REPELLENCY OF VARIOUS OILS AND PINE OIL CONSTITUENTS TO HOUSE FLIES

(DIPTERA: MUSCIDAE)

by

Mwandacha Ezekiel Maganga

BSc. Agriculture (Hons.), University of Nairobi, 1989.

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REPELLENCY OF VARIOUS OILS AND PINE OIL CONSTITUENTS TO HOUSE FLIES (DIPTERA: MUSCIDAE)

Examining Committee:

Chair: Dr. J. Rahe, Professor

Dr. G. Gries, Assistant Professor, Senior Supervisor
Department of Biological Sciences, SFU

Dr. M. Mackauer, Professor,
Department of Biological Sciences, SFU

Dr. K. Slessor, Professor
Department of Chemistry, SFU

Dr. J. Borden, Professor
Department of Biological Sciences, SFU
Public Examiner

Date Approved 19 April 1995

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REPELLENCY OF VARIOUS OILS AND PINE OIL CONSTTTUENTS TO HOUSE FLIES (DIPTERA:MUSCIDAE)

Author:

(signature)

MWANDACHA E. MAGANGA

(name)

20TH APRIL 1995

(date)

ABSTRACT

Comparative repellency of pine, mineral, motor and silicon oil to house flies, *Musca domestica* L., was tested in 10-minute binary choice bioassays, each employing 20 caged, 4-5 day-old flies. Testing the number of flies feeding on 20 *u*l of watery honey solutions (HS) mixed with (treatment) or without (control) 10 *u*l of one of the oils under investigation, only pine oil completely suppressed feeding and remained inhibitory even after 24 hours. Approaching pine oil-treated HS, 95% of flies were repelled at a distance > 6 mm from the source, indicating that recognition of repellent constituents was based on olfaction rather than contact chemoreception.

Analysis of pine oil volatiles by coupled gas chromatographic-electroantennographic detection (GC-EAD) revealed 5 antennally-active compounds, 4 of which were identified by coupled GC-mass spectrometry as myrcene, *para*-cymene, *gamma*-terpinene and linalool.

Repellency of these compounds alone or in combination was demonstrated in a 5-replicate experiment employing one treatment per cage with 50 flies each. At a 10 *u*l dose, significantly lower proportions ($P < 0.05$) of flies fed on HS treated with pine oil or one of the four pine oil constituents. At a 1 *u*l dose only the linalool-treatment inhibited feeding. In binary choice experiments both feeding and oviposition were significantly reduced on linalool-

treated sources. Because fly maggots naturally develop in and rely on microbe-rich organic sources, gravid females may percieve and avoid potential oviposition sites that are rich in antimicrobial compounds such as linalool.

Acknowledgement

I thank Dr. G. Gries for his inspiration, valuable advise and exceptional support throughout this project, Ms. R. Gries for assistance with chemical investigations, Drs. Keith Slessor and M. Mackauer for their worthy advice and support, Mr. A. Syed for assistance with fly rearing, Dr. V. Bourne for assistance with electron microscopy, Dr. J. Borden for his advice and inspiration, and the Kenya high commission for administering the funding. The study was supported by the Canadian International Development Agency (CIDA) and the Natural Sciences and Engineering Research Council of Canada.

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I.0. Introduction

The house fly, *Musca domestica* L. (Diptera: Muscidae), is cosmopolitan (West 1951, Semakula *et al.* 1989) occurring in and around human housing (West 1951, Sacca 1964). It is prolific where man is in close association with livestock (Sacca 1964, Axtell & Arends 1990, Smith & Rutz 1991, Miller 1994). Seasonal occurrence in the nearctic region is determined mainly by temperature, with complete suppression of activity below 7°C (Semakula *et al.* 1989). In most tropical regions, flies remain active throughout the year, producing up to 30 generations per year (Moon & Meyer 1985) with two seasonal peaks based on moisture conditions (West 1951).

The life cycle of house flies may be completed in 6-10 days under optimal temperature and humidity conditions (Axtell & Arends 1990), but may extend over 2 months when conditions are unfavourable (Sacca 1964). Females mate once (Zingrone *et al.* 1959), accepting a mate preferably 3 days postemergence. Males can mate as early as 10-12 h of age (Sacca 1964) but reach sexual maturity 24 h after emergence. They are capable of mating 4-8 females in 24 h (Chang,1965). Gravid females deposit eggs in batches of about 100 (Moon & Meyer 1985), preferably on organic material of attractive odour and 70% moisture level (Fatchurochim *et al.* 1989). Odour is affected by the degree of fermentation which through the heat generated affects the length of the larval

and pupal periods (West 1951, Sacca 1964). Old, completely fermented manure is not attractive to ovipositing females (West 1951).

House flies are important in agriculture because they irritate livestock and working personnel (Fenton & Bieberdorf 1936, Anderson & Poorbaugh 1964, Axtell 1986, Miller 1994), and when they invade urban areas they become nuisance pests in residences and businesses (Axtell & Arends 1990). They are potential mechanical vectors of pathogens which cause human and livestock diseases (Whitehead & Bowers 1983, Youdeowei & Service 1983, Enright *et al.* 1987, Semakula *et al.* 1989). They also deface fixtures and structures by their regurgitate and faeces (Axtell & Arends 1990).

Development of pesticide resistance in house flies (LaBrecque *et al.* 1958, MacDonald *et al.* 1983, Chapman 1985, Price and Chapman 1987) has prompted implementation of physical and biological control measures (Axtell, 1986), including use of (non)electrocuting light-, sticky- and suction-traps (Skovmand & Mouvier 1986, Rutz *et al.* 1988, Tajuddin 1993, Pickens *et al.* 1994), parasites (Morgan *et al.* 1981, Rutz & Axtell 1981, Petersen & Meyer 1983, Smith & Rutz 1986, Mullens *et al.* 1986, Petersen *et al.* 1992), predatory mites (Axtell 1968, Geden & Axtell 1988, Wise *et al.* 1988), nematodes (Geden *et al.* 1987, Mullens *et al.*1987), Muscovy ducks, *Cairina moschata* L. (Glofcheskie &

Surgeoner 1993) and feed-through compounds (Strong 1992, Miller 1994). Semiochemical-based house fly control employs the male-produced pheromone, (*Z*)-9-tricosene (Carlson *et al.* 1971), in combination with adhesive panels, fly paper strips, sugar baits and electric grids (Carlson & Beroza 1973).

Repellents have been suggested as a means to alleviate fly nuisance (Goodhue & Stansbury 1953, Shambaugh *et al.* 1968, Campbell 1983) but are commonly effective only upon contact (Campbell 1983). This thesis reports the results of experiments designed to investigate the chemical identity and bioactivity of candidate repellents/feeding deterrents.

2.0. Comparative Repellency of Various Oils to House Flies (Diptera: Muscidae)

A number of natural, repellent sources have found traditional use against house flies. For example, Ethiopians spread leaves of the pepper tree, *Schinus molle* L., on dining tables (Wimalaratne 1993, Wimalaratne *et al.* 1995) and Kenyans wipe them with motor oil (personal observation). Also repellent to flies are essential oils of many plants, including Indian calamus, *Acorus calamus* L. (Alder & Jacobson 1985), rose geranium, *Pelargonium odoratirum* I'Herit, palmarosa, *Andropogon martini* Vitman, ginger grass, *Cymbopogon martini* L., eucalyptus, *Eucalyptus globulus* Maid *et al.* and citronella, *Cymbopogon winterianus* Jowitt (Osmani *et al.* 1972). Moreover, pine oil, a byproduct of the pulp industry, deters oviposition by the onion maggot, *Delia antiqua* (Meigen) (Javer *et al.* 1987, Ntiamoah 1994). Mineral oil also repels houseflies (Singh & Singh 1991), but biological activity of silicon oil has not yet been demonstrated.

My objectives were to 1) determine which of several oils with known or suggested bioactivity, such as pine, light mineral, commercial motor and silicon oil, is repellent to house flies, and 2) characterize the bioactivity of the most repellent oil.

2.1. Materials and Methods

2.1.1. Test Insects. House flies were maintained at 21-25^{0}C and a 10:14 (light:dark) photoperiod. Cotton wicks partly submerged in a watery skim milk suspension served as oviposition sites for gravid females. Eggs were transferred to, and maggots reared on, artificial media composed of skim milk paste (15 ml) added to a mixture of brewers yeast (25 g), molasses (35 g) and wheat bran (400 g; 3.5% fat, 15% protein, 11.5% fibre) in water (700 ml). Emergent adults were transferred into cages (30 X 30 X 45 cm) and provided with sugar, milk powder and water *ad libitum*.

2.1.2. Bioassay Procedure.

Experiment 1: Relative Repellency of Various Oils. Experiments (Exp.) employed Wimalaratne's (1993) bioassay design with few modifications. Twenty, 4-5 day-old flies (mixed sex) were introduced into a wooden cage (16 X 16 X 13 cm) with a Plexi glass front and plastic screen back and sides, and were starved for 3-4 h prior to bioassay.

In 10-minute binary choice tests replicated 6 times, two microscope coverslips were introduced into the middle of the cage and placed 10 cm apart. Both the control and treatment coverslip carried 20 *u*l of a concentrated honey solution (HS) honey:water (1:1) (vol.:vol.). The solution on the treatment coverslip was mixed with 10 *u*l of the oil under

investigation: Pine oil (Norpine 65, Northwest Petrochemicals, Anacortes, Washington), light mineral oil (Sigma Chemical Co., Mississauga, Ontario), motor oil (Shell Canada Products Ltd., Calgary, Alberta) and silicon oil (Superior Materials Inc., Garden City, New York.). The position of coverslips was alternated and flies replaced in consecutive replicates. Flies feeding on untreated and oil-treated HS were counted for 10 minutes, and the data analyzed using t-tests (Steel & Torrie 1981) with significance level set at $P = 0.05$.

Experiments 2-4: Characteristics of Pine Oil Repellency. The pine oil dose sufficient to inhibit fly feeding completely was determined in Exp. 2 by testing either 10, 1 or 0.1 *u*l of hexane-diluted pine oil in 20 *u*l-HS *versus* untreated HS with equivalent amounts of hexane.
In Exp. 3 control and treated coverslips (prepared as in Exp. 1) were held for 24 h at 22-25°C prior to bioassay to assess persistency of pine oil repellency. The distance over which 10 *u*l of pine oil in 20 *u*l-HS expressed repellency (Exp. 4) was estimated in four 10-minute bioassays each with 20 flies. Behaviour of flies within and outside of 6 and 10 mm radii around the treated HS was video taped. Data from experiments 2 and 3 were analysed using t-tests (Steel & Torrie 1981). The level of significance was set at $P = 0.05$.

2.2. Results and Discussion

Pine oil unlike all other oils, completely suppressed feeding in Exp.1 (Fig. 1). One and 10 ul of pine oil mixed with 20 ul of HS (Exp. 2) equally and completely suppressed feeding (Fig. 2). The later dose inhibited any feeding even after 24 hours (Exp. 3). Approaching pine oil-treated HS in Exp.4, 95.5% of the flies turned away at a distance > 6 mm from the source (Fig. 3), indicating that recognition of repellent constituents was based on olfaction rather than contact chemoreception.

These results confirm the bioactivity of pine oil found for other diptera (Cory 1971, Javer *et al.* 1987, Ntiamoah 1994) and demonstrate that it has both repellent and feeding deterrent properties against house flies, respectively causing flies to make oriented movements away from the source and inhibiting feeding in a place where flies would feed in its absence (Dethier 1960). Behavioural activity of pine oil has also been demonstrated for bark beetles, *Dendroctonus* spp. (Nijholt *et al.* 1981), ambrosia beetles, *Trypodendron* and *Gnathotrichus* spp. (Nijholt 1980) and the white pine weevil, *Pissodes strobi* Peck (Alfaro *et al.* 1984).

Mineral and motor oil also suppressed attraction of flies to HS (Fig. 1), but did not completely inhibit feeding. These oils comprise highly complex chemical mixtures of many

Fig. 1. Mean numbers of flies feeding in Exp. 1 on 20 ul of watery honey solutions mixed with (treatment) or without (control) 10 ul of either pine, mineral, motor or silicon oil. For each of the four, 6-replicate binary choice experiments, significant differences between treatment and control (t-test) are indicated by * for $P < 0.05$ and ** for $P < 0.01$.

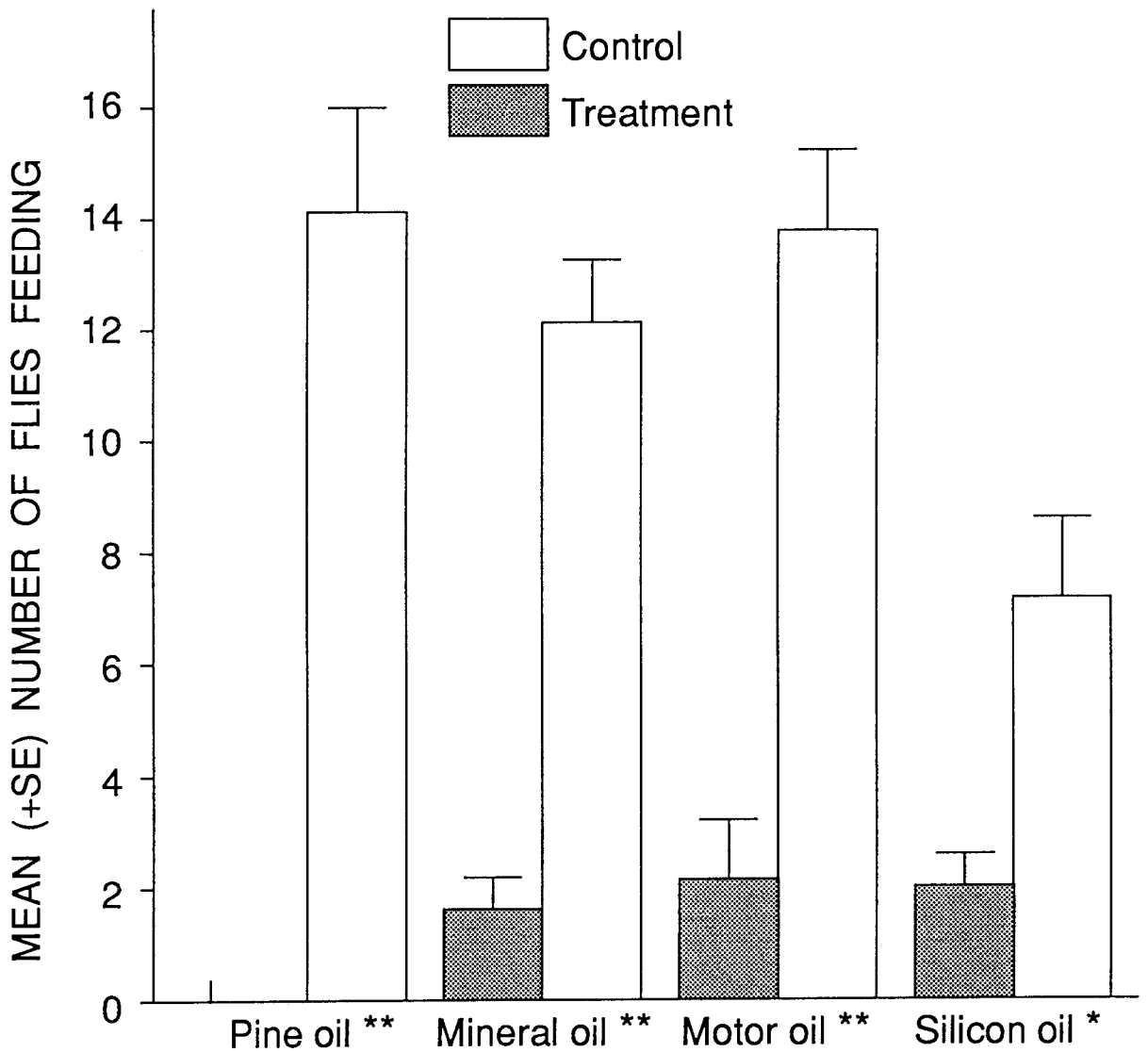

Control
Treatment
MEAN (+SE) NUMBER OF FLIES FEEDING
0
2
4
6
8
10
12
14
16
Pine oil **
Mineral oil **
Motor oil **
Silicon oil *

Fig. 2. Mean numbers of flies feeding in Exp. 2 on 20 *u*l of watery honey solutions mixed with (treatment) or without (control) 10, 1, and 0.1 *u*l of pine oil. The two lower doses were diluted in hexane of which equivalent amounts were also administered to control HS. For each dose with 6 replicates each, differences between treatment and control are highly significant (t-test, $P < 0.01$).

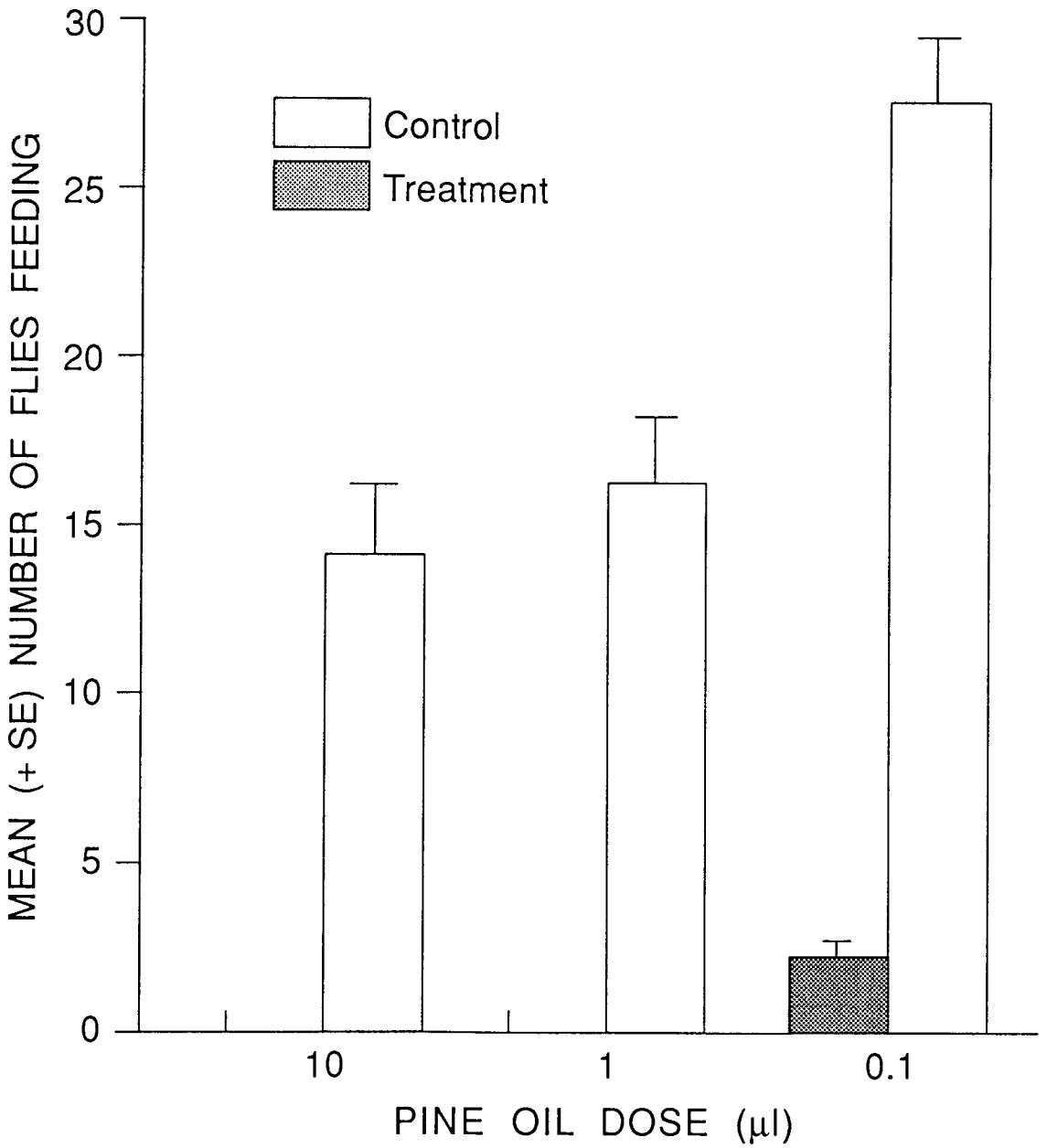
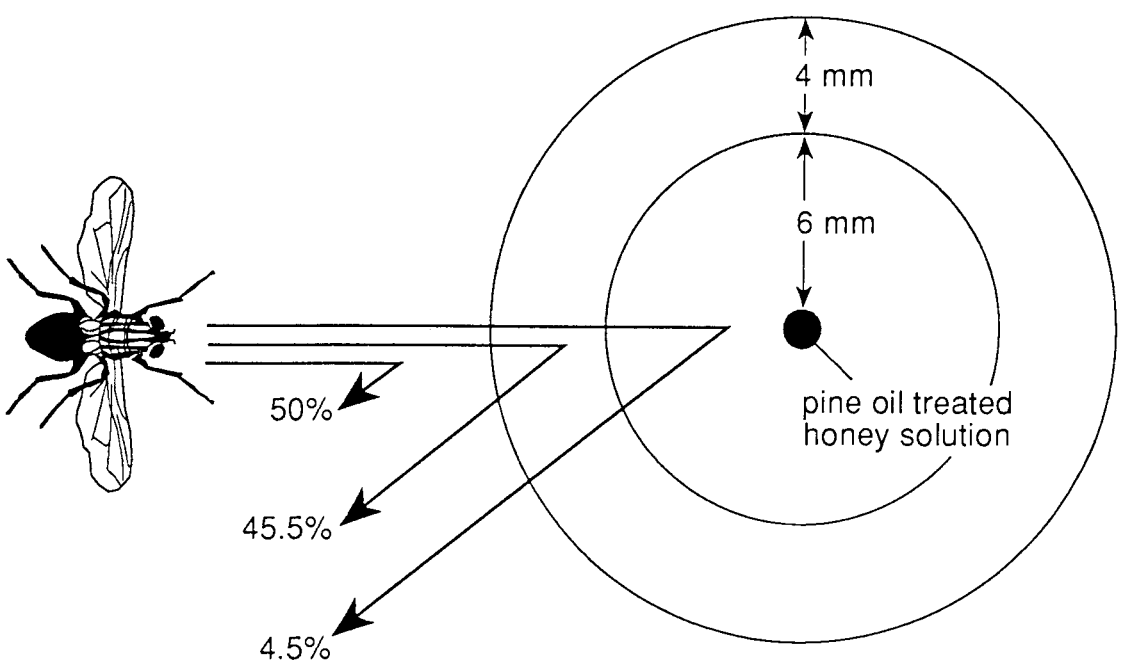

30
25
20
15
10
5
0
MEAN (+ SE) NUMBER OF FLIES FEEDING
Control
Treatment
10
1
0.1
PINE OIL DOSE (µl)

Fig. 3. Effective distance over which 10 ul of pine oil mixed with 20 ul of watery honey solutions repelled approaching flies. Percentages based on responses of 21 flies, video taped in four 10-minute replicates with 20 flies each per cage. No fly ever made contact with treated HS.


4 mm
6 mm
pine oil treated
honey solution
50%
45.5%
4.5%

constituents (Crob *et al.* 1991) some of which have an unpleasant odour. Pine oils in contrast, are less complex and consist mainly of terpenes and terpene alcohols (Nijholt 1980, Alfaro *et al.* 1984, Bell & Harestad 1987), which are associated with chemical defence of plants against phytophagous insects, bacteria and fungi (Rice & Coats 1994).

Limonene, 3-carene and *para*-cymene in pine oil, and *cis*-menth-2-en-1-ol and *trans*-piperitol in the pepper tree, respectively deter oviposition of onion maggots (Ntiamoah 1994) and feeding of house flies (Wimalaratne 1993). Behavioural activity of natural repellents may be associated with individual chemicals warranting the identification of the compound(s) in pine, motor and mineral oil strongly repellent/deterrent to house flies in this study.

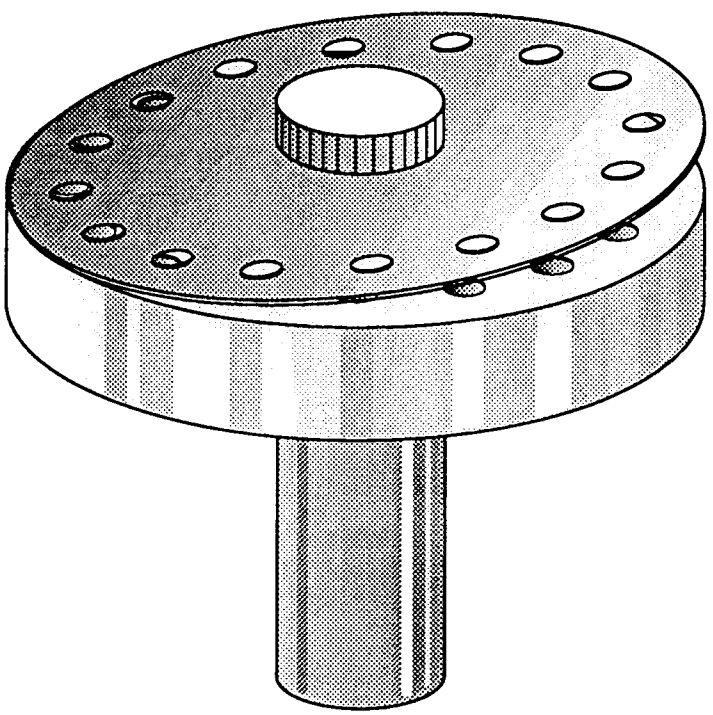
3.0. IDENTIFICATION AND LABORATORY ASSESSMENT OF PINE OIL CONSTITUENTS REPELLENT TO HOUSE FLIES

The objective of this study was to identify pine oil constituent(s) responsible for repelling/deterring house flies from potential feeding and oviposition sources.

3.1. Materials and Methods

3.1.1. Coupled Gas Chromatographic-Electroantennographic Detection (GC-EAD) and Coupled GC-Mass Spectrometric (MS) Analyses. Aliquots of 0.01 ug of hexane-diluted pine oil (Norpine 65, Northwest Petrochemicals, Anacortes, Washington) were subjected to GC (Varian 3400) analyses with both flame ionization detector (FID) and electroantennographic detection (EAD) (Arn *et al.* 1975) on a fused silica column (DB-5; 30 m X 0.32 mm ID, J & W Scientific, Folsom, California). For GC-EAD recordings, a fly with only its head protruding was mechanically immobilized in a Plexi glass block (Fig. 4). Electrodes were prepared as follows: one end of a Pyrex glass tube 1.0 mm O.D and 0.5 mm ID X 2 cm (A-M Systems, Everett, Washington 98704) was drawn by a micro-pipette puller (Industrial Science Associate Inc., New York) to a fine diameter tip. The micro-electrode was then filled with saline (Staddon & Everton 1980) and attached to a microelectrode holder (STR F Wire 1.0 mm, MEHSFW10; World Precision Instruments Inc., Florida) connected to a BNC-2mm-Pin adapter (World Precision Instruments) positioned on a micromanipulator M (Leitz

Figure 4. Plexi glass block developed by Dr. G. Gries for immobilization of flies during GC-EAD analyses. The fly is inserted from below through a hole of appropriate diameter. As the fly's head protrudes, an aluminium plate with corresponding hole(s) is slightly rotated and tightly locked to immobilize the fly's head.



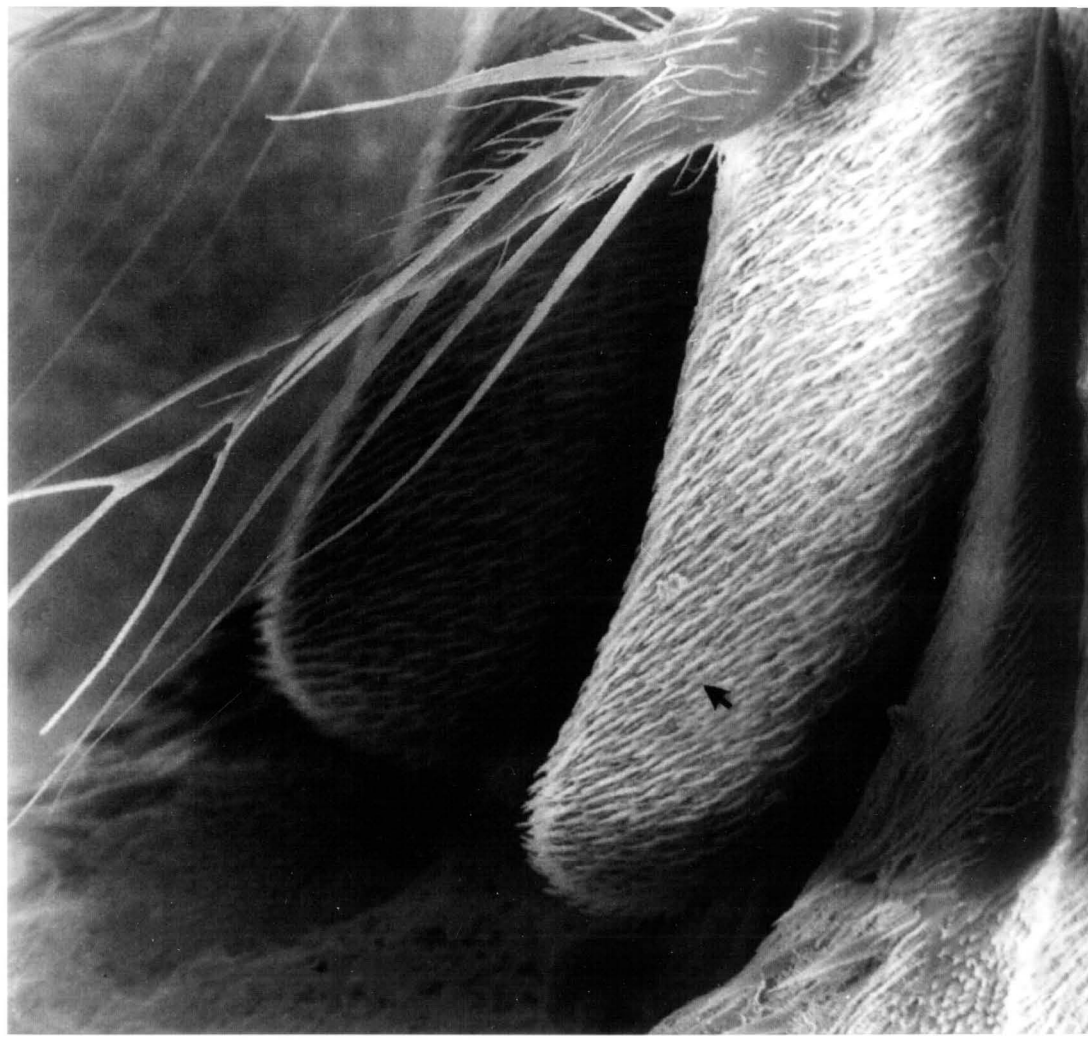
Canada, Willowdale, Ontario). Using an M 10 stereomicroscope and micromanipulator M (Leitz Canada), the recording electrode was micromanipulated to contact the fly's flagellum close to the tip of the arista-bearing side (Fig. 5). The indifferent electrode was inserted into the fly's head between the compound eyes.

Coupled GC-MS (Hewlett Packard 5985B and Varian Saturn II ion trap) both fitted with either the above DB-5 column or an SP-1000 (Supelco Canada Ltd., Oakville, Ontario) coated fused silica column (30 m X 0.25 mm ID) were used for mass spectrometry of antennally active compounds.

3.1.2. Bioassay of EAD-active Pine Oil Constituents

Experiment 1: Comparative Repellency of Antennally-Active Compounds. Five-replicate, no-choice bioassays employed Wimalaratne's (1993) design with few modifications. Fifty 2-3 day-old flies were introduced into cages (16 X 16 X 13 cm), supplied with water and starved for 3 hours. Under red light, one microscope coverslip carrying 20 ul of a watery HS, honey:water (3:1) (vol.:vol.) was then introduced into the middle of each cage. Three treatments (one per cage) were tested concurrently: HS mixed with test chemical(s), an equivalent amount of pine oil, or an equivalent amount of hexane (used to dilute pine oil or test chemical). Purchased myrcene, *para*-cymene (Sigma Diagnostics, Aimco Mississauga, Ontario), *gamma*-terpinene and linalool (Aldrich, Milwaukee, Wisconsin) were tested

Figure 5. Scanning electron micrograph depicting the location of the recording electrode (arrow) during GC-EAD analyses. The indifferent electrode is inserted into the fly's head between the compound eyes.



50μ

individually or in quaternary (1:1:1:1) combination *versus* pine oil at doses of 0.01, 0.1, 1 and 10 *u*l under fluorescent light in the laboratory. After 3 minutes, the number of flies feeding on HS in each cage were recorded.

Experiment 2: Suppression of Fly Feeding on Linalool-treated Sugar Cubes. In a 5-replicate, binary choice experiment with 200, 3 day-old flies per replicate, one untreated sugar cube (1.5 X 1.5 X 1.5 cm, 3.5 + 0.1 mg; B.C. Sugar Refining Co. Ltd. Vancouver, B. C.) and one treated with 10 *u*l of linalool were placed 20 cm apart in the middle of a cage (30 X 30 X 40 cm). The positions of treatment and control cubes were alternated and flies replaced in consecutive replicates. Sugar cubes were weighed prior to and 0.5, 1, 1.5, 2, 3 and 4 h after experiment initiation. Five additional sugar cubes treated with 10 *u*l of linalool were kept in an empty experimental cages and weighed in equivalent time intervals to determine weight loss due to linalool evaporation. These data were used to determine weight losses due to fly feeding.

Experiment 3: Oviposition Deterrency of Linalool. In a 5-replicate, binary choice experiment 200, 2 week-old flies (mixed sex) were exposed to a choice of oviposition substrate comprising two cotton wicks partly submerged in a watery skim milk powder suspension placed 20 cm apart in the middle of a cage (30 X 30 x 40 cm). One wick was untreated and the other treated with 10 *u*l of linalool.

Flies were also provided with milk powder, sugar and water *ad libitum*. Twenty four hours later, eggs deposited on treated and untreated cotton wicks were counted.

Experiment 4: Linalool-Based Suppression of Fly Feeding Under Simulated Operational Conditions. In a 5-replicate, binary choice experiment with 200 flies (mixed sex) per replicate, two trays (40 X 30 cm) covered with 35 g of untreated granulated sugar or sugar treated with 50 ul of linalool were placed 1 m apart in the middle of a growth chamber (4 X 3 X 3 m). One hour and 50 minutes after experiment initiation and 10 minutes prior to counting flies on trays at three 10-minute intervals, sugar in both treatments was sprayed with 1 ml of an attractive solution comprising NH_4OH (2 ml), acetic acid (1 ml) and $(NH_4)_2SO_4$ (2 mg) dissolved in 400 ml of water (Mulla *et al.* 1977).

3.1.3. Statistical Analysis

Numbers of feeding and non-feeding flies in Exp. 1 were summed and placed in 2 X 2 frequency tables (Table 1) (Bliss 1967). Repellency was expressed as a coefficient of association (Jy), calculated as follows:

$Jy = [(ad-bc)/(a+b)(c+d)]$ in which "a", "b", "c" and "d" represent numbers of flies in respective response categories (Table 1). Jy variance is calculated as:

$Var\ Jy = [ab/(a + b)^3 + cd/(c + d)^3]$. Positive and negative Jy-values indicate relative repellency and attraction

Table 1. Frequency (2 X 2) tables of categories of fly responses to honey solutions untreated or treated with pine oil or individual chemicals.

A. Untreated *versus* pine-oil treated HS

GROUP	# Feeding	# Not Feeding	Total
Untreated HS	a	b	a + b
Pine oil-Treated HS	c	d	c + d
Total			N

B. Untreated *versus* chemical-treated HS

GROUP	# Feeding	# Not Feeding	Total
Untreated HS	a	b	a + b
Chemical-Treated HS	c	d	c + d
Total			N

C. Chemical-treated *versus* pine oil-treated HS

GROUP	# Feeding	# Not Feeding	Total
Chemical-Treated HS	a	b	a + b
Pine oil-Treated HS	c	d	c + d
Total			N

respectively. The larger the positive value of Jy, the more repellent is the treatment. Means of weight loss of linalool-treated *versus* untreated sugar cubes (Exp. 2), eggs laid on linalool-treated *versus* untreated oviposition sites (Exp.3) or numbers of flies feeding on linalool-treated *versus* untreated sugar trays (Exp. 4) were analyzed by t-test (Steel & Torrie 1981). For all experiments significance level was set at $P = 0.05$.

3.2. Results

GC-EAD and GC-MS Analyses of Pine Oil.

GC-EAD analyses of pine oil revealed 5 antennally-active compounds (Fig. 6) of which compound 1 was a hexane contaminant. GC-MS of compounds 2-5 indicated that they were myrcene, *para*-cymene, *gamma*-terpinene and linalool (Fig. 7). Identical retention and mass spectrometric characteristics of antennally-active pine oil constituents and authentic standards (Fig. 8) on two columns with different retention characteristics confirmed these structural assignments.

Experiment 1 : *Repellency of EAD-active pine oil constituents*. Except for myrcene, individual chemicals, the quaternary mixture and pine oil at the 10 ul-dose equally suppressed feeding on HS (Fig. 9). At the 1 ul-dose, only linalool suppressed feeding to a greater extent than pine oil. At doses of 0.1 and 0.01 ul there was no significant difference in numbers of flies feeding on treated and untreated HS.

Figure 6. Representative recording of flame ionization detector (FID) and electroantennographic detector (EAD: house fly antenna) responses to 1 ul of hexane-diluted pine oil. Chromatography: Varian 3400 GC fitted with a DB-5 coated, fused silica column (30 m X 0.32 mm I.D.); temperature program: 1 min. at 50^0C, 10^0/min. to 240^0C; injector temperature 240^0C and detector temperature 250^0C. Compounds labelled 1-5 consistently elicited antennal responses.

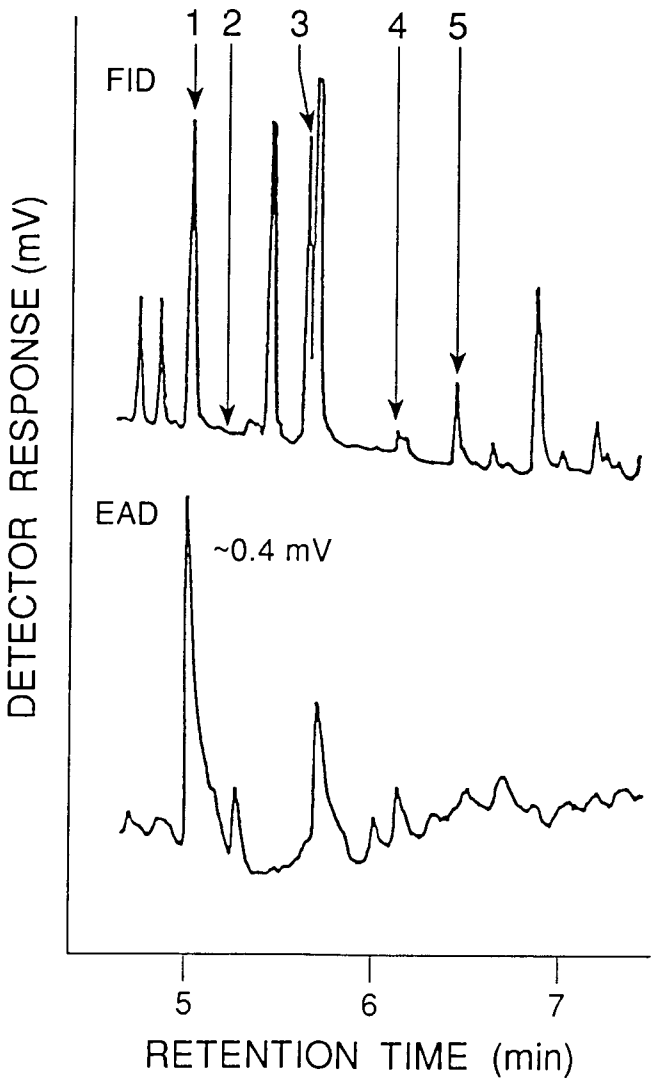

1
2
3
4
5
FID
EAD
~0.4 mV
DETECTOR RESPONSE (mV)
5
6
7
RETENTION TIME (min)

Figure 7. Molecular structure of 4 antennally-active pine oil constituents.

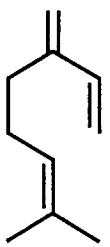
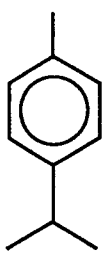
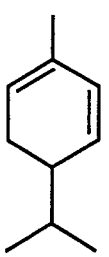
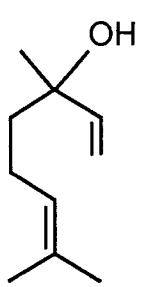
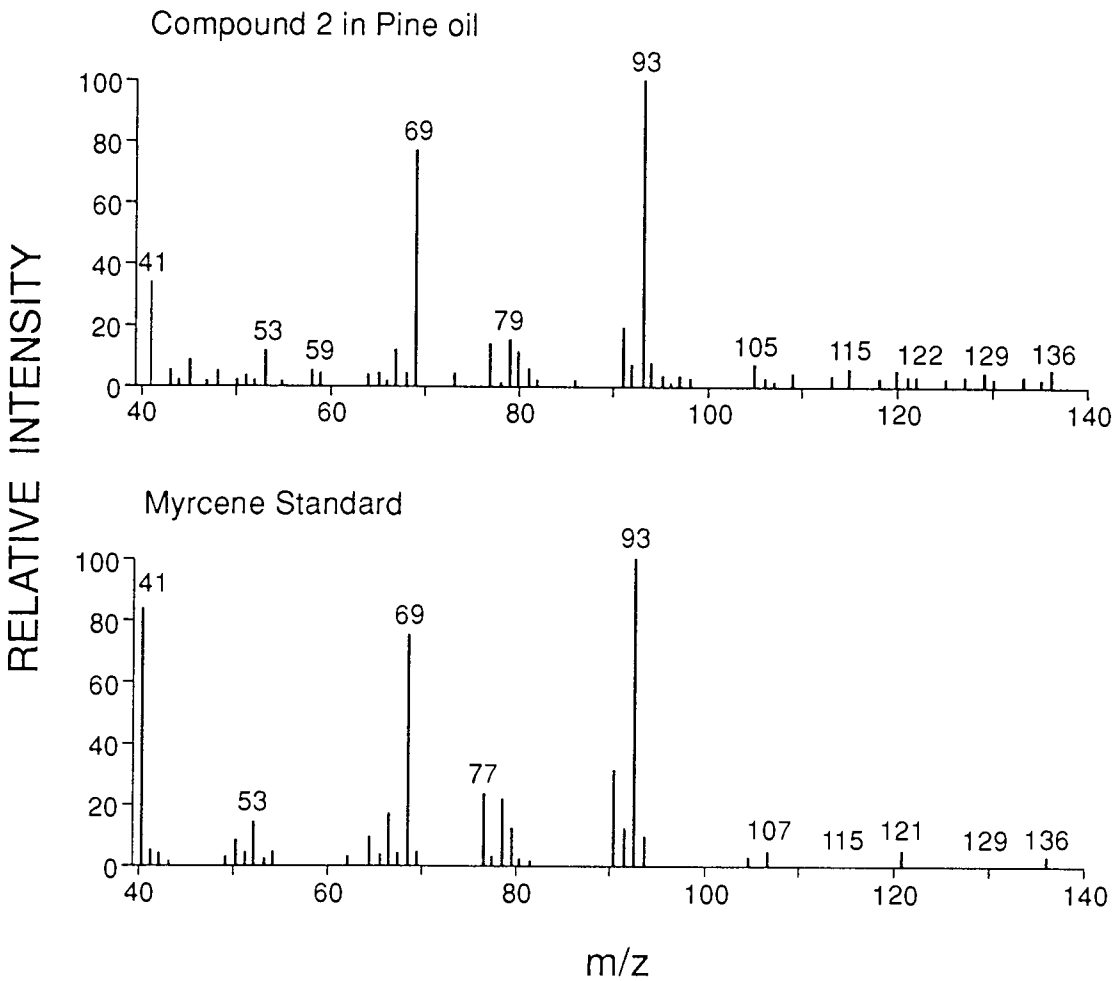
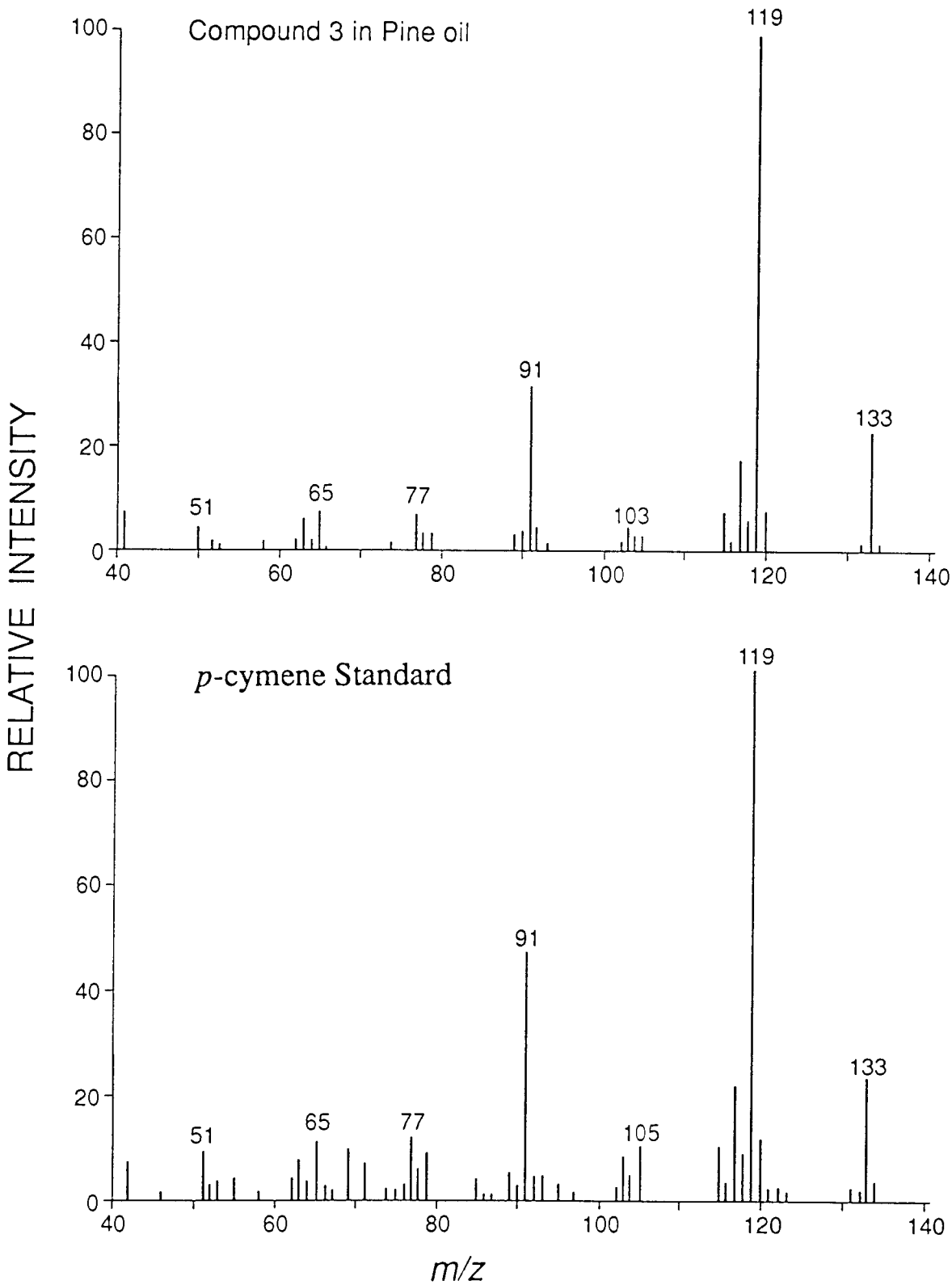
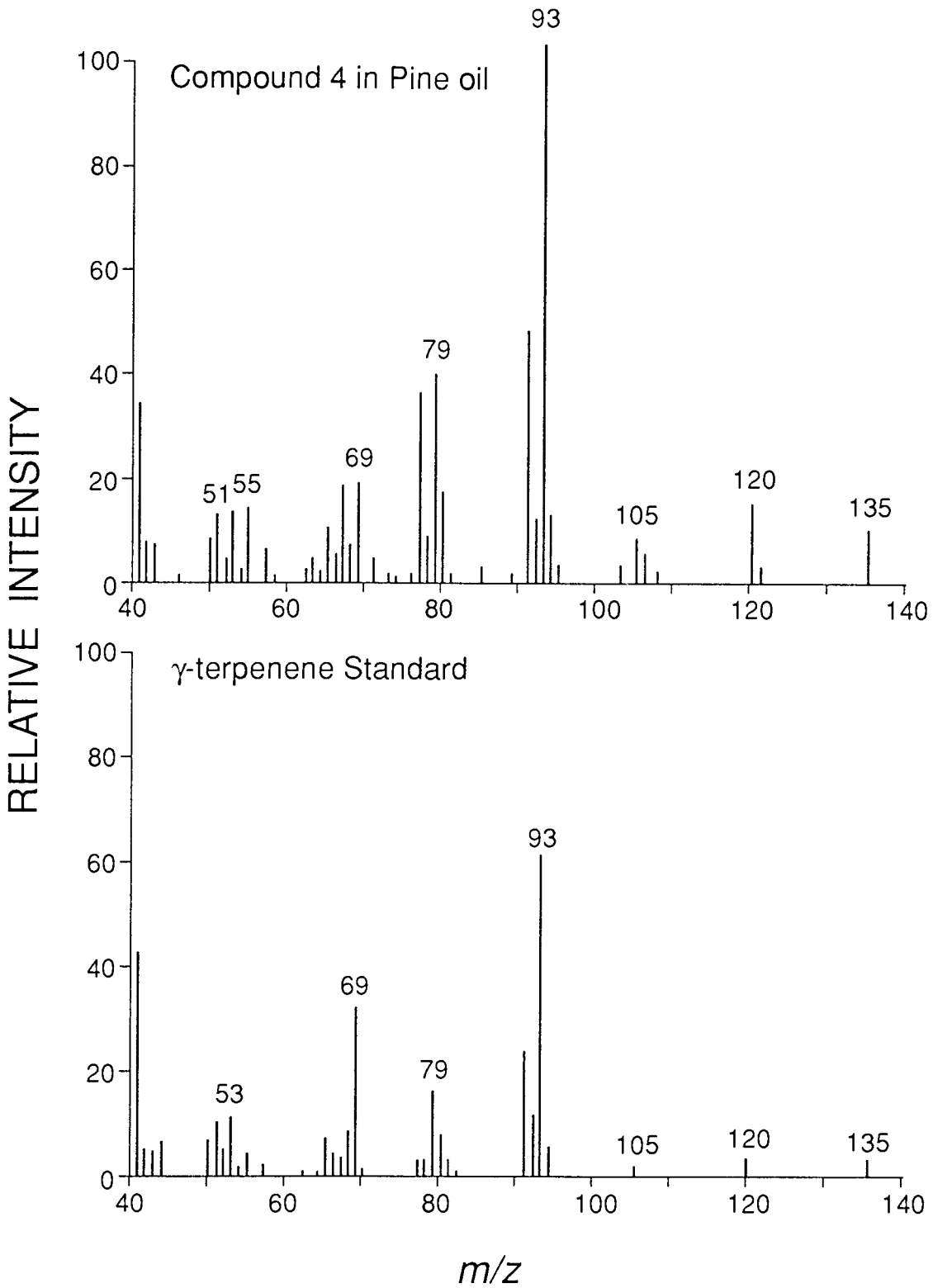
COMPOUND NUMBER	NAME	STRUCTURE
2	MYRCENE 7-methyl-3-methylene-1,6-octadiene	
3	*P*-CYMENE 1-isopropyl-4-methylbenzene	
4	γ-TERPINENE 1,3-*p*-menthadiene	
5	LINALOOL 2,6-dimethyl-1,7-octadien-3-ol	

Figure 8. Electron impact (70 eV) mass spectra of antennally-active pine oil constituents and authentic standards. The spectrum of myrcene was acquired on a Hewlett Packard 5985B GC-MS, whereas spectra of other compounds were taken on a Varian Saturn II ion trap GC-MS. Chromatography: DB-5 and SP-100 columns; temperature program: 1 min. at 50^0C, 10^0C/min. to 240^0C.


Compound 2 in Pine oil
93
69
41
53
59
79
105
115
122
129
136
Myrcene Standard
93
41
69
77
53
107
115
121
129
136
RELATIVE INTENSITY
m/z
100
80
60
40
20
0
40
60
80
100
120
140


Compound 3 in Pine oil
119
91
133
51
65
77
103
100
80
60
40
20
0
40
60
80
100
120
140
RELATIVE INTENSITY
p-cymene Standard
119
91
133
51
65
77
105
m/z


Compound 4 in Pine oil
γ-terpenene Standard
RELATIVE INTENSITY
m/z
93
79
69
55
51
105
120
135
53
100
80
60
40
20
0
40
60
80
100
120
140

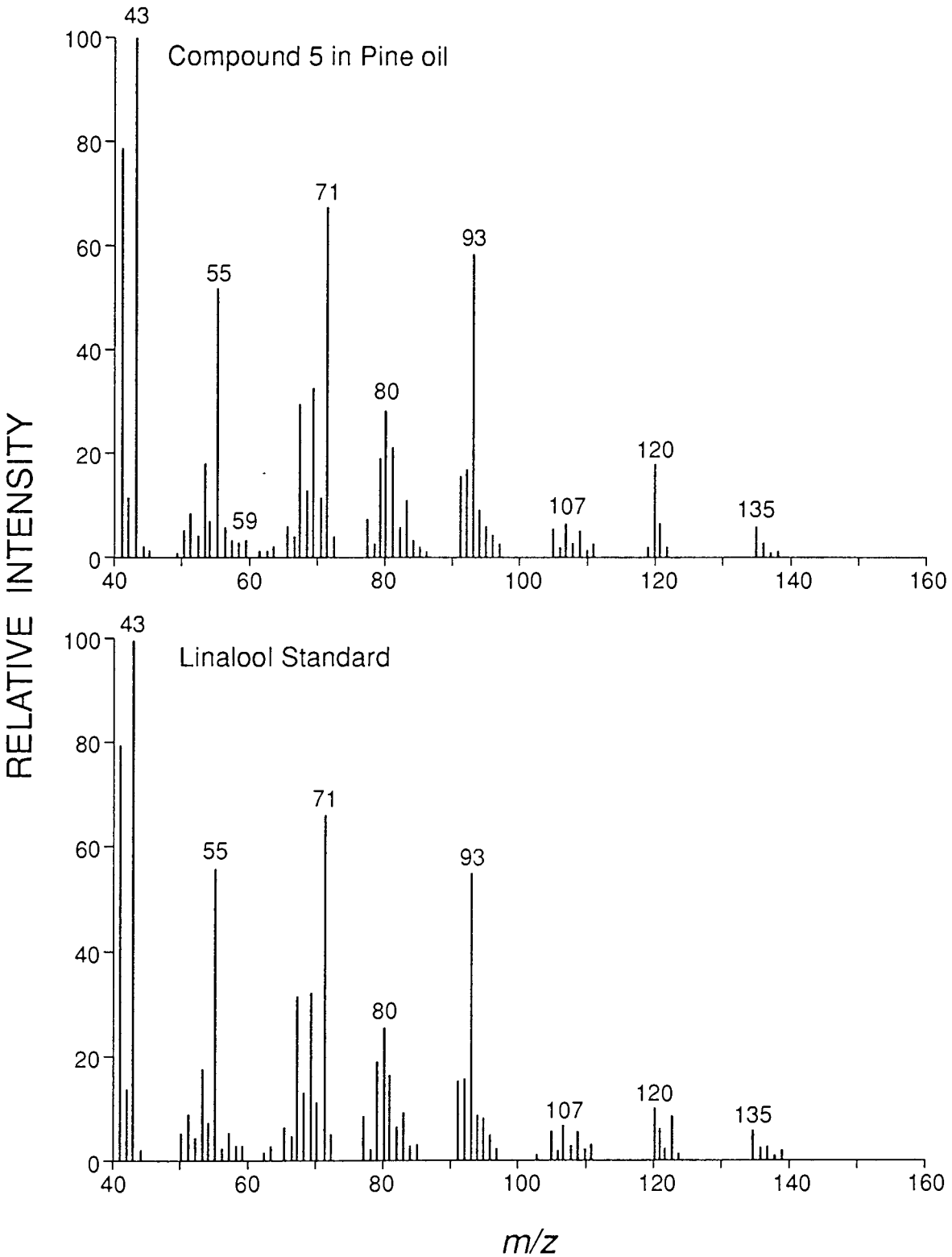
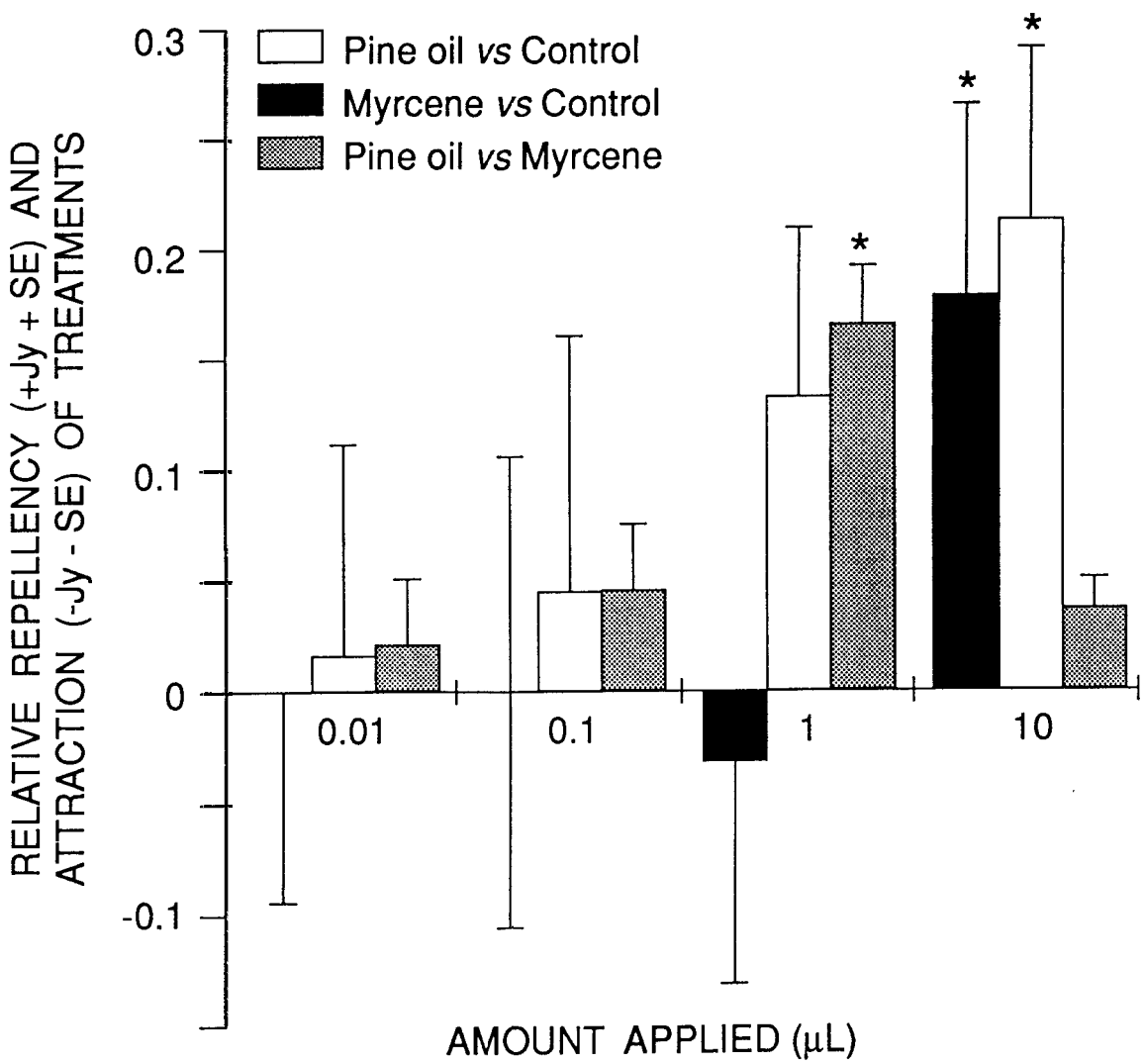
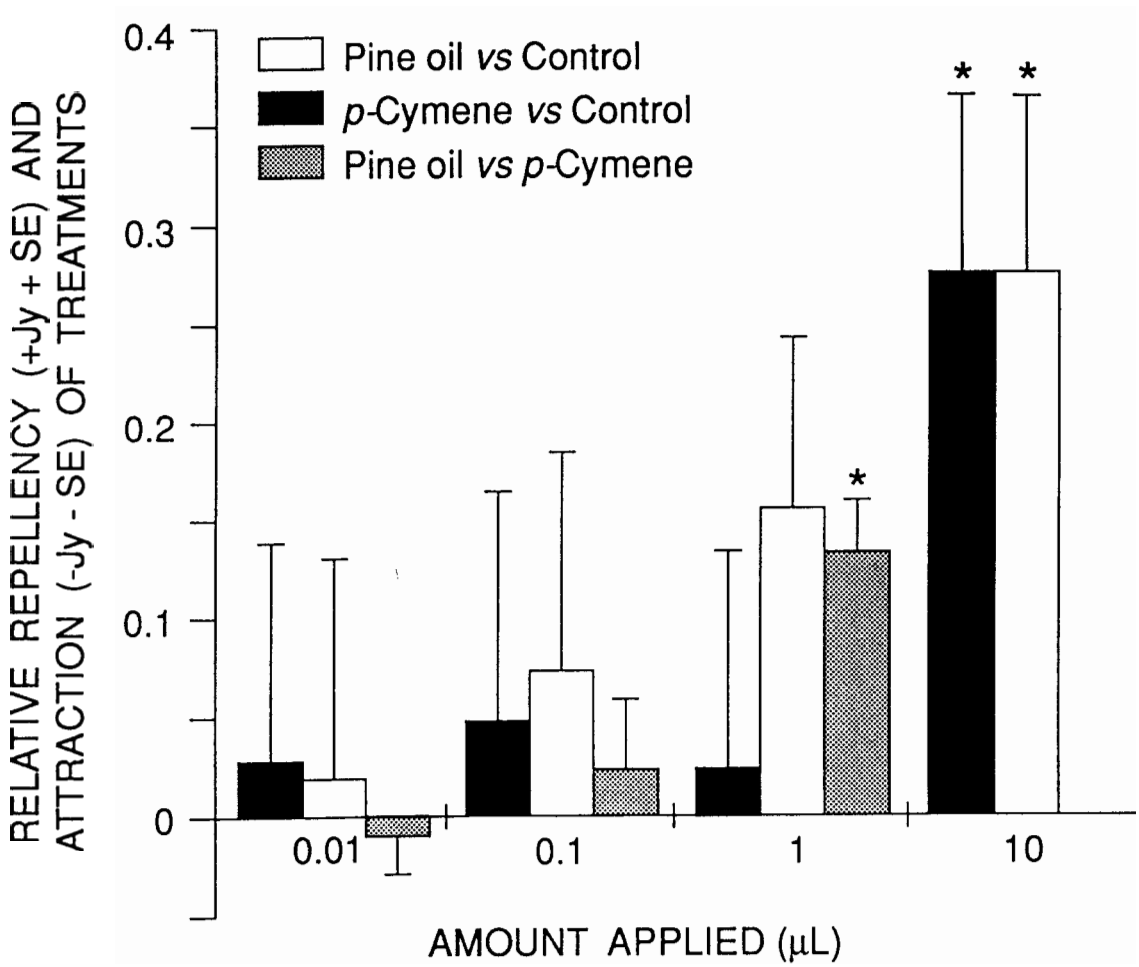
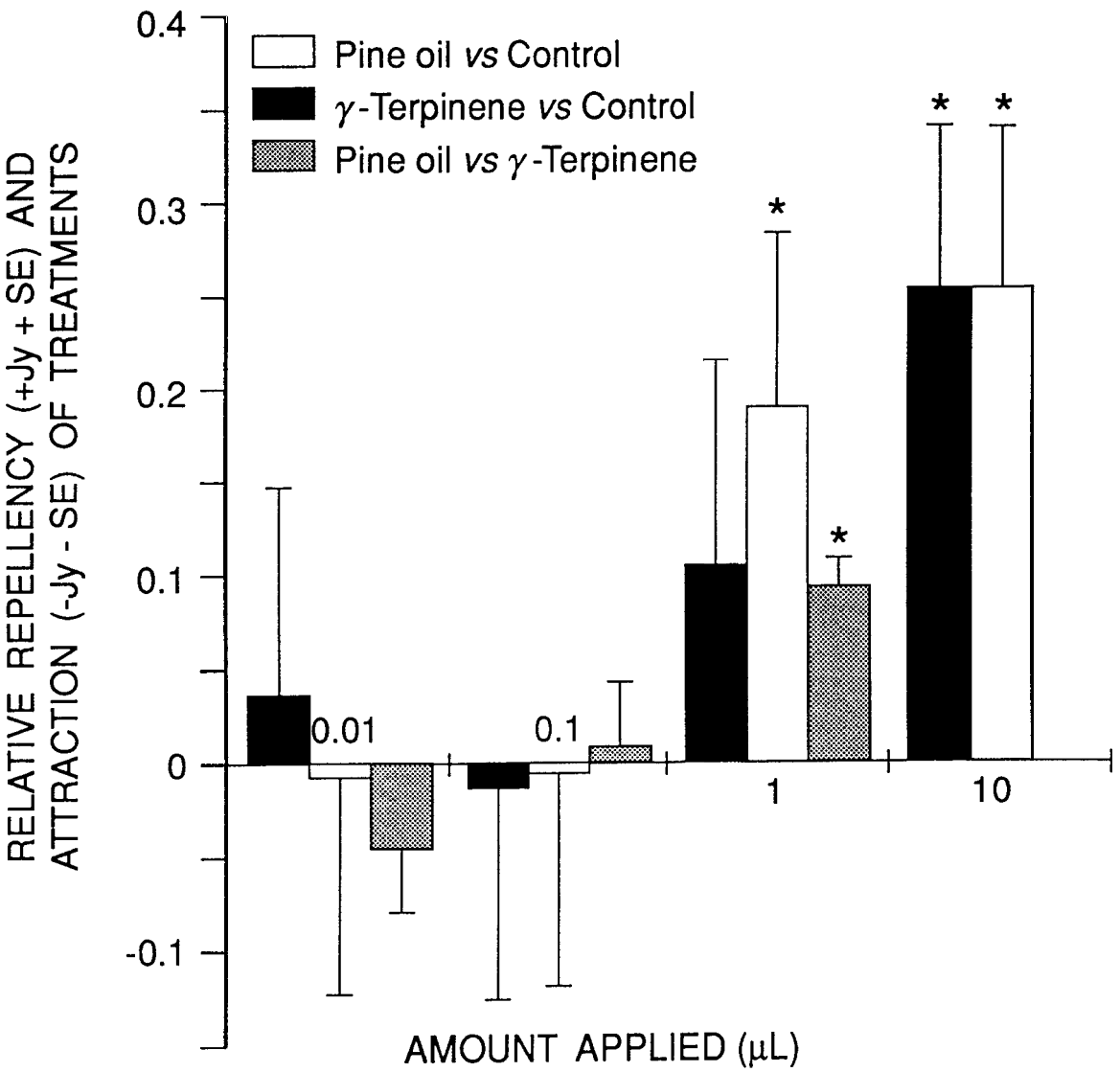
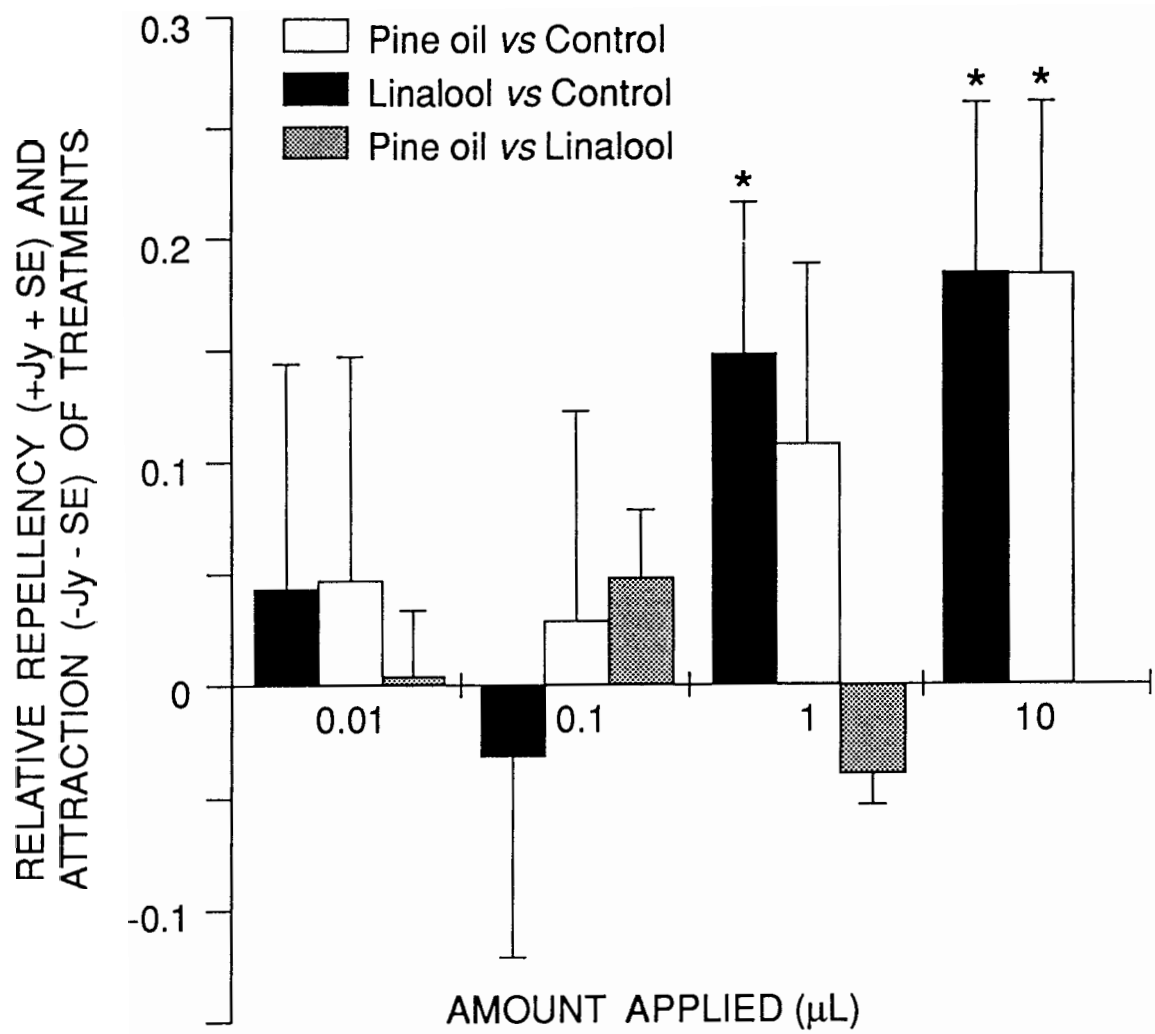

Compound 5 in Pine oil
Linalool Standard
RELATIVE INTENSITY
m/z
43
71
93
55
80
59
107
120
135
100
80
60
40
20
0
40
60
80
100
120
140
160

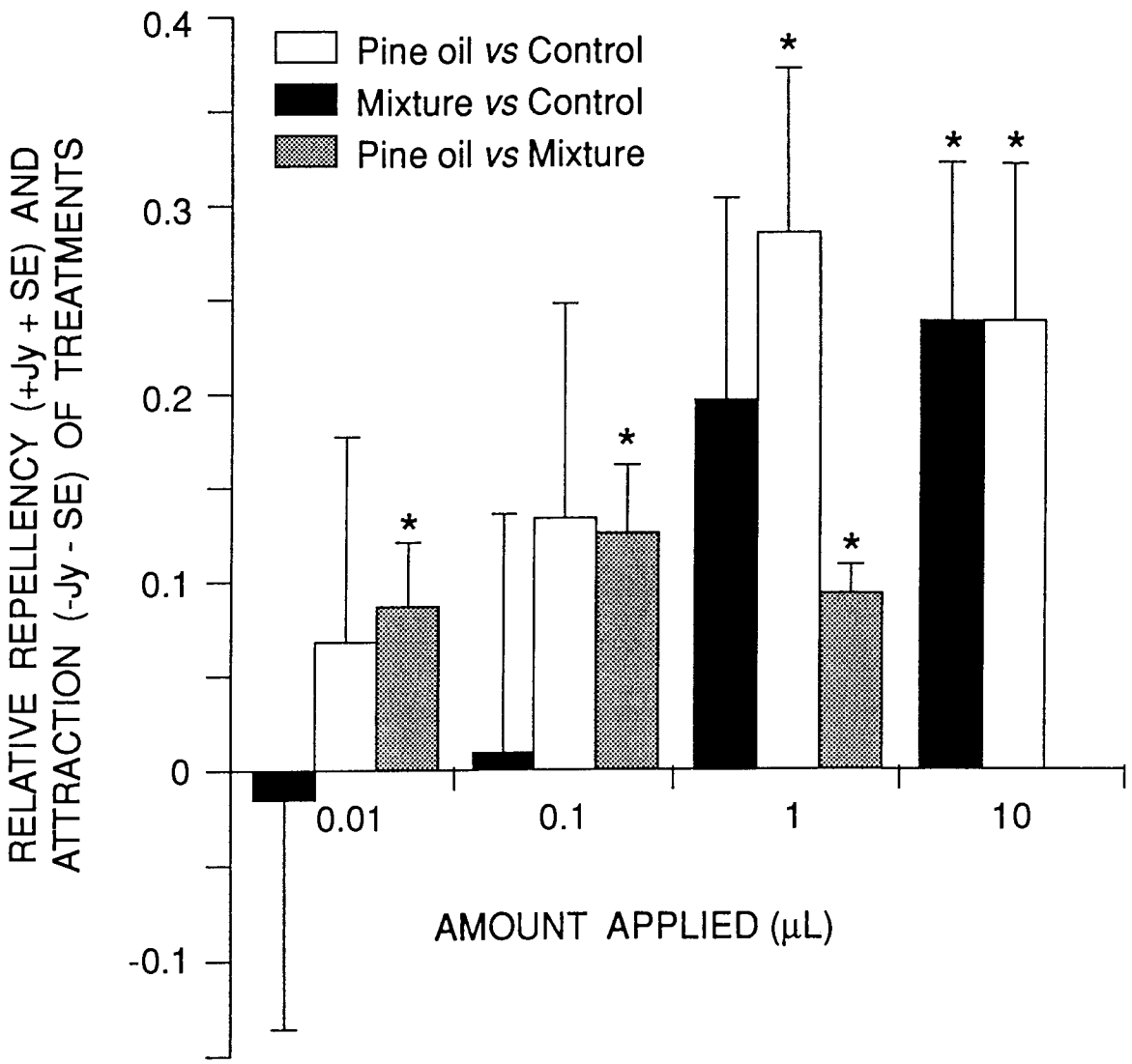
Figure 9. Pairwise comparisons using Jy-values of proportions of flies feeding on a) untreated *versus* pine oil-treated HS, b) untreated *versus* chemical-treated HS and c) pine oil treated *versus* chemical-treated HS at four doses (0.01, 0.1, 1 and 10 *u*l). Jy-values (e.g. for comparison (a) above) are calculated using the formula Jy=(ad-bc)/(a+b)(c+d) in which "a" and "c" are numbers of flies feeding on untreated and pine oil-treated HS respectively, and "b" and "d" are numbers of flies in cages not responding to untreated or pine oil-treated HS, respectively (Bliss 1967). Larger values of Jy represent greater differences in fly responses to the treatments under comparison. Significant differences ($P < 0.05$, n = 5) are denoted by asterisks (*).


Pine oil vs Control
Myrcene vs Control
Pine oil vs Myrcene
RELATIVE REPELLENCY (+Jy + SE) AND ATTRACTION (-Jy - SE) OF TREATMENTS
0.3
0.2
0.1
0
-0.1
0.01
0.1
1
10
*
*
*
AMOUNT APPLIED (µL)


Pine oil vs Control
p-Cymene vs Control
Pine oil vs p-Cymene
RELATIVE REPELLENCY (+Jy + SE) AND ATTRACTION (-Jy - SE) OF TREATMENTS
0.4
0.3
0.2
0.1
0
0.01
0.1
1
10
AMOUNT APPLIED (µL)
*
*
*


Pine oil vs Control
γ-Terpinene vs Control
Pine oil vs γ-Terpinene
RELATIVE REPELLENCY (+Jy + SE) AND ATTRACTION (-Jy - SE) OF TREATMENTS
0.4
0.3
0.2
0.1
0
-0.1
0.01
0.1
1
10
*
*
*
*
AMOUNT APPLIED (μL)


Pine oil *vs* Control
Linalool *vs* Control
Pine oil *vs* Linalool
RELATIVE REPELLENCY (+Jy + SE) AND ATTRACTION (-Jy - SE) OF TREATMENTS
0.3
0.2
0.1
0
-0.1
*
*
*
0.01
0.1
1
10
AMOUNT APPLIED (μL)


Pine oil vs Control
Mixture vs Control
Pine oil vs Mixture
RELATIVE REPELLENCY (+Jy + SE) AND
ATTRACTION (-Jy - SE) OF TREATMENTS
0.4
0.3
0.2
0.1
0
-0.1
0.01
0.1
1
10
AMOUNT APPLIED (µL)
*

Experiments 2-4: Feeding and Oviposition Deterrency of Linalool. Untreated sugar cubes in Exp. 2 were heavily fed on by flies resulting in weight losses over time, exceedingly greater than those of linalool-treated sugar cubes (Fig. 10). Significantly more eggs were oviposited in Exp. 3 on untreated than on linalool-treated cotton wicks (Fig. 11). In growth chambers (Exp. 4), following the application of a feeding stimulant, significantly more flies fed on untreated than on linalool-treated sugar finely spread on tray surfaces (Fig. 13) . Linalool suppressed responses to the feeding stimulant for 20 minutes.

3.3. Discussion

All of the house fly repellents/feeding deterrents identified in pine oil also occur in varying proportions in a wide range of plant extracts, floral volatiles (Henning *et al*. 1992) and essential oils (Eisner *et al*. 1986). It would be of interest to investigate whether any or all of these repellents occur in the oils of *Ocinum gratissinum* L., *Thymus serpyllum* L., *Illicium verum* Hooks. F., *Myristica fragrans* Houtt., and *Curcuma amada* Roxb. all of which are strongly repellent to flies (Singh & Singh 1991).

Repellency/feeding deterrency of terpenoids to house flies at high doses and slight attraction at low doses (Fig. 9) is consistent with previous observations for house flies (Campbell 1983) and for white pine weevils, *Pissodes strobi*

Figure 10. Mean cumulative weight loss due to feeding by house flies in Exp. 2 on paired sugar cubes, one untreated and the other treated with 10 ul of linalool and placed 20 cm apart in cages (30 X 30 X 40 cm) containing 200 three day-old flies. Paired bars are significantly different at each interval (t-test, $P < 0.05$, n = 5).

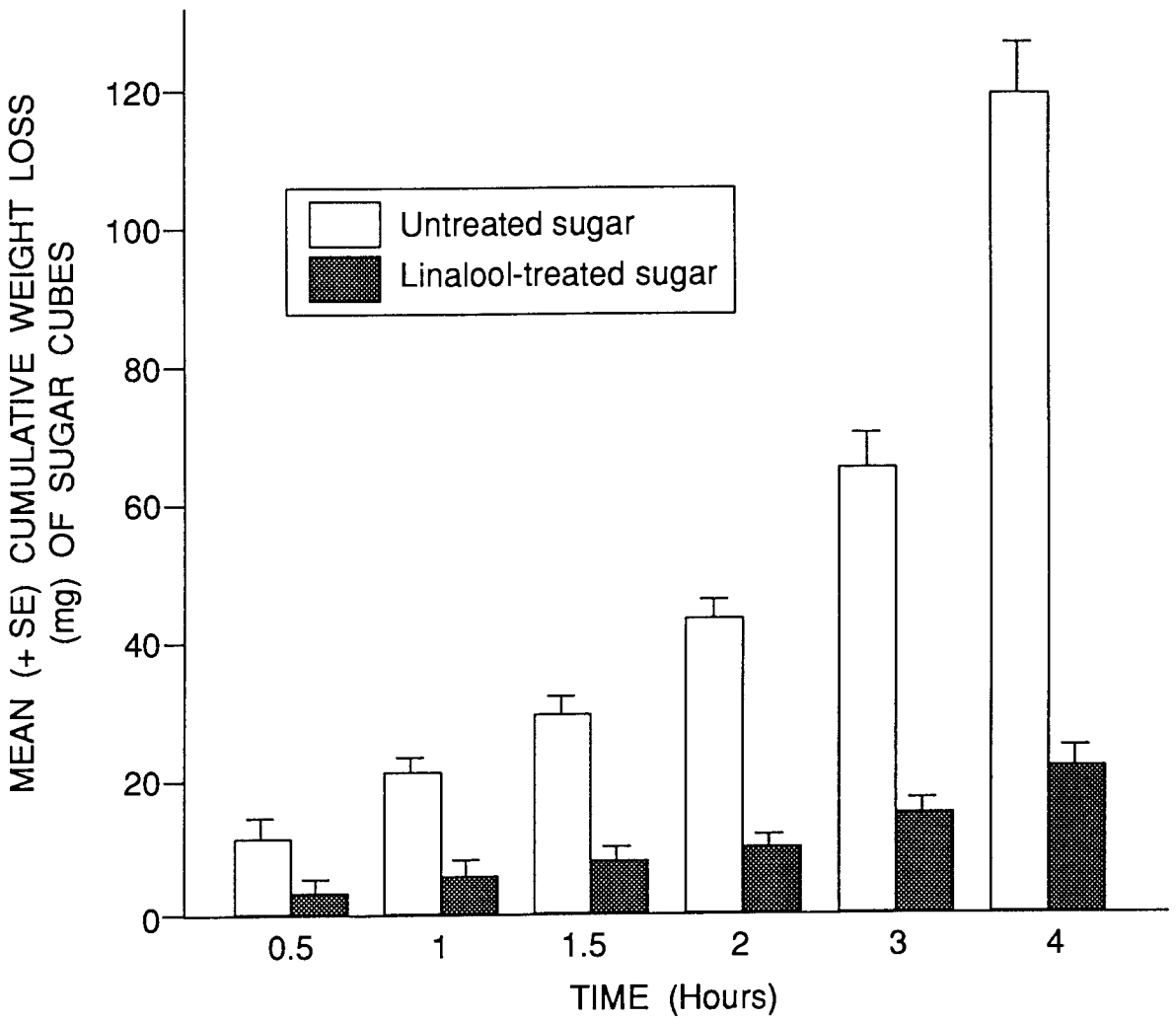

MEAN (+ SE) CUMULATIVE WEIGHT LOSS (mg) OF SUGAR CUBES
120
100
80
60
40
20
0
Untreated sugar
Linalool-treated sugar
0.5
1
1.5
2
3
4
TIME (Hours)

Figure 11. Mean numbers of eggs oviposited by flies on untreated cotton wicks and wicks treated with 10 ul of linalool partially submerged in a watery milk powder suspension. Two hundred, 14 day-old flies of mixed sex used in each of 5 replicates. Differences in ovipostion on treated and control wicks are highly significant (t-test, $P < 0.01$)

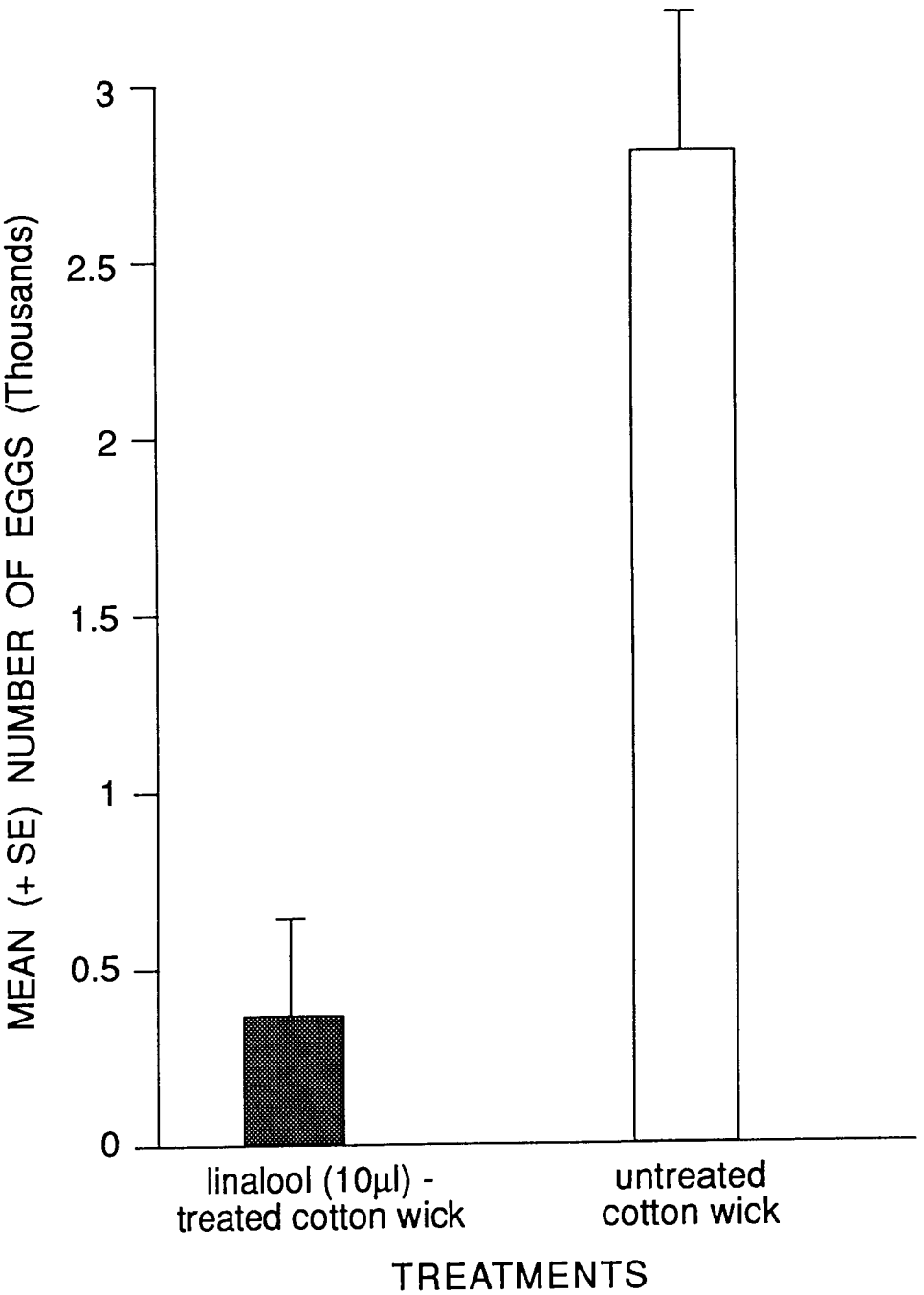
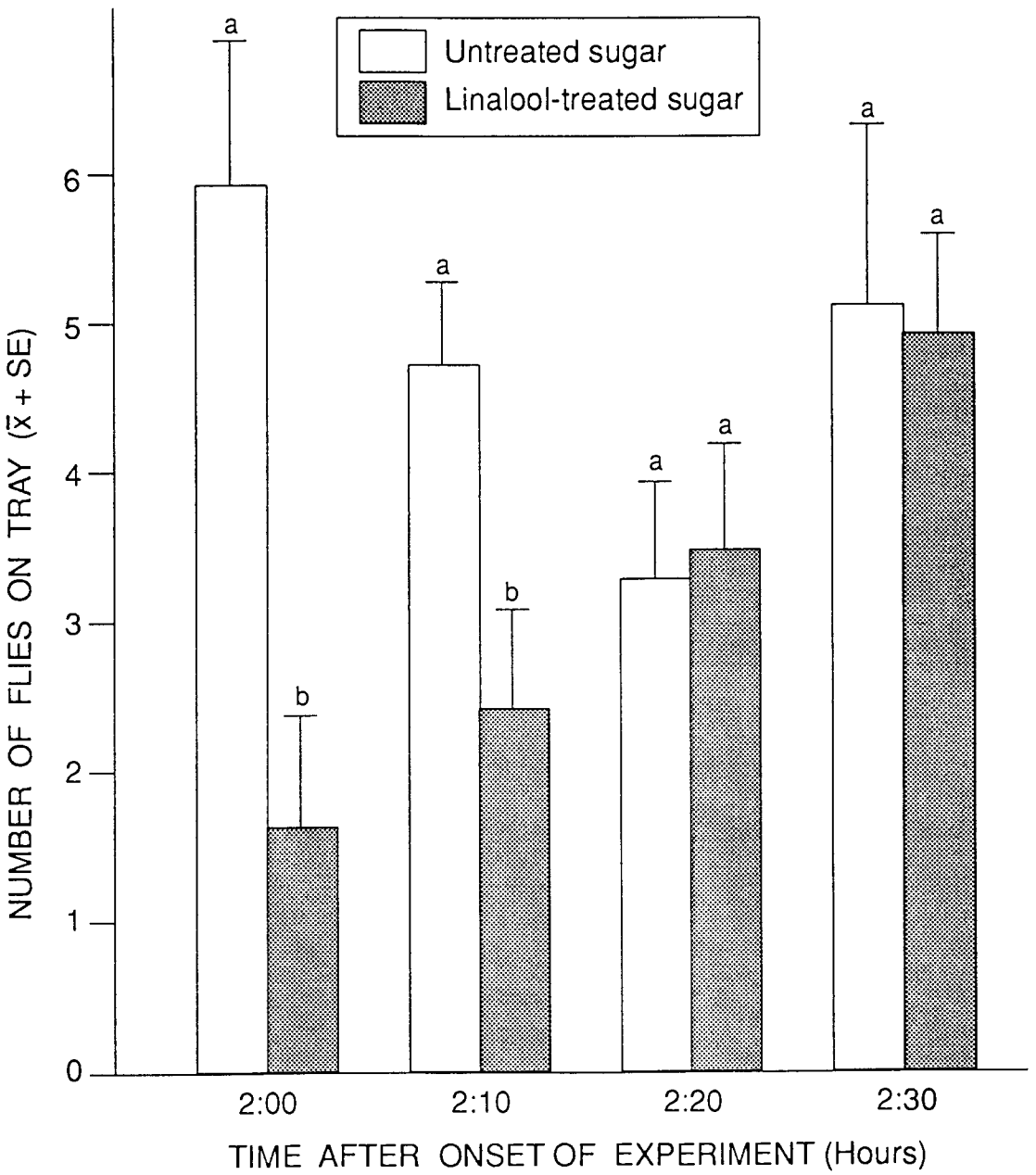

MEAN (+ SE) NUMBER OF EGGS (Thousands)
3
2.5
2
1.5
1
0.5
0
linalool (10µl) - treated cotton wick
untreated cotton wick
TREATMENTS

Figure 12. Feeding responses of 200, 2-3 day-old house flies on paired 30 X 40 cm trays, each containing 35 g of granulated sugar, placed 1 m apart in a growth chambers (4 X 3 X 3 m). The sugar on one tray was untreated and on other sprayed using a syringe with 50 ul of linalool. One ml of an attractant mixture (prepared by dissolving 2 ml of NH_4OH, 1 ml of acetic acid and 2 mg of $(NH_4)_2SO_4$ in 400 ml of water) was sprinkled on both trays 110 minutes after linalool application and the numbers feeding on treated and untreated trays were counted at 120, 130, 140 and 150 minutes (i.e. 2-2.5 h). Paired bars topped by the same letter are not significantly different (t-test, $P < 0.05$, n = 5).


Untreated sugar
Linalool-treated sugar
NUMBER OF FLIES ON TRAY ($\bar{x}$ + SE)
0
1
2
3
4
5
6
a
b
a
b
a
a
a
a
2:00
2:10
2:20
2:30
TIME AFTER ONSET OF EXPERIMENT (Hours)

Peck, (Alfaro *et al.* 1980). The repellent diethyl toluamide (deet) has similar multifuctional effects on yellow fever mosquitoes, *Aedes aegypti* L. (Schreck 1977). Because low doses of certain repellents/feeding deterrents may facilitate orientation to or recognition of food, they must be dispensed at high enough doses to assure effective protection against insects.

Alternatively, only those insect repellents without attraction or stimulatory effect at low doses should be selected for operational use. Linalool appears to be such a compound and is clearly the strongest repellent for house flies in pine oil (Fig. 9). Its superior repellency may be attributed to an allylic hydroxyl group (Fig. 7) (Roadhouse 1953, Garson & Winnike 1968,). Because it effectively suppressed feeding on sugar by house flies both in small cages (Fig. 10) and large growth chambers (semi-operational conditions) (Fig. 12), and also suppressed oviposition (Fig. 11), linalool may have practical potential to manipulate house flies.

Bioactivity of linalool has indeed also been discovered for other insects. While German cockroaches, *Blattela germanica* (L.) (Blattodea: Blattellidae), prefer untreated to linalool-treated diets (Karr & Coats 1992) and mosquitoes are repelled at 0.14 mg of linalool per cm^2 of skin surface (Hwang *et al.* 1985), (S)-(+)-linalool is a male-produced sex pheromone of the cabbage looper, *Trichoplusia ni* (Hubner), and (R)-(-)-

linalool a sex pheromone component of the scarab beetle, *Holotrichia parallela* (Coleoptera: Scarabaeidae) (Leal *et al.* 1993). Consistent with this study, Ntiamoah (1994) identified Myrcene, *para*-cymene and *gamma*-terpinene from the same source of pine oil as bioactive compounds for onion maggots, but did not identify linalool as a feeding or oviposition deterrent. Linalool may indeed be inactive to onion maggots. Alternatively, detection of quantitatively minor constituents such as linalool may not have been possible without a sensitive GC-EAD system as used in this study. Because all antennally-active pine oil constituents showed behavioural activity, identification of the hexane impurity (compound 1, Fig. 6) has been initiated. This compound was not present in the hexane used for dilution of pine oil or test chemicals in behavioural bioassays.

Because linalool is found in varying proportions in a wide range of essential oils, its recognition in food sources and oviposition sites by house flies is likely of adaptive significance. Fly maggots naturally develop in microbe-rich organic material (Levinson 1960), and rearing them in the laboratory on artificial media requires addition of yeast. Artificial diets supplemented with *Escherichia coli* (Migula) Castellani & Chalmers bacteria allow normal development of maggots, whereas lack of bacteria causes 96% mortality (Watson *et al.* 1993). Bacteria or their metabolites therefore seem to constitute an important dietary requirement

for developing house fly maggots (Schmidtmann & Martin 1992). Linalool and other terpenoids, expressing antimicrobial activity against *Staphylococcus aureus* Roenbach, *E. coli* and *Pseudomonas aeruginosa* (Schroeter) Migula (Nguyen *et al.* 1994), may present an olfactory cue to house flies indicating unsuitability of a potential food source or oviposition site. Curiously inhibition of symbiotic caecal microorganisms by pine oil has been suggested as a major cause of its feeding deterrency to voles and snowshoe hares (Bell & Harestad 1987).

Future studies will investigate whether repellent characteristics of linalool may further be enhanced by addition of *cis*-menth-2-en-ol and *trans*-piperitol, recently identified housefly feeding deterrents in the pepper tree, *Schinus molle* (Wimalaratne 1993, Wimalaratne *et al.* 1995). It is also of interest to determine whether the isomeric forms of linalool differ in their bioactivity against house flies. Because effective management of flies may not only require to repel them from one source but also to attract them to and capture them at another, various natural sources will be tested for their attraction to flies. Volatiles of attractive sources will be identified and together with repellents be developed for a "push" and "pull" management strategy for house flies.

4.0. Summary

House flies as mechanical vector of contagious diseases and serious nuisance pests require alternative management strategies where conventional control measures have failed. This is particularly important in livestock rearing facilities and in the tropics with abundant fly populations year round. Because semiochemical repellents (combined with attractants) offer a viable alternative for house fly management, various sources of suggested repellency were analyzed.

Superior fly repellency of pine oil compared to motor, mineral, and silicon oil against *Musca domestica* L. was demonstrated and prompted its analysis by coupled gas chromatographic-electroantennographic detection (GC-EAD) and coupled GC-mass spectrometry. Four pine oil constituents elicited responses by fly antennae and were identified as myrcene, *para*-cymene, *gamma*-terpinene and linalool. Of these, linalool was superior and as effective as pine oil in repelling flies from feeding sites.

Because fly maggots naturally develop in and rely on microbe-rich organic sources, olfactory recognition by gravid females of antimicrobial linalool in oviposition sites may be of adaptive significance and may explain superior repellency of linalool.

In future studies, linalool should be tested in combination with previously identified repellents.

Furthermore, volatiles from attractant sources should be identified and together with repellents be developed as a "push" and "pull" management strategy for house flies.

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