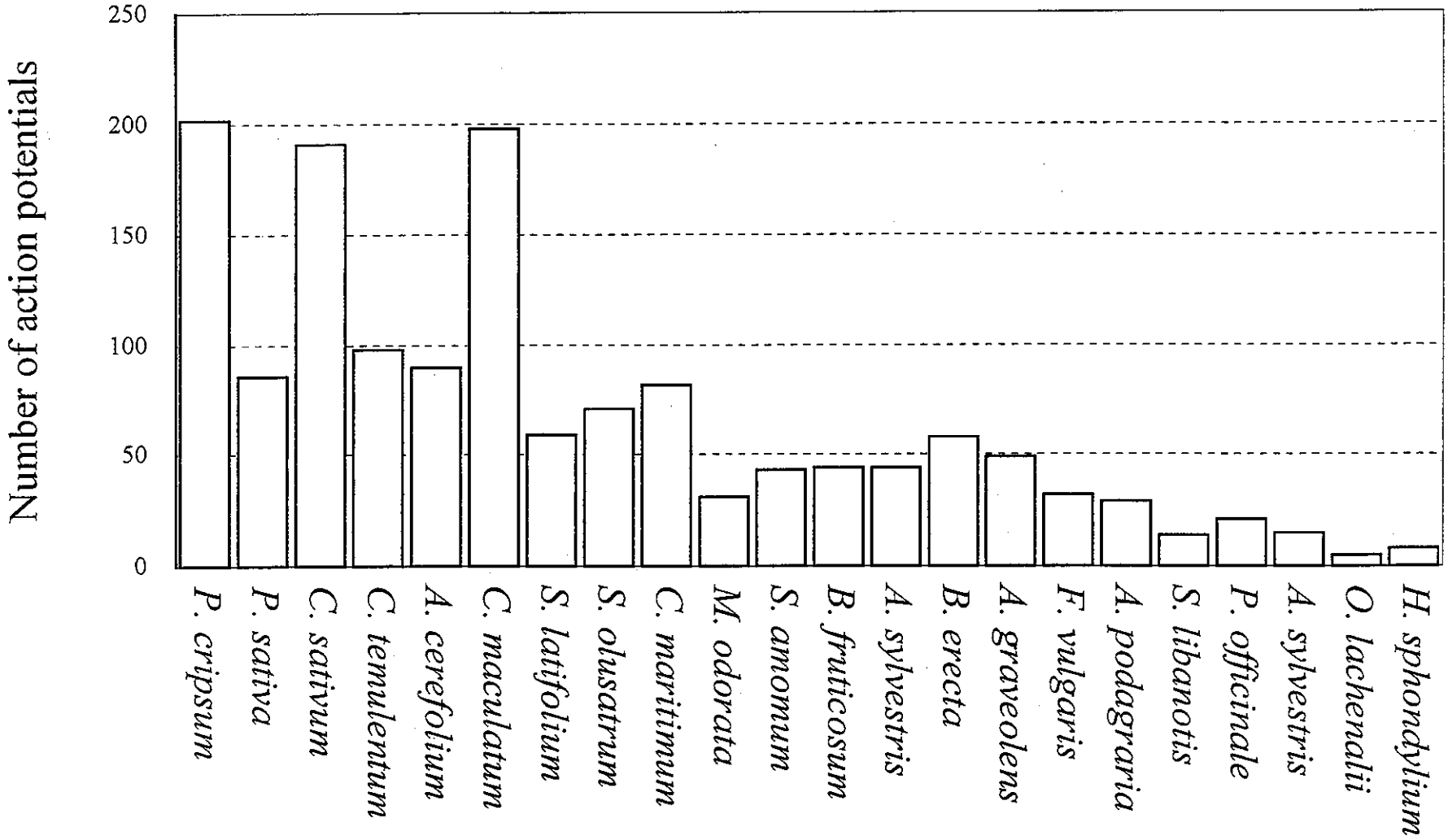


Fig. **4** Effect of volatile components from extracts of Apiaceae species on the electrophysiological activity of the olfactory nerve of *D. reticulatum*

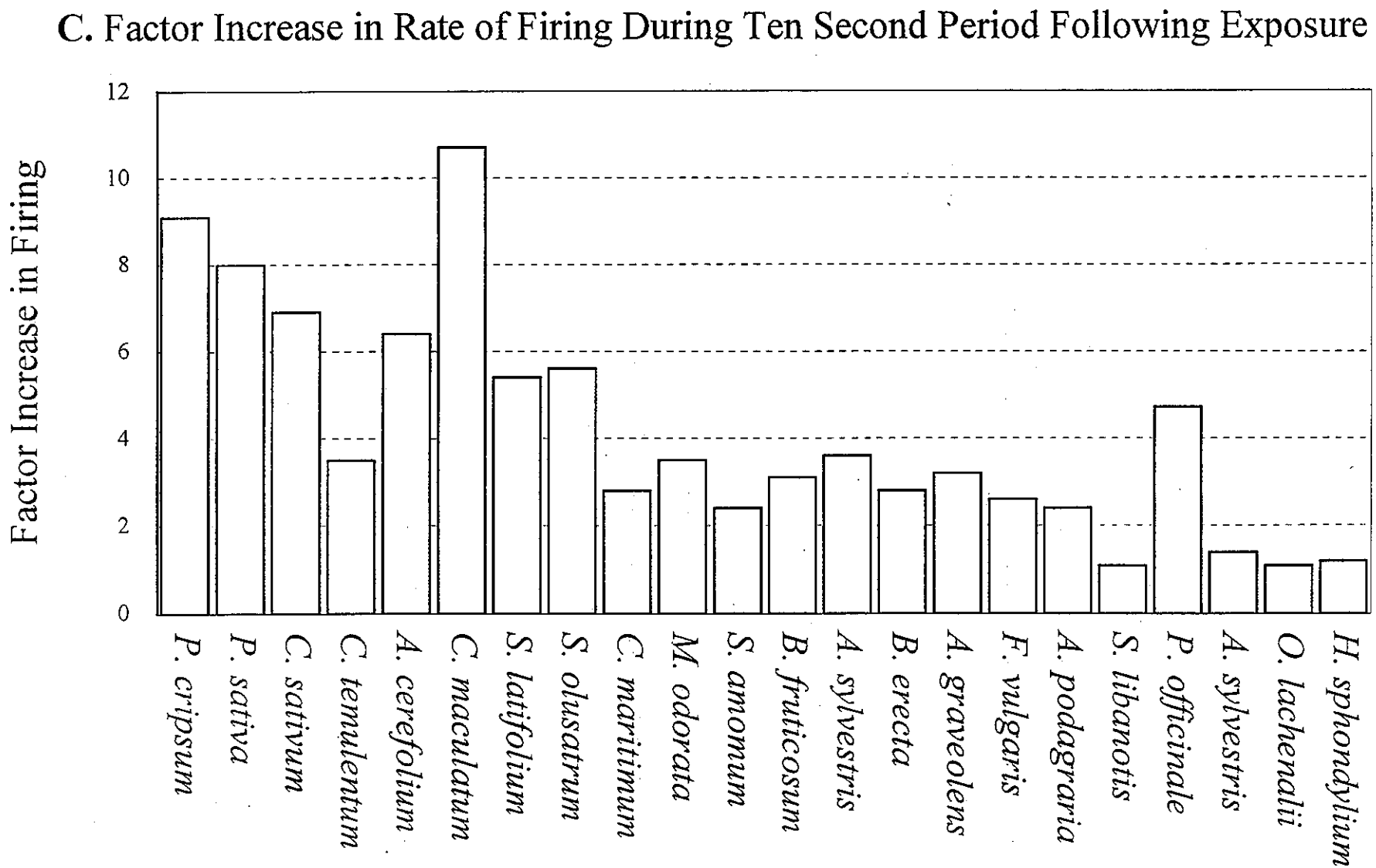


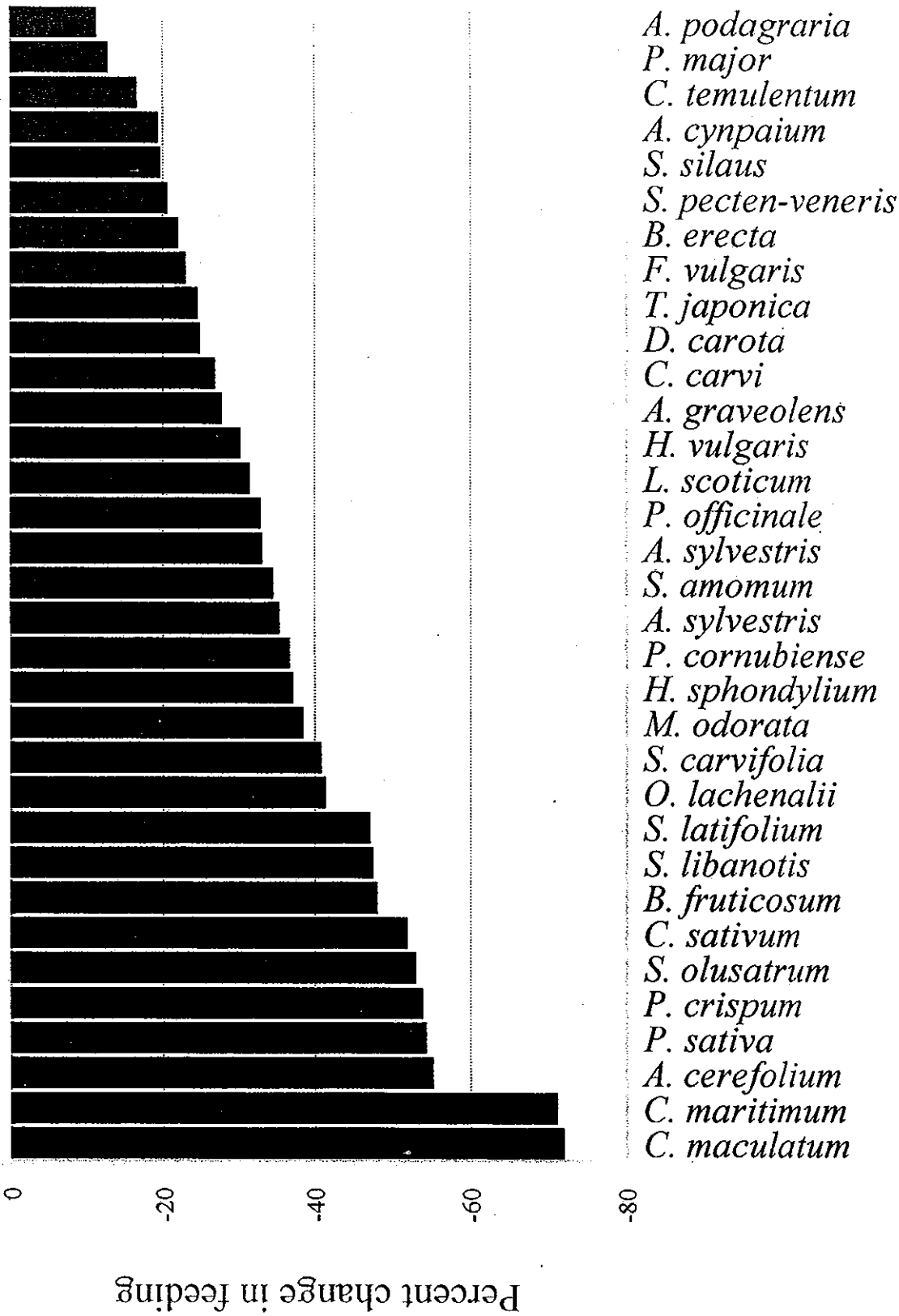
Table 5 Feeding responses to hexane solvent, sucrose and extract of *B. chinensis*.

Test Material	% Change in Feeding
hexane solvent	6.0±11.0%
hexane solvent	8.0±8.3%
1.5% sucrose solution	22.6±9.3%
methanol extract of *B. chinensis*	-5.0±11.6%

Dosing with methanol extract of chinese cabbage (*B. chinensis*), which is readily eaten by *D.reticulatum*, did not alter feeding (-5.0±11.6%) (Table 5). The effect of Apiaceae extracts on feeding behaviour in *D. reticulatum* is shown in Fig 5. Dotted lines on this figure separate species whose extracts fall into four different categories of activity. Extracts from *C. maculatum* and *C. maritimum* reduced feeding by the largest amount (60-80%). Extracts from the next set of species including *A. cerefolium*, *P. crispum* and *C. sativum* all reduced feeding by a large amount (over 40%), while species such as *M. odorata*, *O. lachenalii* and *S. olusatrum* reduced feeding to a smaller extent. The final group included species such as *D. carota*, *C. temulentum* and *A. cynapium* which induced only a low level of antifeedant activity.

Tests on extracts of *C. maritimum*, *A. cerefolium*, *C. maculatum* and *P. crispum* were repeated at different times of the year to detect any seasonal variation in feeding behaviour in *D. reticulatum* (Table 6). All tests were conducted with the same extract of plant material collected on the same day, thereby eliminating the possibility of variation in

Fig **5.** Effect of adding extracts of Apiaceae species on weight of food eaten by *D. reticulatum* under standard conditions



chemical composition within the plant. Extracts were stored in airtight glass vials at a temperature of -20°C.

Extracts of *C. maculatum* were tested separately on four occasions within a 6 month period. Similar results were obtained on each occasion, indicating that feeding behaviour did not change over time and that the pellet feeding bioassay gave consistent results.

Table 6 Feeding reduction on successive tests with *C. maculatum, A. cerefolium, C. maritimum* and *P. crispum* on *D. reticulatum.*

Apiaceae Extract	Date Tested	% Reduction in Feeding
C. maculatum	31/10/95	-72±9.6
	12/11/95	-70±6.8
	28/11/95	-60±6.0
	24/02/96	-66±7.3
A. cerefolium	2/11/95	-55±6.2
	24/02/96	-54±8.3
C. maritimum	22/10/95	-71±5.4
	18/12/95	-65±6.6
P. crispum	02/09/95	-54±7.4
	11/12/95	-53±6.6

5.3 RELATIONSHIP BETWEEN NEUROPHYSIOLOGICAL AND ANTIFEEDANT ACTIVITY OF APIACEAE EXTRACTS

Extracts of Apiaceae elicited a range of electrophysiological responses from *D. reticulatum*, with some species such as *C. maculatum, C. sativum* and *P. crispum* inducing intense electrical activity in the nerve preparation and reducing feeding by up to 72%.

Although the results of themselves did not demonstrate that both effects were caused by the same compounds, comparison of the a neurophysiological and behavioural data indicated a relationship. In the group of plants which reduced feeding by at least 47%, the correlation between electrophysiological and antifeedant activity was noticeable, except in the cases of *C. maritimum, B. fruticosum* and *S. libanotis*. Extracts which reduced feeding by less than 25% were generally either inactive or produced only a low level of activity in the electrophysiological assay, while those with intermediate antifeedant activity showed no consistent patterns.

Using non-hierarchical cluster analysis, all 22 species of Apiaceae which were active at both a neurophysiological and behavioural level were grouped in terms of their similarities in response to four variables:

- Reduction in feeding in *D. reticulatum* due to extract
- Duration of nerve response induced in response to extract
- Total number of AP's produced in response to extract
- Increase in frequency of firing during first ten seconds after stimulation by extract.

The resultant grouping of species identified *C. sativum, P. crispum* and *C. maculatum* as the most active antifeedant extracts (Table 7).

Table 7 Groupings of extracts obtained from the initial classification by non-hierarchical cluster analysis

Group 1	Group 2	Group 3
C. sativum	*C. temulentum*	*B. erecta**
P. crispum	*A. cerefolium*	*P. officinale*
C. maculatum	*P. sativa*	*M. odorata**
	S. latifolium	*A. podagraria**
	S. olusatrum	*S. amomum**
	C. maritimum	*S. libanotis*
		*B. fruticosum**
		*A. sylvestris**
		*F. vulgaris**
		*A. sylvestris**
		H. sphondylium
		A. graveolens
		O. lachenalii

*species which swapped groups to optimise criteria tested

This relationship was also examined by another multivariate analysis technique, principal components analysis (PCP), a multivariate ordination method which aims to display most of the original variability in a data set in as small a number of dimensions as possible (Digby & Kempton, 1987). In this case, extracts which did not induce any activity in the electrophysiology nerve preparation were disregarded. A data matrix was constructed for the remaining 22 extracts for all four variables (Table 8).

Table 8 Data matrix containing values for four variables for the 22 active Apiaceae extracts analysed using principal components analysis.

Extract	Variable 1 (RF)	Variable 2 (Dur)	Variable 3 (NOE)	Variable 4 (Freq)
1. *B. erecta*	-22	41.3	58	2.8
2. *P. officinale*	-33	19.4	21	4.7
3. *C. temulentum*	-16	99.1	98	3.5
4. *C. sativum*	-52	101.3	191	6.9
5. *A. cerefolium*	-55	88.5	90	6.4
6. *M. odorata*	-38	48.0	31	3.5
7. *A. podagraria*	-11	27.3	29	2.4
8. *S. amomum*	-35	48.1	43	2.4
9. *P. crispum*	-54	170.0	202	9.1
10. *S. libanotis*	-47	23.1	14	1.1
11. *B. fruticosum*	-48	46.8	44	3.1
12. *A. sylvestris*	-33	11.2	15	1.4
13. *P. sativa*	-54	142.0	86	8.0
14. *S. latifolium*	-47	77.0	59	5.4
15. *A. sylvestris*	-35	42.0	44	3.6
16. *F. vulgaris*	-23	33.0	32	2.6
17. *S. olusatrum*	-53	53.0	71	5.6
18. *H. sphondylium*	-37	5.0	8	1.2
19. *A. graveolens*	-28	34.0	49	3.1
20. *O. lachenalii*	-41	10.0	5	1.1
21. *C. maculatum*	-72	77.3	198	10.7
22. *C. maritimum*	-65	49.3	82	2.8

Reduction in feeding due to extract (**RF**); Duration of nerve response from extract (**Dur**); Number of AP's produced in response to extract (**NOE**); Increase in frequency of nerve firing during the first ten seconds after stimulation (**Freq**).

Both cluster and principal components analysis indicated that extracts of the three species, *C. maculatum, P. crispum* and *C. sativum*, differed from the other extracts and induced high levels of neurophysiological activity together with marked antifeedant effects. These three extracts were therefore studied in greater detail to identify the active components responsible for this activity (Section 5.4).

5.4 IDENTIFICATION OF ACTIVE COMPONENTS OF EXTRACTS

Extracts were analysed using linked GC/MS. This method is described in detail by Rose (1990). The spectrum produced by the three most active extracts indicated the possible identities of various components which were subsequently confirmed by a series of GC peak enhancements or co-injections. This was done for each of the three extracts and the identified components were then re-tested on the electrophysiological nerve preparation, at the concentration at which they occurred within the extract. This was then repeated on a further two nerve preparations. Active components were then re-screened in the pellet feeding bioassay.

5.4.1 GC/MS TRACE FOR *CONIUM MACULATUM*

The GC/MS spectrum and series of peak enhancements led to the identification of 7 major volatile components present within extract of *C. maculatum* (Fig 6), six of which induced activity in the tentacle nerve preparation (Fig 7). The olfactory chemoreceptors of *D. reticulatum* however, were unable to detect either E or Z isomers of β-ocimene, when applied at a concentration of 3.8mg/ml. The active components were then tested in the pellet feeding bioassay at the concentration at which they occurred within the plant extract, calculated through GC analysis (Fig 8). This revealed that one particular chemical accounted for almost all antifeedant activity of the extract, reducing feeding by -68.5 ± 5.4%. Remaining compounds all elicited some level of antifeedant activity, with the components β-pinene and β-caryophyllene, which were present in only minute quantities (15 and 9ng/μl in a 25g/ml extract), reducing feeding by up to 44.3 ± 8.5%.

As the active components within the extracts had now been identified, they were tested in the feeding bioassay at the same concentration to identify those with the greatest potential to reduce damage to crops in the field. All components were tested at 1mg/ml

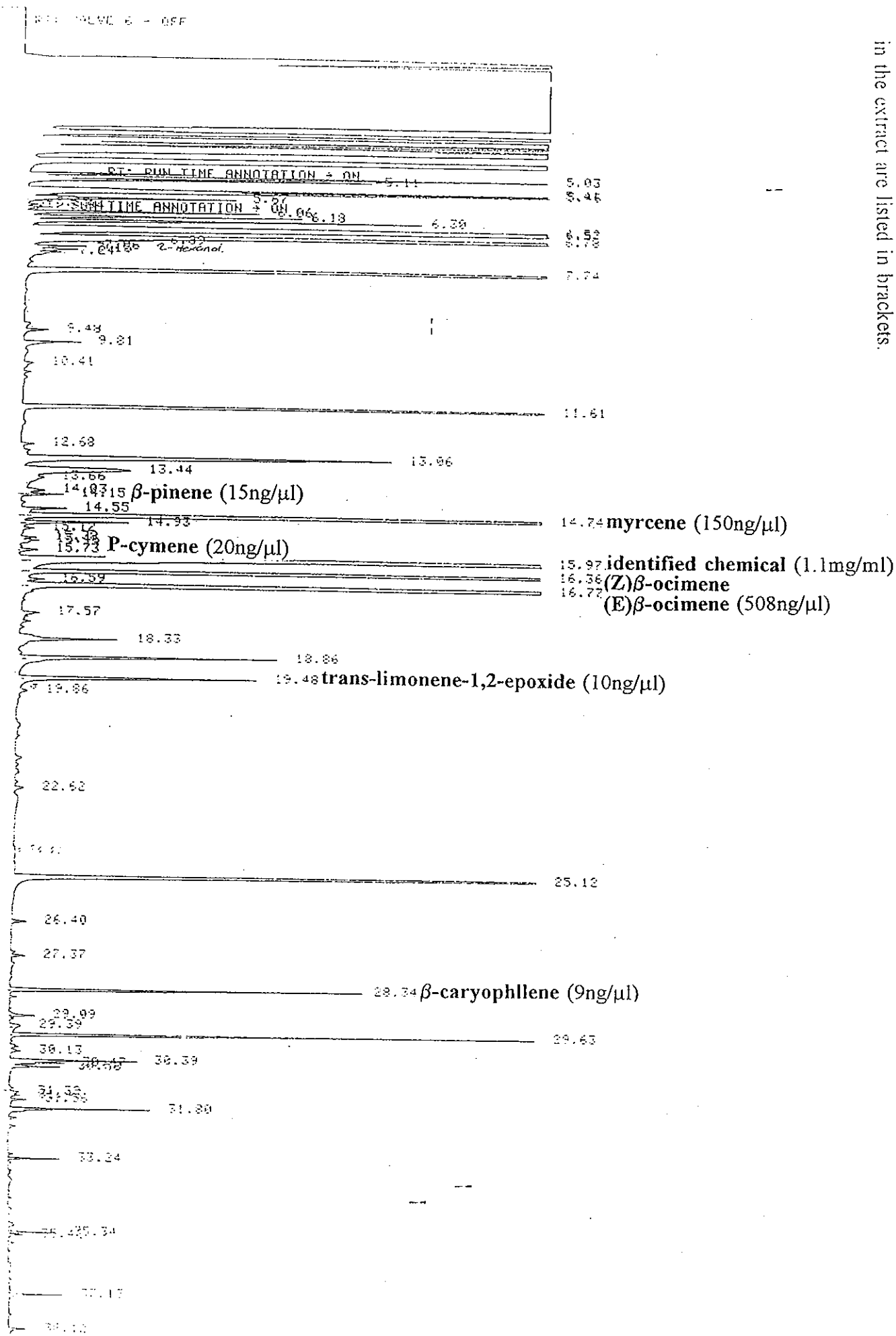
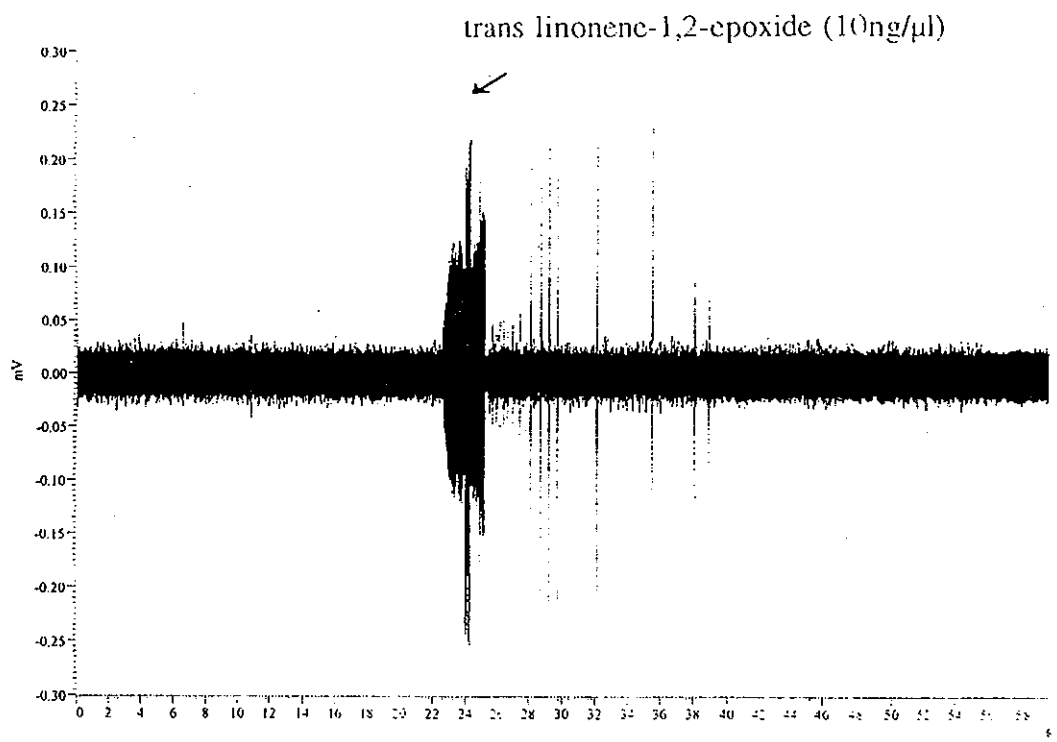
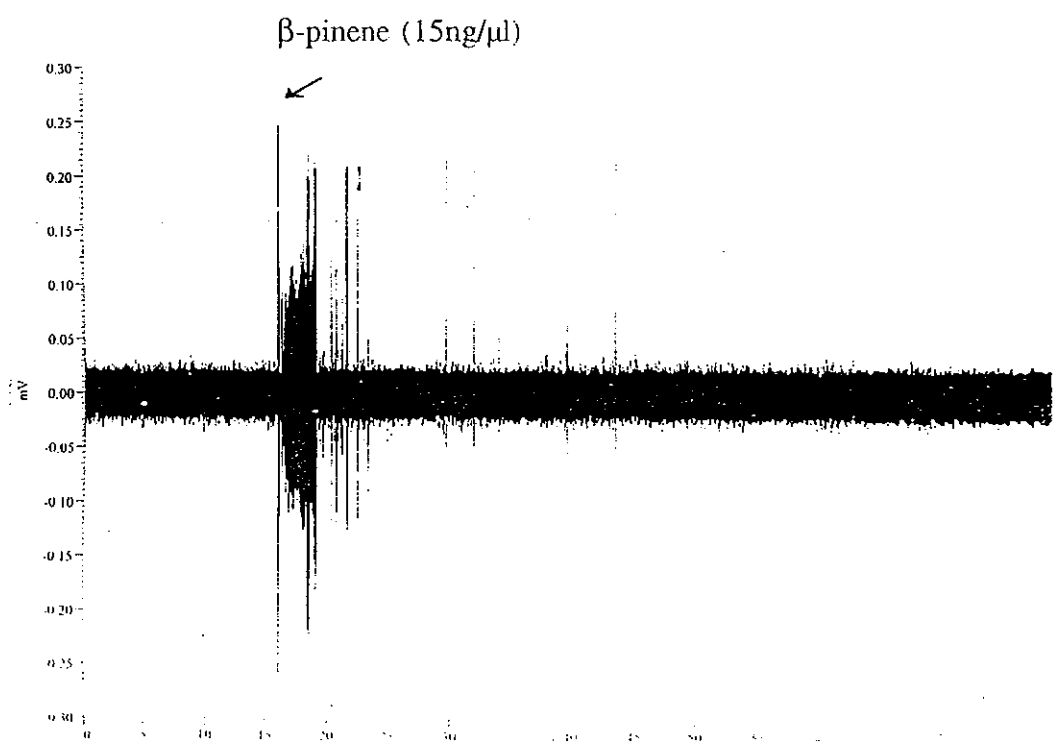


Fig **6** GC trace produced for extract of *C. machilanum*, concentrations at which they occur in the extract are listed in brackets.

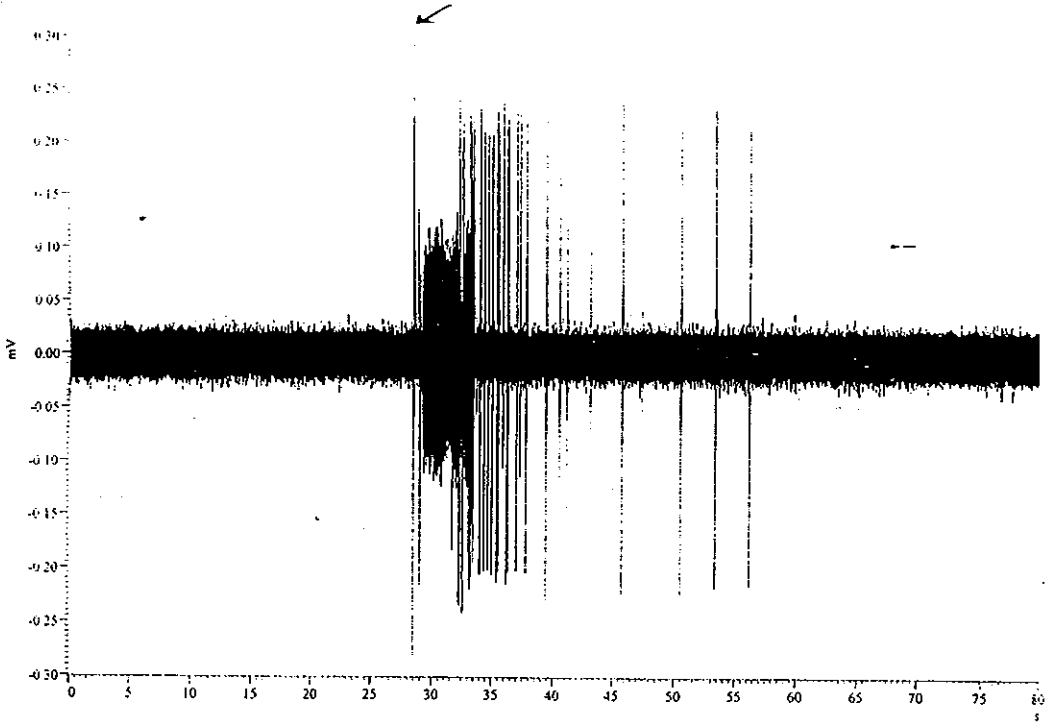
Fig **7** Electrophysiological traces from the olfactory nerve preparation of *D. reticulatum* in response to components isolated from extract of *C. maculatum*.



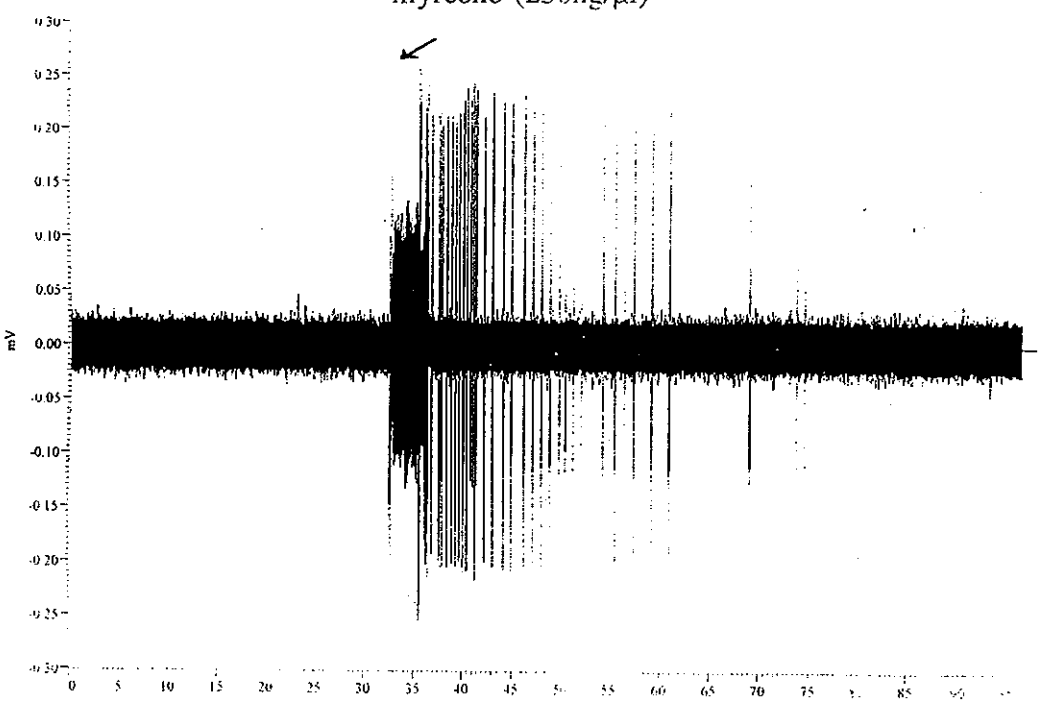


7

P-cymene (150ng/µl)

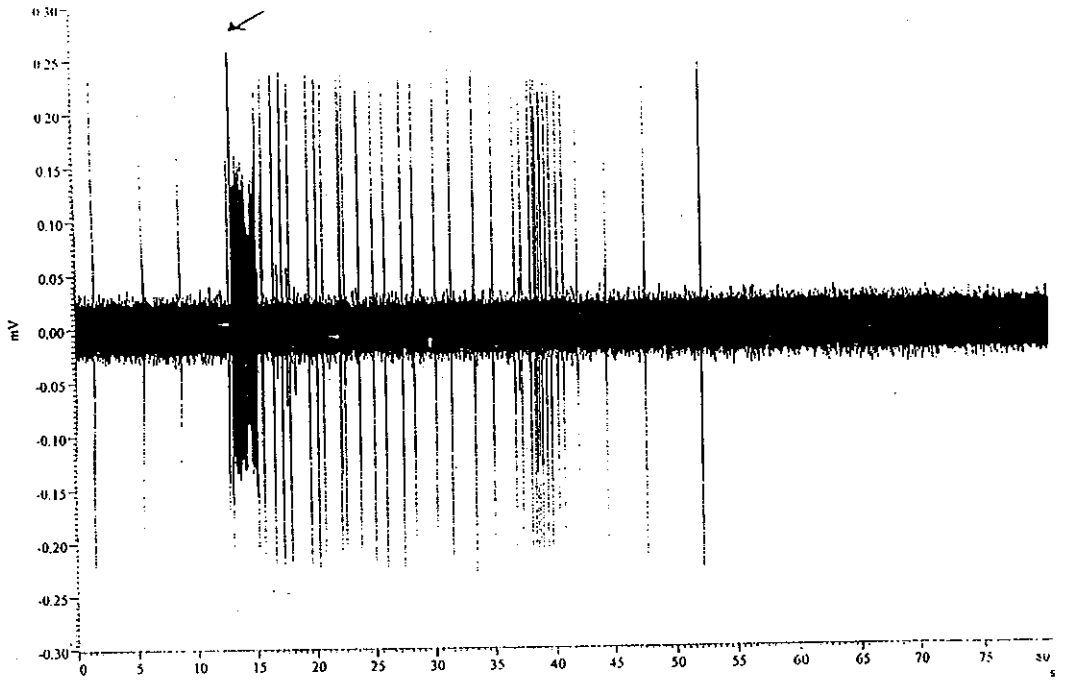


myrcene (250ng/µl)



7

identified chemical (1.1mg/ml)



β-caryophyllene (8.9ng/µl)

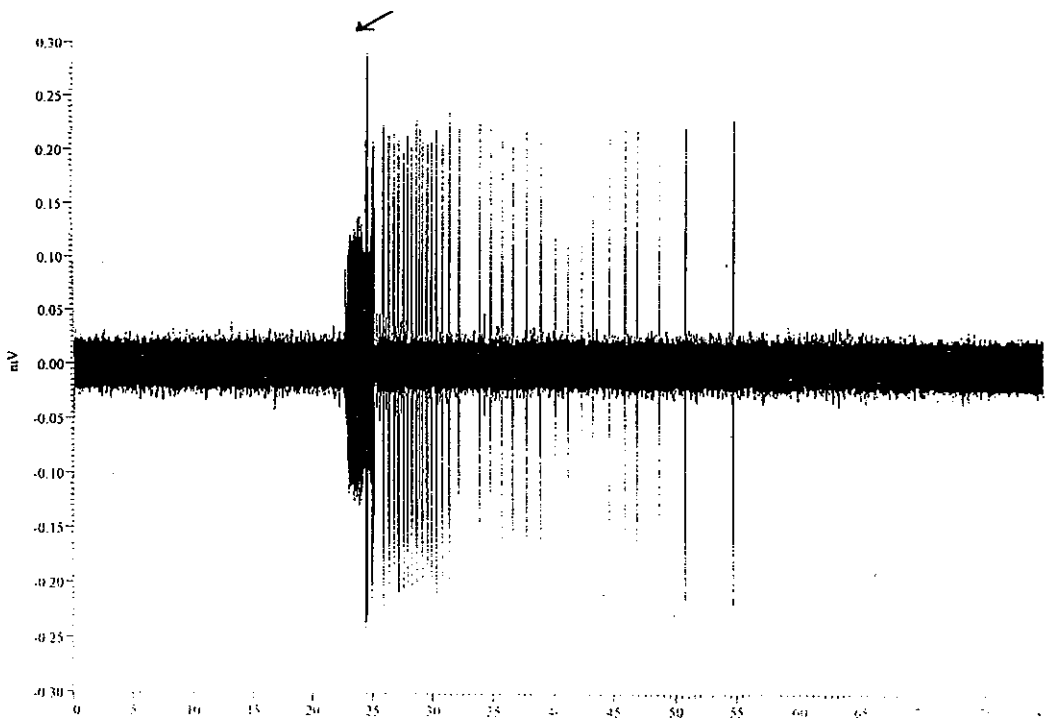
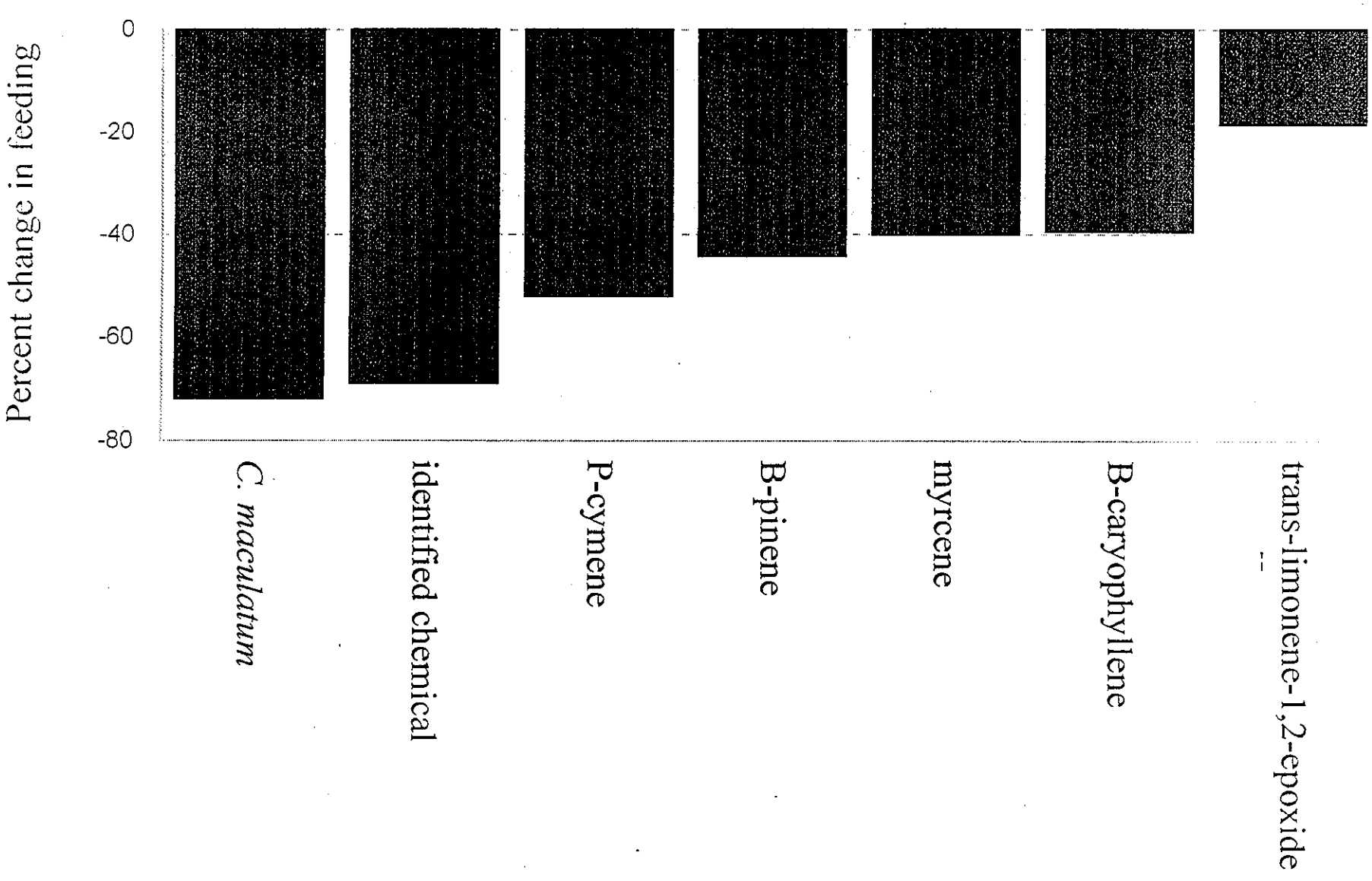


Fig 8 Effect of components isolated from *C. maculatum*, on feeding behaviour in *D. reticulatum* (tested at the concentration found within the extract)



(Fig 9). Increasing the concentration of test material applied to the pellets increased the level of antifeedant activity, but the identified chemical from hemlock remained the most active one.

5.4.2 GC/MS TRACE FOR *CORIANDRUM SATIVUM*

The GC/MS spectrum for *C. sativum* led to the identification of five aldehydes (Fig 10). As 2-tetradecenal was unavailable commercially screening was confined to the remaining four. Smaller peaks at this stage were left unidentified as the past trace for *C. maculatum* had demonstrated that the major peak accounted for the majority of antifeedant activity.

All four aldehydes induced electrical activity in three slug tentacle nerve preparations and were also found to reduce feeding to a similar extent in the behavioural bioassay, with 2-undecenal having a slightly greater effect (Fig 11).

5.4.3 GC/MS TRACE FOR *PETROSELINUM CRISPUM*

The GC/MS trace and co-injections for extract of *P. crispum* identified four major components present in the extract: myrcene, D3-carene, myristicin and 1,3,8-P-menthatriene (Fig 12). Menthatriene was unavailable commercially and attempts to synthesise this compound did not produce enough for screening. D3-carene was inactive on the electrophysiological nerve preparation, while myrcene had already been identified in the extract of *C. maculatum*, where a 1mg/ml solution reduced feeding by 40±7.8%. Myristicin induced electrical activity in the nerve preparation when applied to chemoreceptors at a concentration of 180ng/µl. Feeding bioassay tests demonstrated that when myristicin was applied to wheat flour pellets at a concentration of 1mg/ml, feeding was reduced by 69±4.3% (Fig 11).

Fig **9** Effects of components isolated from *C. maculatum*, on feeding behaviour in *D. reticulatum* (tested at 1.0mg/ml)

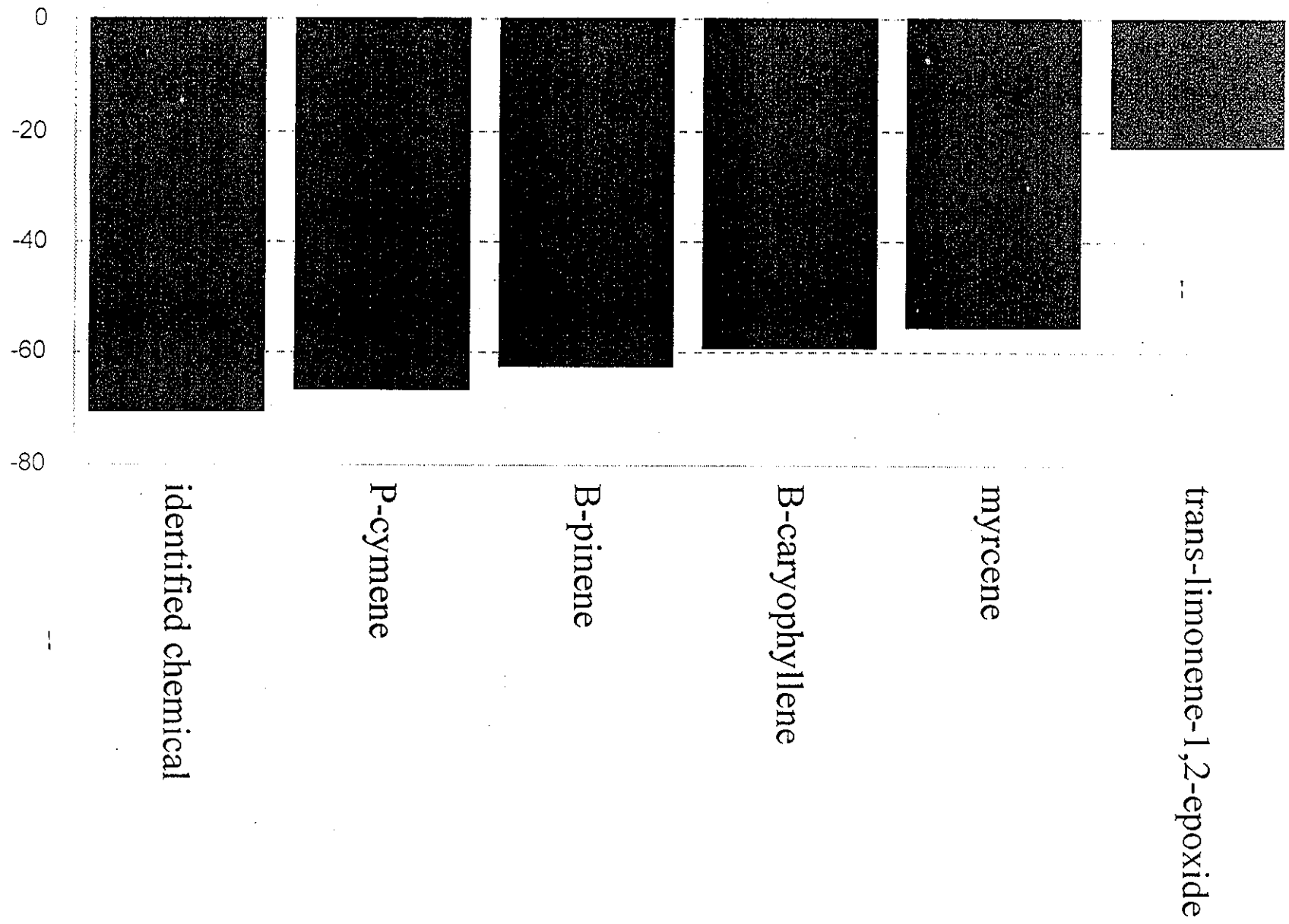


Fig **10** A section of the GC trace produced for extract of *C. sativum*, concentrations at which they occur in the extract are listed in brackets.

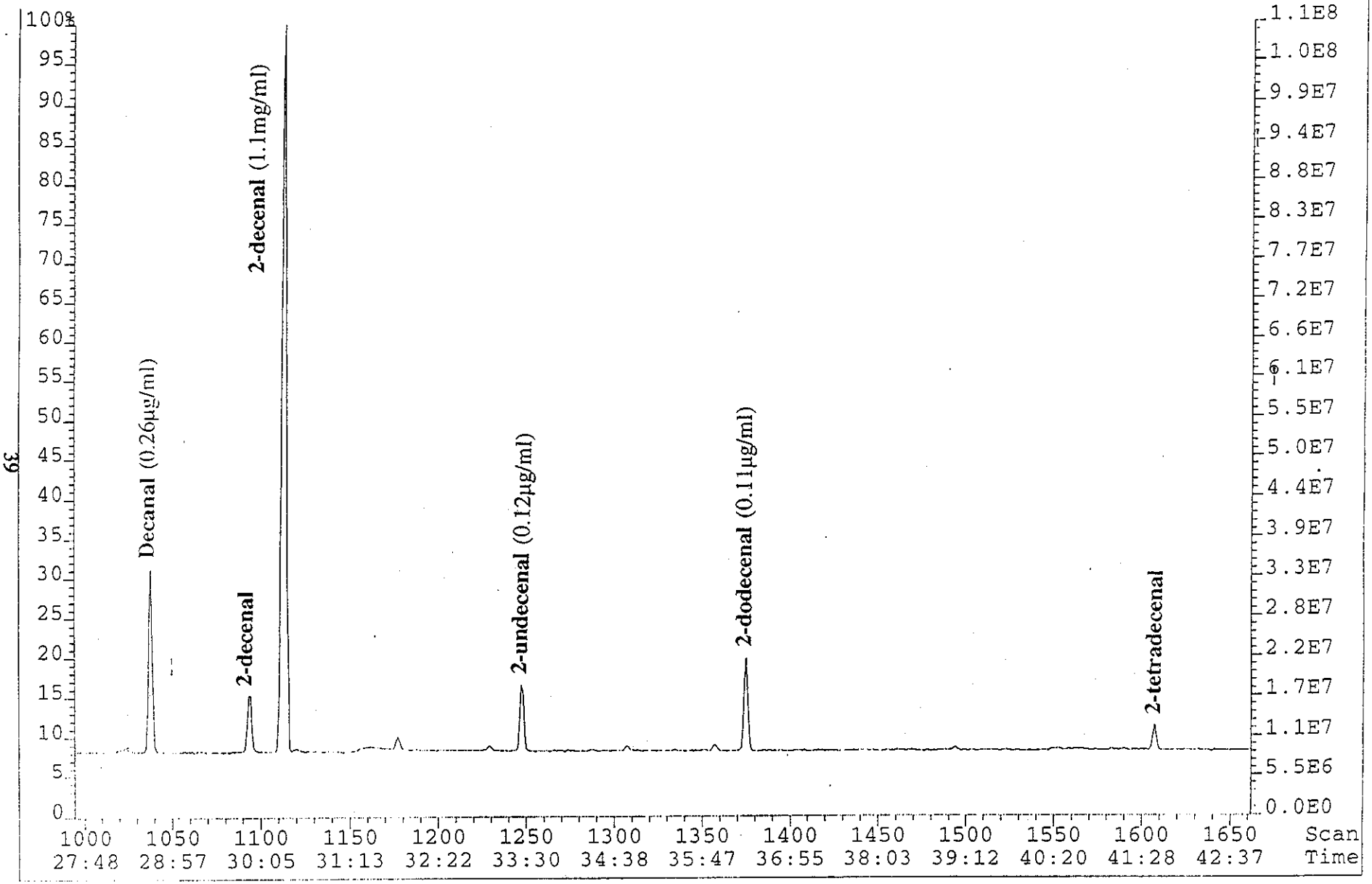


Fig **11** Effect of components isolated from *C. sativum* and *P. crispum*, on feeding behaviour in *D. reticulatum* (tested at 1.0mg/ml)

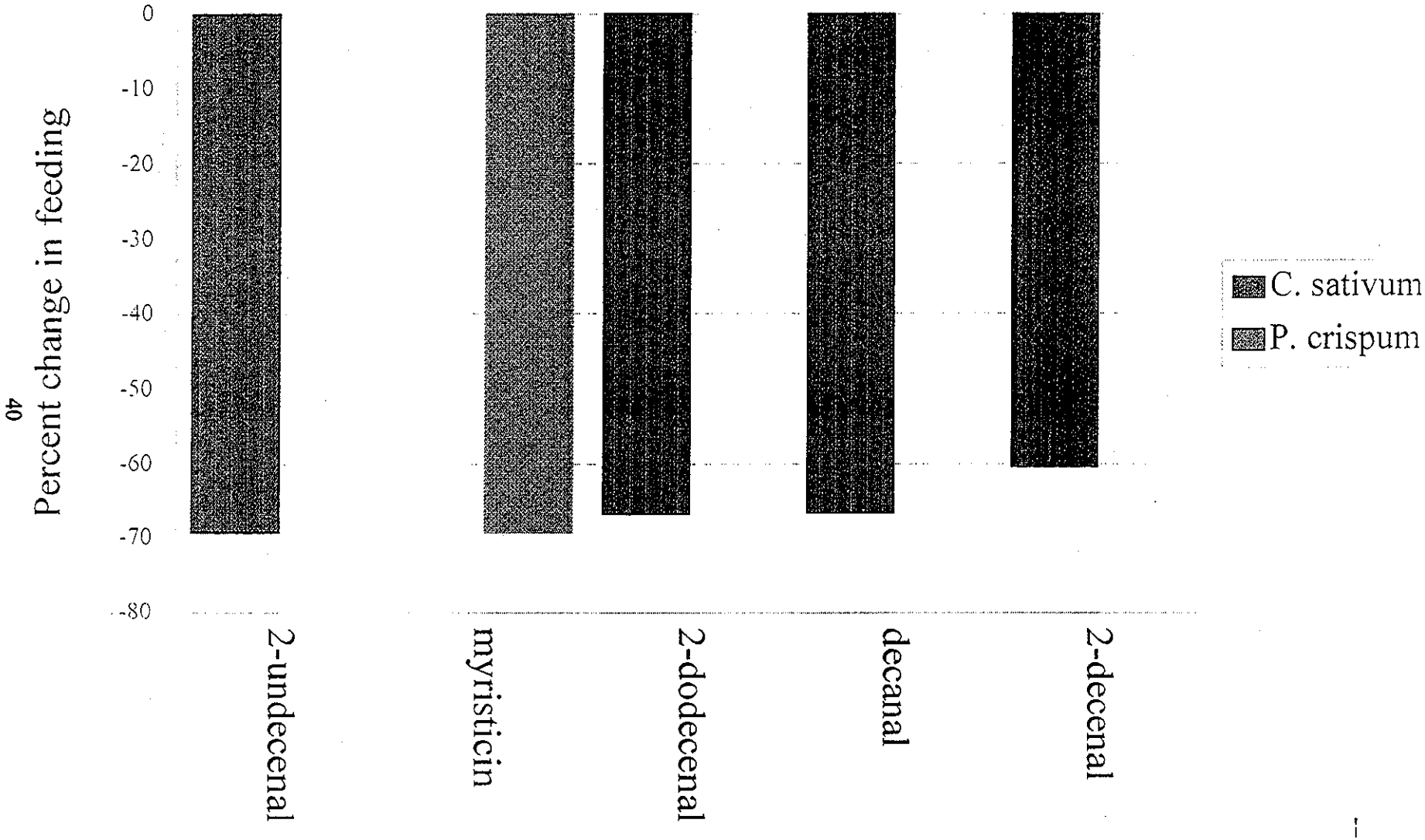
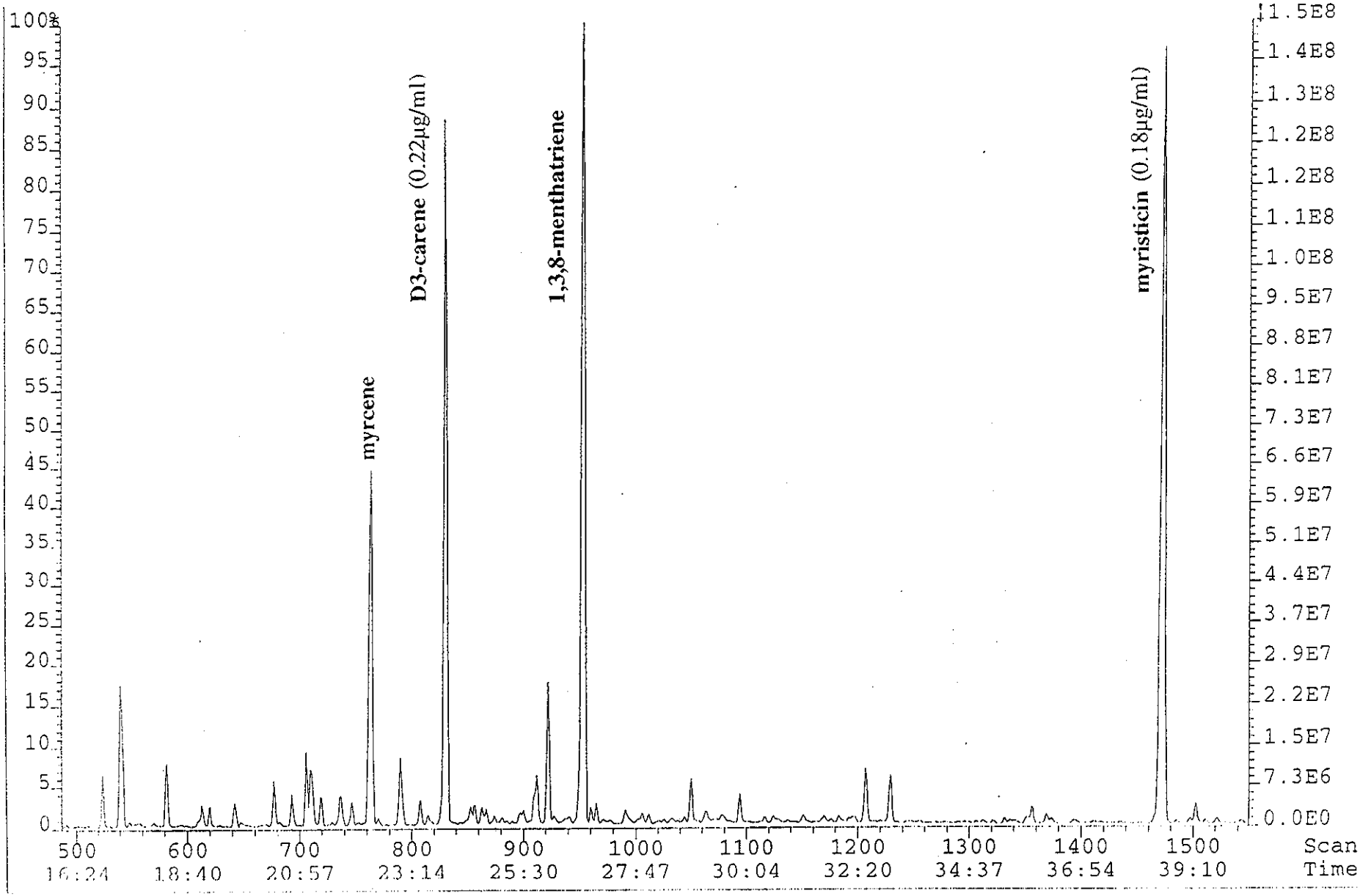


Fig **12** A section of the GC trace produced for extract of *P. crispum*, concentrations at which they occur in the extract are listed in brackets.



5.5 ASSESSMENT OF HEMLOCK ANTIFEEDANT AS A PLANT PROTECTANT SPRAY

The most potent antifeedant found, the major active component in hemlock extract, was tested at increasing rates as a spray application to young Chinese cabbage plants under conditions of extreme slug grazing pressure. Paired plants, sprayed and unsprayed, were exposed to 30 starved adult *D. reticulatum* over a 24 hour period. A reduction in feeding damage occurred at 18 μgcm^{-2} and at the highest rate tested, 54 μgcm^{-2}, slug feeding on the treated plants was completely prevented. At this rate slight discolouration of the leaves was observed, indicating the onset of phytotoxic effects (Table 9).

When the test was repeated at the 18μgcm^{-2} rate with a further nine replicate pairs of plants the protectant effect was confirmed (Table 10).

Table 9 Effect of spaying C. maculatum antifeedant chemicals at increasing rates on slug damage to Chinese cabbage plants exposed to 30 D. reticulatum over 24 hours.

		Visual Score of Damage to Foliage							
		2μgcm^{-2}	U	6μgcm^{-2}	U	18μgcm^{-2}	U	54μgcm^{-2}	U
Rep.1	Leaf 1	3	3	3	3	1	4	0(P)	3
	Leaf 2	2	3	1	3	0	3	0(P)	2
Rep. 2	Leaf 1	3	3	1	3	0	4	0(P)	3
	Leaf 2	2	2	3	1	1	3	0(P)	3

Plants had 2 leaves of roughly equal size and each test was replicated twice. After 24 hours leaves were given a visual score relating to the area of leaf consumed. 4 = All leaf consumed; 3 = ≥ 50% of leaf consumed; 2 = approximately 50% of leaf consumed; 1 = ≤ 50% of leaf consumed; 0 = leaf undamaged; P = signs of phytotoxicity; U = untreated plant and T = treated plant.

Table 10 Effect of spraying C. maculatum antifeedant chemical at 18μgcm^{-2} on Chinese cabbage plants exposed to 30 D. reticulatum over 24 hours.

		Visual Score of Damage to Foliage at 18µgcm^{-2}									
	Replicate		1	2	3	4	5	6	7	8	9
T	Leaf 1	2	0	1	1	1	1	0	0	1	1
	Leaf 2	2	1	0	2	1	0	0	0	0	0
U	Leaf 1	5	3	3	2	3	4	4	4	4	3
	Leaf 2	4	3	4	4	3	3	4	4	3	4

Plants possessed only 2 leaves of roughly equal size and each test was replicated. After 24 hours leaves were given a visual score relating to the area of leaf consumed. 4 = total leaf consumed; 3 = ≥ 50% of leaf consumed; 2 = approximately 50% of leaf consumed; 1 = ≤ 50% of leaf consumed; 0 = leaf undamaged; P = signs of phytotoxicity; U = untreated plant and T = treated plant.

6. CONCLUSIONS AND IMPLICATIONS

This study has shown that slugs can detect and react to a range of secondary metabolites found in plant tissues. Although present in very small amounts these chemicals have a profound effect on such basic behaviour as feeding, which presents an opportunity for the development of an alternative strategy for protecting crop plants from these pests.

Of the 33 plant species tested, extracts of 22 affected slug feeding. The eleven chemical antifeedants identified included alcohols, aldehydes and alkaloids, suggesting that slug feeding behavoiur may be manipulated by chemicals with different physiological and toxicological characteristics, some of which may have the correct combination of properties for practical application.

Only the most active antifeedant chemical, an alkaloid found in hemlock, was so assessed and then only as a foliar spray. This compound has significant mammalian toxicity which would appear to disqualify it for commercial development. However, if deployed as a seed treatment on, for example, winter wheat, it would address the major UK slug problem and

might do so at application rates low enough to avoid any toxic risk.

In the tests with this compound on brassica foliage, slug feeding was completely prevented at rates of 50 μg cm^{-2} active ingredient. If comparable activity were shown as a wheat seed treatment this would be equivalent to less than 0.1% of seed weight. For foliar application less toxic antifeedants would be required which may be found among the other classes of antifeedant chemicals already identified or present in species not yet tested.

Slug control currently relies on three synthetic molluscicides formulated as baits, which give unreliable control and put non-target species at risk. This study has identified eleven naturally-occurring antifeedants, one of which has shown useful activity under artificial conditions. Appropriate formulation and deployment of these chemicals or of others yet to be isolated may offer a new way to tackle this particularly intractable pest problem.

7. ACKNOWLEDGEMENTS

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The Home-Grown Cereals Authority is a public body set up by the Cereals Marketing Act 1965. A number of important amendments to the Act were made by the Agriculture Act 1986 and the Cereals Marketing Act (Application to Oilseeds) Order 1989. The Act, as amended, defines the Authority's functions, constitution and the specific functions which it may undertake for the purpose of improving the production and marketing of home-grown cereals and oilseeds. In 1990 the HGCA Oilseeds Levy Scheme was introduced to fund research and development.

As well as sponsoring research and development in relation to both cereals and oilseeds, the Authority's other functions are:-

- providing a market information service for cereals and oilseeds;
- developing UK cereals exporting capabilities;
- promoting increased consumption of cereal based products in the home market and overseas.

The Authority is funded principally by levies paid by growers of cereals and oilseeds and by cereal dealers and processors.

The Authority administers its R&D function with the assistance of two Advisory Committees, one dealing with cereals and the other with oilseeds R&D. Cereals growers, dealers and processors all contribute in differing proportions to the funding of cereals R&D and all these sectors are represented, therefore, on the R&D Advisory Committee for Cereals. The R&D Advisory Committee for Oilseeds represents the interests of oilseed growers who are the sole funders of oilseeds R&D.

Details of subject areas of interest to both committees are published in strategy documents. Reports of all funded R&D are also published and promoted within the industry.

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