IDENTITY AND BIOACTIVITY OF OVIPOSITION DETERRENTS IN PINE OIL FOR THE ONION MAGGOT, *Delia antiqua* (MEIGEN) (DIPTERA: ANTHOMYIIDAE)

By

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THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF PEST MANAGEMENT

in the Department

of

Biological Sciences

SIMON FRASER UNIVERSITY

August 1994

APPROVAL

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Degree: **Master of Pest Management**

Title of Thesis:

IDENTITY AND BIOACTIVITY OF OVIPOSITION DETERRENTS FOR THE ONION MAGGOT, DELIA ANTIQUA (MEIGEN) (DIPTERA: ANTHOMYIIDAE), IN PINE OIL

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Date Approved 11 August 1994

Title of Thesis/Project/Extended Essay

Identity and Bioactivity of Oviposition

Deterrents for the Onion Maggot, Delia Antiqua (Meigen)

(Diptera: Anthomyiidae), in Pine Oil

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Abstract

The oviposition deterrent properties of pine oil (Norpine 65, Northwest Petrochemicals, Anacortes, Washington) for the onion maggot, *Delia antiqua* (Meigen), were verified using a two-choice bioassay with onion oil as an attractive control. The principal deterrent activity of this pine oil was found to reside in three monoterpenes, 3-carene, limonene, and *p*-cymene which were the primary constituents identified in the most deterrent of two fractions made by preparative gas chromatography of distilled pine oil. At a release rate of 220, 320, and 320 µg per 24 h these monoterpenes respectively caused 73.2, 65.4 and 56.3% deterrency of oviposition, in two-choice bioassays, while the ternary mixture released at 320 ug caused 88.6% deterrency. The ternary mixture also caused 62.5% deterrency in a no-choice bioassay. Of eight other monoterpenes tested for deterrency, myrcene, α-phellandrene, α-terpinene, β-phellandrene, γ-terpinene, terpinolene, and β-pinene were significantly deterrent in declining order, while α-pinene was inactive. The ternary mixture was released from glass capillary tubes or flexible plastic tubes in bioassays that challenged caged females to oviposit around the base of 35 potted onion seedlings with release devices placed on the soil surface. The most effective deterrency (85.3%) was achieved at a release rate of 280 µg per 24 h per pot if plastic tube devices were deployed 24 h before the treated pot was exposed to *D. antiqua* females. Deterrency of oviposition on potted onion seedlings was significant, but low (11.7-63.2%) if female *D. antiqua* were given only a treated pot. Because of incomplete efficacy, a monoterpene-based deterrent formulation would be best used operationally if combined with other deterrents, or if it were integrated with some other tactic.

Acknowledgement

My sincere, heartfelt thanks and deep appreciation go to Dr. John H. Borden, Professor and Director of Chemical Ecology Research Group, first and foremost for giving me the chance to enter the program, his excellent supervision, assistance and most importantly being a source of inspiration during the course of study and preparation of this thesis. I am highly indebted to him for his great support and kindness.

I thank Drs. Harold Pierce Jr. and Gerhard Gries for their guidance in analytical chemistry, assistance, helpful suggestions and review of the thesis. I would also like to thank Francois Bellevance for assistance with statistical analyses, Akbar Syed for providing onion maggot, Robert S. McDonald for his advice and numerous friends and colleagues who have made my stay in Canada joyous and rewarding.

The greater part of my research was funded by Dr. John Borden and part by Simon Fraser University Graduate Student Fellowship, Teaching Assistantship, H. R. MacCarthy Graduate Scholarship, Sidney Hogg Memorial Graduate Scholarship and Vancouver Horticultural Society Bursary to the author.

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1.0 Introduction

The onion maggot, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae), is an important pest of onion, *Allium cepa* L. (Lilales: Amarylidaceae); it has few other hosts (Harris and Svec 1976; Loosjes 1976; Liu and McEwen 1980; Finch and Eckenrode 1985; Finch *et al.* 1986a).

In most of Canada and the northern USA, *D. antiqua* has three generations per year (Liu *et al.* 1982). Females are mated at 6-7 days after eclosion and can lay up to 50 eggs (Miller and Cowles 1990). Vernon and Borden (1979) demonstrated that under optimal laboratory conditions females reared on an artificial diet can produce several hundred eggs. Eggs are laid just below the soil surface near the base of plants. Direct damage is caused by the larvae which feed on the bulb for up to two weeks while developing through three larval instars before pupating in the soil. An estimated 0.1 to 0.2 million pupae can overwinter per ha of onions (Finch and Eckenrode 1985). Trap catches as high as 48 adult onion maggots per trap per day have been recorded in one commercial onion field in New York State (Finch *et al.* 1986b). Crop protection efforts are directed mainly toward control of spring generation maggots because a single maggot can destroy several small seedlings (Kendall 1932, Workman 1958, Loosjes 1976, Miller and Cowles 1990). Damage caused by second and third generation larvae is less extensive, because it is difficult for larvae to enter the mature onion bulb (Finch and Eckenrode 1985), and as an onion increases in size it may be able to absorb many more larvae than a seedling (Dindonis and Miller 1980a). Mature onion bulbs which survive attack have low market value since they become distorted due to secondary infections including onion white rot, *cepirorum* Berk., and onion smut, *Urocystis magica* Pass. Ovipositing females are involved in the spread of bacterial rot from infected to uninfected onions. The third generation causes no economic damage, but becomes a reservoir of overwintering pupae (Drummond 1982, Finch and Eckenrode 1985) from which arise the first generation in the following year.

Current pest management procedures against onion maggots include the use of granular insecticides at planting, insecticidal drenches to control adult and laval populations and removal or burning of cull piles from onion fields (McEwen *et al.* 1970, B.C. Min. Agric. and Food 1992). Without the soil treatment, crop loses will exceed 70% in Canada (Madder and McEwen 1981). Repeated applications of chemical insecticides have been costly (Vernon *et al.* 1987), and have resulted in development of resistance of onion maggot to several organophosphorus insecticides including parathion, diazinon and malathion (Harris and Svec 1976; Harris 1977; Harris *et al.* 1982; Finch *et al.* 1986a). Other concerns are that recommended insecticides lack the persistence to reduce mid- and late-season pest populations, that human health is at risk (Morris *et al.* 1984), and that there will be environmental disruption caused by heavy use of broad-spectrum toxicants (Vernon *et al.* 1987).

These concerns have caused emphasis to be placed on investigations into alternative methods of onion maggot control. The increasingly restrictive guidelines for the use of insecticides in agriculture in many farming areas in North America, and increasing demand for food free of insecticide residues, emphasizes the urgent need for the development of alternative strategies to protect onions from damage by onion maggots (Miller and Cowles 1990).

Various cultural options have been used for onion pest management, including crop rotation, trap crops and disposal of cull piles (Finch and Eckenrode 1985; Finch *et al.* 1986b; Finch 1989). These methods may be costly or impractical. Integration of flooding with the use of the parasitoids, *Aphaereta pallipes* (Say) and *Aleochara bilineata* (Gyllenhal), has been used to achieve some control on an experimental basis (Whistlecraft and Lepard 1989). However, potential problems arise with the use of flooding as it affects non-target and beneficial insects in agricultural ecosystems. Monitoring of populations in sticky traps reflecting attractive wavelengths (Judd *et al.* 1988) has been effective in British Columbia in improving the timing of insecticidal applications and in reducing their number (Vernon *et al.* 1987). Recently, Vernon and Mackenzie (1993) have shown that vertical barriers can reduce movement of onion maggot adults into planted areas.

One other potential method is to use naturally occurring deterrents to prevent oviposition by onion maggot females. Oviposition behavior in many insects occurs in response to chemical stimulants from the host-plant. Examples of oviposition stimulants include: glycosides from tomato, *Solanum esculentum* (Mill)., for the tomato horn worm, *Manduca quinquemaculata* (Howorth) (Yamamotu and Fraenkel 1960); allyl-isothiocyanate from cabbage, *Brassica nigra* (L.), for the diamondback moth, *Plutella xylostella*(Curt.) (Matsumoto 1970); lecithin from potato for the colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Gripson 1958); saponis and urease from soybeans for the bruchids, *Bruhus pisorum* L. and *B. rufimanus* (Boheman) (Applebaum *et al.* 1965); and methyl-iso-eugenol from carrot leaves for the carrot rust fly, *Psila rosae* F. (Beruter and Stadler 1971; Judd *et al.* 1985a, b).

Oviposition stimulants have also been reported for the Anthomyiidae. Cabbage maggots, *Delia radicum* (L.), were stimulated to oviposit by sinigrin and ß-phenylethylamine (Trayneir 1965), and onion maggots were similarly stimulated by a number of onion compounds including dipropyl disulfide, *n*-propyl mercaptan (Matsumoto and Thorsteinson 1968; Vernon *et al.* 1977), methyl propyl disulfide, *cis*-propenyl propyl disulfide and *trans*-propenyl propyl disulfide (Pierce *et al.* 1978). Larvae are attracted to methyl disulfide; their development has been associated with microorganism-infected onions (Matsumoto and Thorsteinson 1968a; Ellis *et al.* 1979; Dindonis and Miller 1981b; Schneider *et al.* 1983; Miller *et al.* 1984). Larval development is important in determining adult onion maggot reproductive success; thus gravid females identify suitable host plants by chemical and physical stimuli that characterize the plant (Miller and Strickler 1984).

Judd and Borden (1988) demonstrated long range orientation to onion odors in host location by adult onion maggots. At close range several physical and physiological factors, along with odor, influence oviposition (Harris and Miller 1982; Harris and Miller 1988; Mowry *et al.* 1989). Prior to oviposition, onion maggot females assess their environment through vision, olfaction and taste, while running along the plants or over the foliar surfaces (Harris and Miller 1984). Subsequent ovipositor probing at the soil-plant interface is correlated with the number of eggs laid (Harris and Miller 1988). Egg load lowers the acceptance threshold level (Dethier 1982) of female onion maggots (Harris and Miller 1988), and they may lay several eggs in clumps (Cowles and Miller 1992). Clumped oviposition may be stimulated by an oviposition pheromone as well as volatiles released by bacteria-infected onions (Judd and Borden 1993).

Disruption of oviposition behavior could be a potential tool for preventing plant damage. Miller and Cowles (1990) proposed the concept of stimulo-deterrent diversion (SDD) for onion maggot control in which the combined use of stimulant diversions and deterrents applied to the host plant would prevent female maggots from lowering their acceptance threshold for treated plants. The practical prospect of such a tactic is improved by the fact that unlike most other insects, which will not oviposit in the absence of the host-plant, e.g. fruit flies, *Dacus* spp. (Fitt 1986), and *D. radicum* (Nair and McEwen 1975, 1976), onion maggot females will oviposit in the absence of host odor in cracks and crevices of cages and on moist surfaces.

Oviposition deterrency, i.e. the capacity to prevent continous oviposition or to enhance its termination (Dethier 1947), has been reported for a number of insects. Female apple maggots, *Rhagoletis pomenella* Walsh., and *R. fausta* Curan., employ a marking (epideictic) pheromone to deter other females from laying eggs in the same fruit (Prokopy 1972, 1975). Like-wise, an oviposition deterrent pheromone has been reported in the cement that causes eggs of the sorghum shoot fly , *Atherigona soccata* Rondani, to adhere to leaves (Ogwaro 1978; Raina 1981). Several species of moths have oviposition deterrent pheromones associated with freshly laid eggs (Schoonhoven *et al.* 1981) or larval frass, e.g. Egyptian cotton leaf worms, *Spodotera littoralis* (Boisd.) (Hilker 1985). Poirier and Borden (1991, 1992) demonstrated the presence of oviposition deterrent pheromone associated with eggs of the obliquebanded leafroller, *Choristoneura rosaceana* Harris. Zimmerman (1979, 1980, 1982) demonstrated the occurrence of an oviposition deterrent pheromone in *Delia* spp., as females are deterred from oviposition when exposed to a mass of freshly laid eggs on flower buds of the perennial herb, *Polemonium foliosissimum* Gray.

In addition to pheromones, the use of plant derived oviposition deterrents has considerable promise. Coumarin obtained from several plant families (Leung 1980) deterred oviposition by the diamondback moth (Tabashnik 1985) and the cabbage butterfly, *Pieris rapae*

(L.) (Tabashnik 1987). Oviposition by female *P. rapae* was deterred by extracts of the crucifer, *Erysimum cheiranthodes* (Renwick and Radke 1985, 1987), and by cardenolides from *E. cheiranthodes* (Dimock *et al.* 1991). Oviposition deterrents that showed potential efficacy against onion maggots in experiments include: hydrated been extract (Wiens *et al.* 1978); black pepper, chili powder, ginger, red pepper and paprika (Cowles *et al.* 1989); cinnamaldehyde (Miller and Cowles 1990); citronella, terpinene (Cowles *et al.* 1990) and pine oil (Javer *et al.* 1987). Recent advances in chemical ecology could make the cost of production and application of deterrents competitive with chemical insecticides (Ho *et al.* 1992).

Pine oil was originally described as the mixture of isomeric secondary and tertiary, cyclic terpene alcohol's obtained by distillation of pines, *Pinus* spp. (Nijholt 1980; Richmond *et al.* 1985). The term is also used to describe synthetically produced hydrocarbons (Richmond 1985). The cheapest and most abundant pine oil available today is a by-product of wood pulping from pulp mills, and is a complex mixture of varying amounts of monoterpenes and other natural products (Nijholt 1980; Nijholt *et al.* 1981; Alfaro *et al.* 1984; Javer *et al.* 1987). Its composition is dependent on the species of conifers used. Pine oil has been used in the manufacture of disinfectants and deodorants (Laake *et al.* 1926), as a flotation agent in the mining processes, and as a paint additive (Richmond 1985).

Pine oil was found to be an effective repellent for the screw worm, *Cochliomyia hominivorax* (Coquerel.), under a range of conditions (Laake *et al.* 1926). Application of pine oil to the bark of apple trees resulted in almost total mortality of overwintering codling moth larvae, *Cydia pomenella* (L), without any visible damage to the tree over a three year period (Headlee 1929). As a non-insecticidal repellent, and when mixed with pyrethrum, it effectively repelled houseflies, *Musca domestic*a L., and hornflies, *Haematobia irritans* (L), pests of dairy cattle (Freeborn *et al.* 1934). Pine oil has also been demonstrated to be an oviposition deterrent for yellow fever mosquitoes, *Aedes aegypt*i (L.) (Ho *et al.* 1992), and a feeding deterrent for mammals (Bell and Harestad 1986), and white pine weevils, *Pissodes strobi* Peck (Alfaro *et al.* 1984). It can also be used to inhibit attacks on otherwise suitable hosts by two species of ambrosia beetles, *Trypodendron lineatum* (Oliv) and *Gnathotricus sulcatus* (LeConte) (Nijholt 1980; Dubbel 1992), and bark beetles, e.g. southern pine beetles, *Dendroctonus frontalis* Zimmerman (O'Donnel *et al.* 1986) and mountain pine beetles, *D. ponderosae* Hopkins (Richmond *et al.* 1985). Against the onion maggot, Javer *et al.* (1987) determined that a concentration of 0.09% pine oil in hexane caused 50% oviposition deterrency in laboratory bioassays.

My objectives were:

1. to isolate and identify the semiochemicals in pine oil that deter oviposition by *D. antiqua*;
2. to determine the bioactivity of related chemicals;
3. to evaluate the potential efficacy of the active constituents in simulated field experiments; and
4. to determine whether the constituents of pine oil are active against another root infesting dipteran, the cabbage maggot, *D. radicum*.

2.0 Evaluation of the Bioactivity of Dipropyl disulfide, Onion Oil and Onion as Control Oviposition Stimulants.

Dipropyl disulfide forms one of the major volatile components of onion plants. In various host selection studies it has been an effective oviposition stimulant (Niegisch and Stahl 1956; Matsumoto and Thorsteinson 1968b; Vernon *et al.* 1977). Studies of oviposition behavior of onion maggots have demonstrated that cut pieces of onion bulb can be effective and consistent oviposition stimulants (Vernon *et al.* 1977, 1981; Wiens *et al.* 1978; Dindonis and Miller 1981b; Ishikawa *et al.* 1981; Javer *et a*l. 1987; Judd and Borden 1993). However, Pierce *et al.* (1978), using a sensitive laboratory bioassay for measuring the oviposition response of *D. antiqua* found that dipropyl disulfide could elicit only 17% of the oviposition response to captured onion volatiles. Studies on the olfactory response of onion maggots in the field, have demonstrated the involvement and importance of dipropyl disulfide in the long range search and acceptance phases of host selection (Judd and Borden 1989).

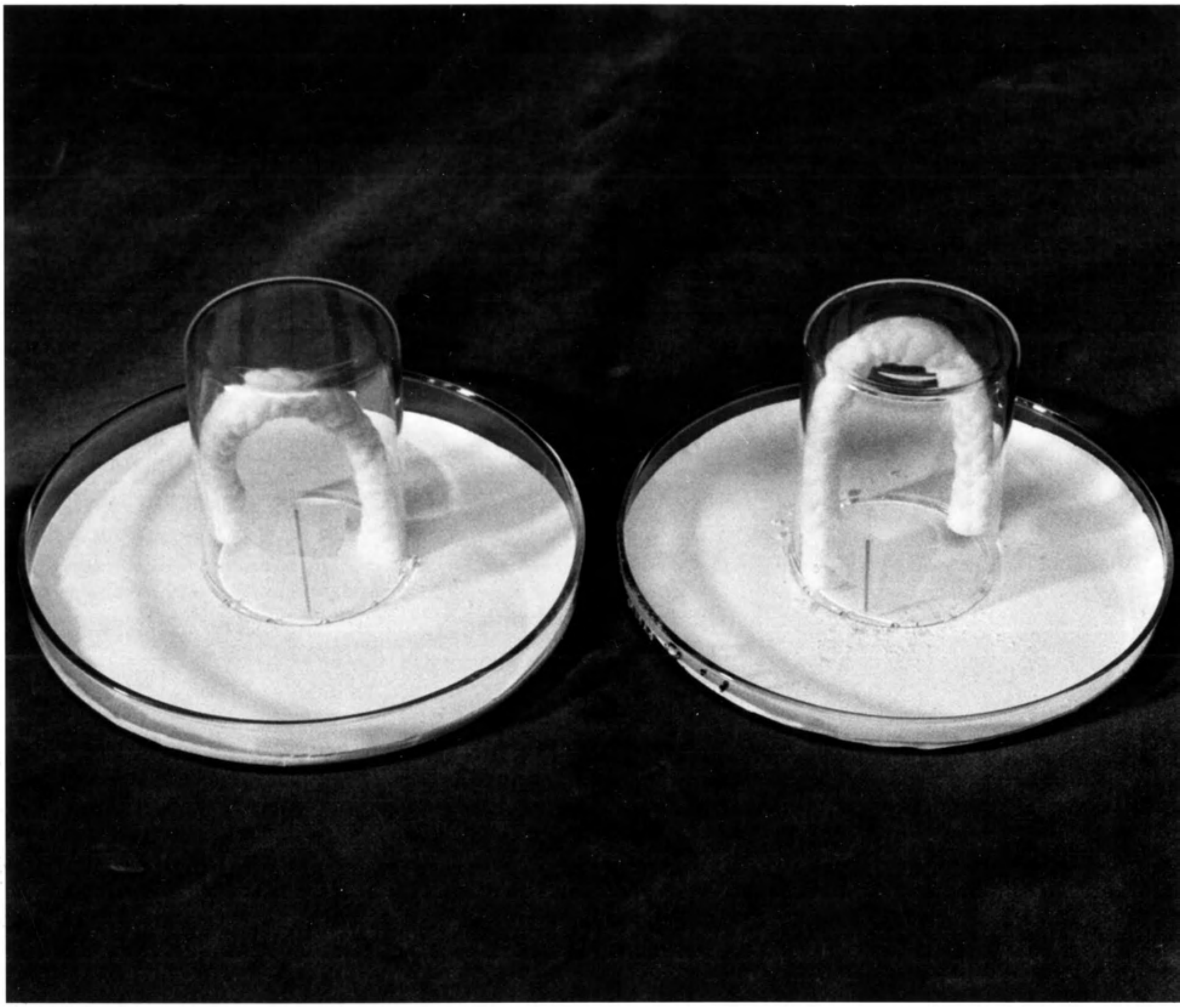
My objective was to obtain a reliable and efficient laboratory bioassay stimulus for oviposition by *D antiqua.* This bioassay was considered necessary as a positive control for stepwise identification of all possible oviposition deterrent constituents of pine oil .

2.1 Materials and Methods

2.1.1 Oviposition Bioassay

A two-choice bioassay was used following the basic design developed by Vernon *et al.* (1977). The floor of each of two 14.5 cm diam. petri dish bioassay stations per replicate was comprised of four discs of stacked Whiteman No. 1 filter paper (15 cm diam.) kept moist by wet dental cotton rolls placed inside an inverted autoclaved 100 mL glass "beaker" manufactured from pieces of straight-sided 5.5 cm diam. glass tubing, 7.5 cm long, open at one end and closed at the other. (Fig 2.1). Volatile stimuli were released from 50 μL glass capillary tubes open at one end and taped inside the inverted beaker.

Figure 2.1. Modified glass "beaker" oviposition stations set up for a two-choice bioassay. Note stimuli in capillary tubes taped to the inside wall of the inverted beakers.



Volatiles escaped from 10 V-shaped grooves (1 mm wide) cut in the rim of the inverted 100 mL beaker. Most females oviposited through these grooves onto the filter paper substrate. Bioassays were conducted in 25 x 25 x 45 cm wooden-frame cages, with nylon mesh screen walls and ceiling and a Plexiglas front, containing 15 gravid females 15-20 days old obtained from cultures maintained as described by Vernon et al. (1977) and Vernon and Borden (1979). Access to water in the cages was provided by a moist dental cotton roll. Prior to a bioassay, females were held at 21^{o}C for 24 h with access to water and food (Ticheler 1971) but without host odor. Bioassays were conducted at the Biological Science Trailer equiped with florescent lighting at 21-26 ^{o}C, 50-60% R.H and 16:8 L:D for 24 h, after which all eggs were counted. To facilitate egg counting, the petri dishes containing eggs were flooded with water, gently agitated, and the eggs decanted onto a fine nylon mesh. Bioactivity of experimental stimuli was evaluated by comparing the number of eggs laid on experimental stations with the numbers laid on solvent or unbaited control stations. Paired oviposition stations were placed 8 cm apart at the center of the cage. The positions of the treatment and control stations were alternated between replicates.

For no-choice experiments the bioassay was modified so that experimental and control stations were placed in separate cages. A three-choice bioassay had three oviposition stations per cage.

2.1.2. Experiments

A paired-stimulus, 11-replicate dose-response experiment was conducted to test the responsiveness of females to dipropyl disulfide. Five experimental treatments, 100% dipropyl disulfide and 10, 1, 0.1, and 0.01 dipropyl disulfide diluted in undecane, were compared with an undecane control. A control experiment was also set up with undecane-baited stations compared with unbaited control stations. Release rates were standardized by adjusting the level of liquid stimuli to 1 cm below the open top of the 50 μL capillary tubes.

Onion oil (Kalsec Inc., Kalamanzoo, Michigan) and onion were evaluated for oviposition stimulation in similar experiments. For onion oil, the control oviposition station had an empty glass capillary tube while the experimental tube contained 50 μL of undiluted onion oil. The release rate of onion oil was 309 μg per 24 h as determined by differences in weights of the loaded capillary tubes before and after the experiment. Onion slice stimuli were cut pieces (5 g) of a medium-sized onion bulb, suspended in a cheese cloth bag inside the inverted glass beaker; control stations had no onion piece.

2.1.3. *Statistical Analysis*

In order to stabilize the variance, all data were transformed by square roots (Zar 1984). Data for responses to dipropyl disulfide were subjected to analysis of variance (ANOVA) (SAS System for Statistical Analysis 1987), while means for onion oil and onion were compared by t-tests (Zar 1984). In all cases $\alpha=0.05$. Percent stimulation (deviation from an expected 50:50 distribution of eggs) was calculated by ((n/2)-a)/n/2 x 100, where n=the total number of eggs at both stimulus and control stations and a= the number of eggs laid at the stimulus station.

2.2 Results and Discussion

Dipropyl disulfide at all concentrations elicited a poor oviposition response from female onion maggots (Table 2.1). Although there were from 76 to 124 more eggs laid at the experimental than control stations there was no significant difference in response among the five doses. These results confirm that dipropyl disulfide stimulates oviposition by onion maggots, but indicate that its use in oviposition studies may not be reliable. More consistent and higher responses can be obtained by combining dipropyl disulfide with non-chemical host selection factors, e.g. by using surrogate "plants" that mimic the shape, size and color of onion plants (Harris and Miller 1983, 1984; Harris *et al.* 1987). Alternatively, a volatile stimulus that presents a more complete onion odor than dipropyl disulfide could increase the reliability of the inverted beaker bioassay (Vernon *et al.* 1977; Pierce *et al.* 1978).

Table 2.2 indicates that both the pieces of onion bulb and onion oil elicited strong oviposition responses by onion maggot females. These results confirm that both onion bulbs and onion oil could provide an effective and reliable stimulus for oviposition deterrent bioassays. Onion oil compared effectively with an onion piece and was selected for standard bioassays because its release rate could be controlled.

Table 2.1 Oviposition response by female *Delia antiqua* to 100 % dipropyl disulfide and to dipropyl disulfide at lower dilutions in undecane. Eleven replicates, 15 females, 15-20 days old, per cage for each dose.

Concentration (%) in 50 μL capillary tube	Mean number of eggs laid per female ($\bar{X} \pm SE$)[a]
100.00	1.41 ± 0.33
10.00	0.83 ± 0.25
1.00	1.10 ± 0.26
0.1	0.76 ± 0.19
0.01	1.24 ± 0.27
undecane	0.01

[a] No difference in number of eggs laid among experimental treatments, ANOVA, $P > 0.05$.

Table 2.2. Comparison of oviposition response by female *Delia antiqua* to cut pieces of onion and onion oil stimuli in two-choice bioassays, 15 females , 15-20 days old, per cage.

Treatment	Number of replicates	Number of eggs/female ($\bar{X} \pm SE$) Stimulus	Control	t-test probability, stimulus *vs* control	Percent stimulation of oviposition compared to expected 50:50 distribution ($\bar{X} \pm SE$)[a]
Onion slice (5g)	10	15.8 ± 1.7	0.32 ± 2.2	0.0001	96.7 ± 1.6
Onion oil in 50 μl capillary tube	8	19.4 ± 4.5	0.34 ± 0.2	0.0001	96.0 ± 2.1

[a] Calculated as in section 2.1.3. Stimulation not significantly different between treatments, t-test, $P >$ 0.80.

3.0 Identification of Oviposition Deterrent Constituents in Pine oil

The objectives of this project were: a) to verify the oviposition deterrent capability of the pine oil used by (Javer et al. 1987); b) to isolate and identify the bioactive constituents; c) to validate the identifications by bioassay of authentic compounds and d) to determine the bioactivity of related compounds found in coniferous trees (Funes et al. 1973; von Rudloff 1974).

3.1 Materials and Methods

3.1.1 Bioactivity of Pine oil

The ability of distilled pine oil (Norpine 65, Northwest Petrochemicals Ltd., Anacortes, WA) to deter the oviposition by female onion maggots in response to onion oil was evaluated using the two-choice bioassay (section 2.1.1). The release rate of pine oil in this and subsequent experiments was 246 μg per 24 h, as determined by the difference in weights of the loaded capillary tubes before and after the experiments. The experiment had 12-replicates, in which experimental stations contained one tube with onion oil and one with pine oil and control stations had only onion oil. A similar 16-replicate experiment was set up evaluating the ability of pine oil to deter oviposition in response to dipropyl disulfide.

3.1.2 Isolation and Identification of Bioactive Constituents[1]

Analysis of volatiles was done with Hewlett-Parkard 5830 and 5890 gas chromatographs equipped with capillary inlet systems, flame-ionization detectors, and a glass column (30 m x 0.5 mm ID) coated with SP-1000 (Supelco Canada Ltd.,Oakville, Ontario) or a fused silica column (15 m x 0.25 mm ID) coated with DB-1 (J & W Scientific Inc., Folsom, CA). The injection port and detector temperatures were 260^{o} and 270^{o} C, respectively. A Hewlett-Packard 5895B GC/MS/DS with a fused silica column (30 m) (0.25 mm ID) coated with DB-1 (J & W

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[1] Analytical chemistry done by H. D. Pierce Jr., Department of Chemistry, Simon Fraser University.

Scientific Inc., Folsom, CA.) inserted directly into the ion source was employed for coupled gas chromatography-mass spectrometry (GC-MS). The injection port, transfer line, and ion source were 260, 250 and 200^{o} C, respectively. Helium was the carrier gas for GC and GC-MS.

A Varian 1700 gas chromatograph fitted with a stainless steel column (1.52 m x 0.64 cm OD) packed with 25% SE-30 on Chromosorb A (60/80 mesh) was used for preparative separation of distilled pine oil. Helium was the carrier gas, and the injection port and detector temperatures were 260 and 300^{o} C, respectively. Two fractions were collected, the first containing limonene as a major constituent and all earlier-eluting compounds, and the second containing all compounds eluting after limonene. The two fractions were tested for bioactivity in seven- and eight-replicate experiments respectively, in the two-choice bioassay.

A ternary mixture of synthetic monoterpenes (Aldrich Chemical Co., Milwaukee, WI) was prepared based on the relative proportion of the three main constituents in fraction 1 as follows: 300 µL limonene, 200 µL 3- carene and 100 µL *p*-cymene. Two-choice bioassay experiments with 7-15 replicates were done testing the three monoterpenes individually and the ternary mixture against onion oil controls. A 12-replicate no-choice experiment tested the ternary mixture with onion oil and onion oil controls in separate cages.

Two three-choice experiments with seven and eight replicates, respectively, tested the ternary mixture and fraction 1 with onion oil, and the ternary mixture and distilled pine oil with onion oil, against onion oil controls. Release rates determined by weight loss (n=10) for *p*-cymene, limonene, 3-carene and the ternary mixture were 220, 320, 320, and 320 µg/24 h, respectively. Purity of all monoterpenes was ≥ 95%.

3.1.3 Bioactivity of Related Monoterpenes

Eight other synthetic monoterpenes (purity ≥ 95 %) (Aldrich Chemical Co., Milwaukee, WI) were also tested for deterrent activity in six-replicate, two-choice experiments. These were α-pinene, ß-pinene, myrcene, terpinolene, α-terpinene, γ-terpinene, α-phellandrene and ß-phellandrene. Release rates determined by weight loss (n=10) for the eight monoterpenes were :290, 300, 320, 300, 310, 310, 320 and 330 μg/24 h, respectively.

3.1.4 Statistical Analysis

All data were transformed by square root to stabilize the variances before they were subjected to t-tests or ANOVA followed by the Bonferroni and Student-Newman-Keuls (SNK) test for comparison between means (Zar 1984; SAS System for Statistical Analysis 1987). In all cases α=0.05. Percent deterrency was calculated as in section 2.1.3.

3.2 Results and Discussion

3.2.1 Bioactivity of Pine Oil

Pine oil reduced oviposition by female onion maggots in response to onion oil by 72% at a release rate of 200 μg per day (Table 3.1). The deterrency was, however, low when pine oil was paired with dipropyl disulfide, probably because dipropyl disulfide alone was an inferior attractive control. These results confirm those of Javer et al. (1987) with respect to the deterrent properties of pine oil and support the use of onion oil as control stimulus in bioassays.

3.2.2 Identification of Oviposition Deterrent Constituents in Pine Oil.

Fraction 1 caused almost complete deterrency of oviposition by female onion maggots (Table 3.2), suggesting that it might contain most of the volatile constituents active in pine oil. Although fraction 2 caused 61% deterrence in oviposition, much of the deterrent activity was probably caused by residual limonene carried over from fraction 1.

Analysis of fraction 1 by GC and GC-MS revealed that it consisted primarily of three major components, 3-carene, limonene and *p*-cymene (Fig. 3.1). Fraction 2 contained over 50 compounds including 5% limonene. All three of the major constituents of fraction 1 caused highly significant deterrency in oviposition (Table 3.3). The ternary mixture was more active than *p*-cymene or limonene, while the most potent monoterpene, 3-carene caused deterrency that

Table 3.1 Comparison of oviposition deterrent effect when pine oil was combined with dipropyl disulfide and onion oil controls in two-choice bioassay, 15 females, 15-20 days old per cage.

Treatment[a]	Number of replicates	Mean number of eggs laid per female ($\bar{X}$ ± SE): Stimulus	Control	t-test probability, stimulus *vs* control	Percent deterrency of oviposition compared to 50:50 distribution ($\bar{X}$ ± SE)[b]
Pine oil with dipropyl disulfide	16	1.57 ± 0.42	3.81 ± 1.18	0.0875	42.9 ± 12.0
Pine oil with onion oil	12	2.94 ± 0.74	17.73 ± 2.35	0.0001	72.8 ± 5.85

[a] Pine oil, dipropyl disulfide and onion oil were in separate 50 μL capillary tubes.

[b] Calculated as in section 2.1.3. Deterrency significantly different between treatments, t-test, P<0.05.

Table 3.2 Comparison of oviposition deterrent effect of two fractions of pine oil with onion oil control.

Treatment	Number of replicates	Mean number of eggs laid per female ($\bar{X} \pm SE$) Stimulus	Control	t-test probability, stimulus *vs* control	Percent deterrency of oviposition compared to 50:50 distribution ($\bar{X} \pm SE$)[a]
Fraction 1 with onion oil	7	0.46 ± 0.15	26 ± 3.45	0.0001	96 ± 1.15
Fraction 2 with onion oil	8	4.83 ± 1.05	19.89 ± 2.05	0.0001	61.45 ± 5.79

[a] Calculated as in section 2.1.3. Deterrency significantly different between treatments, t-test $P < 0.0001$.

Figure 3.1 Chromatogram of fraction 1 of Norpine 65 showing the three major constituents.

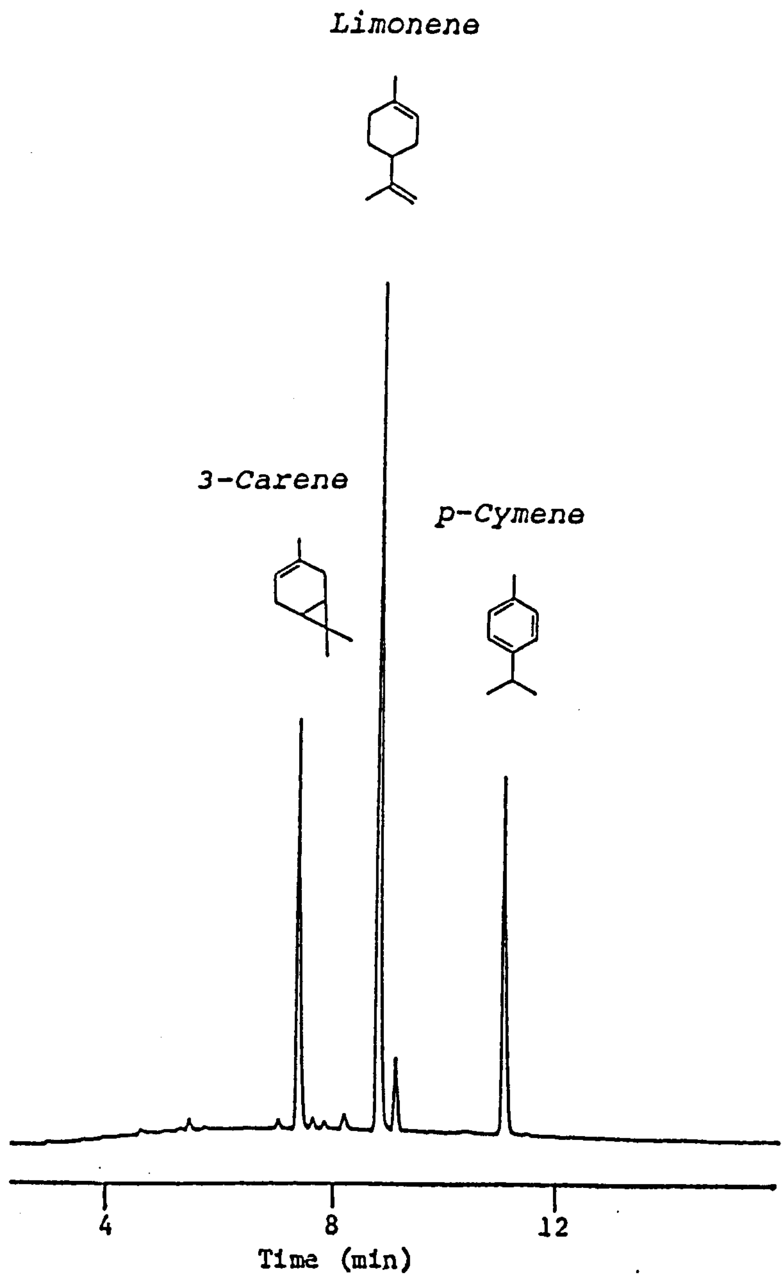

Limonene
3-Carene
p-Cymene
4
8
12
Time (min)

Table 3.3 Oviposition deterrency of three major monterpenes in fraction 1 tested alone and in combination in comparison to onion oil controls in two-choice bioassays 15 females, 17 days old, per cage.

Treatment	Release rate (mg/24 h)	Number of replicates	Mean number of eggs laid per female ($\bar{X}\pm$ SE)		t-test probability, stimulus *vs* control	Percent deterency of oviposition compared to expected 50:50 distribution ($\bar{X}\pm$ SE)[a]
			Stimulus	Control		
Choice-bioassay						
p-cymene	0.22	7	3.63 ± 1.26	12.17 ± 1.92	0.0004	56.34 ± 12.16a
limonene	0.32	15	3.91 ± 0.59	18.42 ± 1.71	0.0001	65.39 ± 4.32a
3-carene	0.32	8	1.88 ± 0.39	13.69 ± 2.15	0.0009	73.23 ± 5.41ab
Ternary mixture	0.32	7	0.97 ± 0.24	16.03 ± 2.72	0.0002	88.60 ± 3.31 b
No-choice bioassay						
Ternary mixture	0.32	12	3.65± 0.54	16.89 ± 1.75	0.0001	62.54 ± 5.87

[a] Calculated as in section 2.1.6. Mean percents followed by the same letter are not significantly different, Bonferroni t-tests, $P < 0.05$.

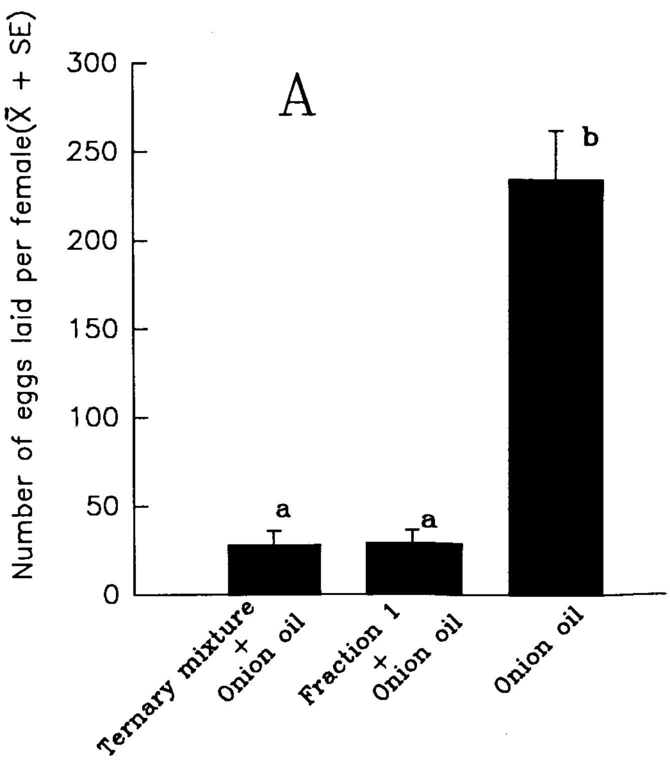
was intermediate between that caused by the mixture and the other individual components. In the no-choice bioassay experiment the deterrent activity of the ternary mixture was reduced to 62.5 %. The increased numbers of eggs at the treated stations may be explained by the fact that gravid females were deprived of any opportunity to oviposit at a control station (Javer et al 1987; Cowles and Miller 1989).

At release rates of 451 and 246 μg per 24 h, respectively, fraction 1 and distilled pine oil would have released the constituents of the ternary mixture at approximately 320 and 200 μg per 24 h, respectively. Because these rates are comparable to the rate of 320 μg per 24 h for the ternary mixture, the monoterpenes in fraction 1 apparently accounted for almost all the deterrent activity of the fraction 1 and raw pine oil. The hypothesis that the deterrent activity of the ternary mixture would account for the deterrent activity in both fraction 1 and pine oil was upheld by the results of the three-choice bioassay experiments (Fig. 3.2).

3.3.3 Bioactivity of Related Monoterpenes

All the monoterpenes with exception of α-pinene significantly deterred oviposition by female onion maggots (Table 3.4). Myrcene caused more deterrency than any other monoterpene except 3-carene (Table 3.3). These results indicate that several conifer monoterpenes possess oviposition deterrent capability, and suggest that a more complete blend than the ternary mixture may be a superior oviposition deterrent formulation for future applications.

Figure 3.2. Comparison of oviposition deterrency in three-choice bioassays between the ternary mixture and fraction 1 of pine oil (A), and between the ternary mixture and distilled pine oil (B), in contrast to onion oil controls. Seven replicates, 15 females, 18 days old, per cage. Bars with same letter are not significantly different, Bonferroni test, $P < 0.0001$.


Number of eggs laid per female($\bar{X}$ + SE)
300
250
200
150
100
50
0
A
a
a
b
Ternary mixture + Onion oil
Fraction 1 + Onion oil
Onion oil

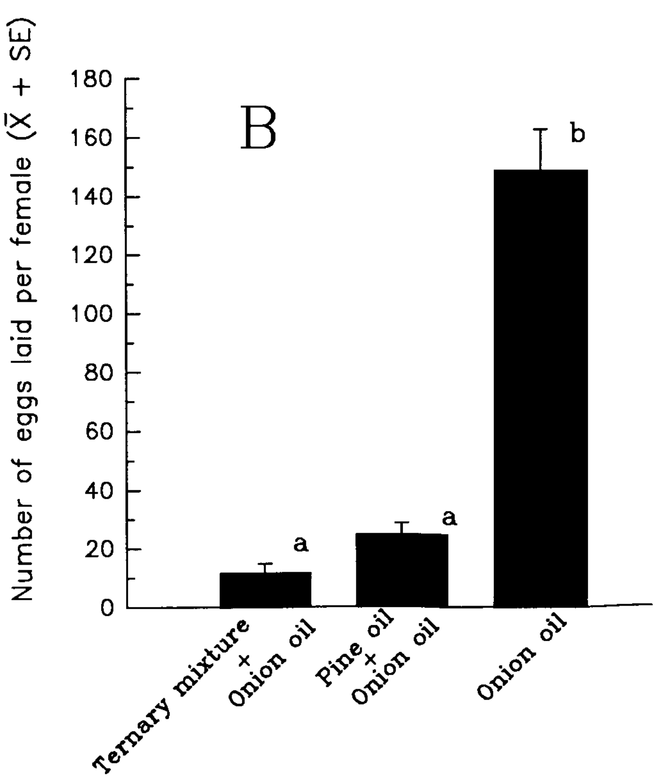

Number of eggs laid per female ($\bar{X}$ + SE)
180
160
140
120
100
80
60
40
20
0
B
a
a
b
Ternary mixture + Onion oil
Pine oil + Onion oil
Onion oil

Table 3.4. Ranked oviposition deterrency in two-choice bioassay with onion oil as control, of major conifer monoterpenes not found in significant amounts in Norpine 65, six replicates,15 females, 17 days old, per cage.

Treatment	Number of eggs per female ($\bar{X}$ ± SE) Stimulus	Control	t-test probability stimulus *vs* control	Precent deterrency of oviposition compared to expected 50:50 distribution ($\bar{X}$ ± SE)[a]
α-pinene	6.24 ± 3.49	6.94 ± 1.76	0.8507	21.6 ± 19.46a
ß-pinene	4.47 ± 1.03	11.47 ± 0.9	0.0029	45.68 ± 9.40ab
terpinolene	4.37 ± 1.18	13.92 ± 2.78	0.0117	50.83 ± 8.01ab
γ-terpinene	0.83 ± 0.18	3.72 ± 1.22	0.0591	54.67 ± 6.99ab
ß-phellandrene	1.16 ± 0.30	4.88 ± 1.57	0.03591	54.81 ± 5.31ab
α-terpinene	5.09 ± 1.37	18.39 ± 3.34	0.0049	56.41 ± 7.71ab
α -phellandrene	2.74 ± 1.18	13.29 ± 1.22	0.03228	59.47 ± 9.82ab
myrcene	1.88 ± 0.48	10.89 ± 3.39	0.0337	68.16 ± 6.23b

[a] Calculated as in section 2.1.3 Mean percents followed by the same letter are not significantly differrent, SNK test, $P < 0.05$.

4.0. Deterrency of Oviposition in Response to Onion Seedlings

Deterrency of oviposition in response to an onion oil stimulus in a laboratory bioassay does not necessarily indicate that response to actual onion plants in the field would be deterred. This is because onion plants offer visual as well as chemical stimuli, both of which are important in eliciting host selection behavior and ultimately oviposition, by *D. antiqua* females (Harris and Miller 1983, 1984; Harris *et al.* 1987). Therefore, choice and no-choice bioassays experiments to examine the effect of the ternary mixture on oviposition behavior of onion maggot females were conducted using potted onion seedlings in a green house.

4.1 Material and Methods

4.1.1 Bioassay Procedure

Onion seeds, *Allium cepa* L. variety golden globe, were planted in black plastic pots (13 x 13 x 6 cm). The seeds were scattered onto a metal screen (1.5 mm mesh) laid over top soil (4 cm deep). A 1 cm deep layer of washed sand was placed over the screen. Thus the onion plants grew up through the sand, but had their roots in contact with the soil through the mesh (Fig 4.1).

This procedure allowed eggs laid in the sand around the base of the plant to be recovered by lifting the screen with the sand and seedlings away from the top soil, immersing them in water, and collecting the floating eggs on a nylon mesh for counting. The seedlings were thinned to 35 plants per pot, and were watered daily. Four week old seedlings were used in the bioassay. Bioassays were conducted in 60 x 35 x 50 cm wooden-frame cages, with nylon mesh screen walls and Plexiglas ceiling and front, containing 20 gravid females 15-20 days old obtained from cultures as described in Section 2.1.1. The cages were maintained in a green house at approximately 23° C, 60 % RH and 16L: 8D. Stimuli were tested for 24 h, after which eggs were counted.

The ternary mixture was released from capillary tubes as described in Section 2.1.2 or was formulated according to the proportions in raw pine oil (Fig. 3.1) into pieces of plastic tubing (4 cm long) by PheroTech Inc. Delta, B.C. Each tube release device contained 12.8 mg

Figure 4.1 Two-choice bioassay set up for evaluating oviposition deterrency of pine oil constituents challenged by onion seedlings.

of the active ingredients consisting of 50% limonene, 33.3% 3-carene and 16.7% *p*-cymene and after curing for two weeks at room temperature the device released the blend of materials at 140 μg per 24 h as determined by weight loss before and after the experiment. Capillaries or tube release devices were placed directly on the soil surface at the base of the plants. In two-choice bioassays, treatment and control pots with onion seedlings were placed 43.5 cm apart on the diagonal axis of the cage floor. In no-choice bioassays, the single pot was centered.

Females accepted the onion plants readily and could be seen running over the green and moist foliage, and probing their ovipositor at the plant soil interface in characteristic behavior (Harris and Miller 1988; Cowles and Miller 1990, 1992).

4.1.2. Experiments

Two dose-response, choice-bioassay experiments were run testing 1-5 plastic tube devices or combinations of 25 and 50 μL capillary tubes in one seedling pot against an untreated pot in the same cage. A third two-choice experiment allowed two plastic tube devices to remain in the treatment pots for 24 h before they were exposed to female *D. antiqua.* In two no-choice experiments the above treatments were tested in single onion pots, with the control pots in separate cages. The numbers of replicates are given in Tables 4.1 and 4.2. In each experiment, one replicate was run per day with the cages arranged in a randomized complete block.

4.1.3 Statistical Analysis

Data in the choice and no-choice experiments were transformed by square root and log (X+1), respectively, to stabilize the variances before they were subjected to t-test or ANOVA followed by the SNK test for comparison between means at $\alpha=0.05$ (Zar 1984).

4.2 Results and Discussion

The ternary mixture caused significant dose-dependent oviposition deterrency to potted onion seedlings in both two-choice and no-choice bioassays (Tables 4.1, 4.2) with comparable deterrency for the plastic tube and capillary release devices. The remarkably strong >85% deterrency achieved by the two plastic tube release devices tested 24 h after being placed in the onion seedling pot (Table 4.1) suggests that there may have been sufficiently large amounts of monoterpenes released over 24 h for them to be adsorbed onto the cuticle of plants. This would have made the entire seedling "crop" repellent rather than just the base of the plants and sand near the release devices.

The deterrency was weaker in the no-choice experiments, (Table 4.2) than in the two-choice experiments (Table 4.1), exceeding 60% only at the high release rate of 1240 μg per 24 h from capillary tubes, and not being significant at one dose. Deterrency in the no-choice experiments might have been greater had the devices been deployed for 24 h prior to commencement of the experiments.

There was sufficient deterrency at levels comparable to those found for other agents (Wiens *et al.* 1978; Cowles *et al.* 1989; Miller and Cowles 1992) to justify continuing research and development of the active constituents in pine oil. Efficacy might be improved by incorporation of additional monoterpenes (Table 3.3), combining monoterpenes with other known deterrents, using deterrents in combination with arresting barriers (Vernon and MacKenzie 1993), and development of a stimulo-deterrent strategy, (Miller and Cowles 1992) whereby pine oil volatiles were deployed in one area of a field and attractants were deployed in another area.

The very effective release rate of 280 μg per 24 h in the 24 h delay experiment (Table 4.1) is equivalent to a daily release rate of 16.6 mg per m^2 or 166 g per ha. Given the prospect of some damage to a commercial crop with a deterrent alone, it would have to be very cheap to be released at such a high rate and to offset the damage that did occur. Alternatively, a monoterpene-based deterrent might be a very useful product for the home gardener who may

Table 4.1 Oviposition deterrency of ternary mixture of limonene, 3-carene and *p*-cymene in two-choice bioassays assessing effect of stimulus against onion seedlings control, 20 females,15-20 days old per cage.

Release method	Release rate (µg/24h)	Number of replicates	Number of eggs laid per female ($\bar{X} \pm SE$)		t-test probability, stimulus *vs* control	Percent deterrency of oviposition compared to expected 50:50 distribution ($\bar{X} \pm SE$) [a]
			Stimulus	Control		
Capillary tubes	150	7	1.66 ± 0.46	6.18 ± 1.66	0.0371	54.66 ± 7.65a
	310	7	5.73 ± 1.44	18.64 ± 2.66	0.0030	52.60 ± 8.71a
	620	7	2.85 ± 0.33	10.30 ± 0.80	0.0002	56.05 ± 5.73a
	930	7	0.89 ± 0.39	4.47 ± 0.88	0.0018	73.25 ± 7.76b
	1240	7	0.97 ± 0.30	13.06 ± 2.00	0.0010	84.36 ± 5.49c
Plastic tubes						
	140	6	5.65 ± 0.87	12.29 ± 1.04	0.0014	39.75 ± 9.22a
	280	6	3.52 ± 0.78	8.63 ± 0.85	0.0036	44.73 ± 8.81a
	420	6	6.30 ± 1.40	18.02 ± 2.86	0.0056	48.71 ± 10.82a
	560	6	2.85 ± 0.33	10.30 ± 0.80	0.0002	56.64 ± 7.55b
	700	6	3.13 ± 0.81	20.86 ± 3.46	0.0045	70.71 ± 8.10c
Plastic tube, 24 h equilibration	280	9	0.48 ± 0.19	7.93 ± 2.08	0.0059	85.33 ± 4.05

[a] Calculated as in Section 2.1.3. Mean percents within an experiment followed by the same letter are not significantly different, SNK-test, $P < 0.05$.

Table 4.2 Oviposition deterrency in ternary mixture of limonene, 3-carene and *p*-cymene in no-choice bioassays assessing effect of stimulus against onion seedlings, six replicates, 20 females, 15-20 days old per cage.

Release method	Release rate (μg/24 h)	Number of eggs laid per female ($\bar{X} \pm SE$) Stimulus	t-test probability, stimulus *vs* control	Percent deterrency of oviposition compared to expected 50 :50 distribution ($\bar{X} \pm SE$)[a]
Capillary tubes	Control	28.07 ± 3.41		
	150	20.53 ± 3.23	0.0235	16.28 ± 5.52a
	310	17.69 ± 3.13	0.1483	23.28 ± 10.64a
	620	13.73 ± 3.98	0.0309	38.93 ± 13.37ab
	930	9.56 ± 2.34	0.0009	51.54 ± 6.66ab
	1240	7.07 ± 1.62	0.0004	63.21 ± 6.67b
Plastic tubes	Control	22.10 ± 1.61		
	140	17.84 ± 2.17	0.0354	11.67 ± 3.19a
	280	17.11 ± 2.54	0.0359	15.16 ± 7.12a
	420	12.51 ± 1.65	0.0001	28.89 ± 2.79ab
	560	9.33 ± 1.88	0.0001	42.41 ± 7.60ab
	700	8.00 ± 2.63	0.0006	53.63 ± 12.41b

[a] Calculated as in section 2.1.3. Mean number of eggs within an experiment followed by the same letter are not significantly different, SNK-test, $P < 0.05$.

tolerate some damage and high expense to produce a "crop" without the use of conventional chemical pesticides.

5.0 Effect of Ternary Mixture on Oviposition by Cabbage Maggot, *Delia radicum* (L.).

The cabbage maggot, *Delia radicum* (L) (Diptera: Athomyiidae), is a major root-infesting pest of cruciferous crops throughout most of North America (Simser 1992; Walgenbach 1993). As for onion maggots, control measures are directed towards the most damaging first generation larvae. Current control methods include soil drenches with fensulfothion, carbofuran and chlopyrifos (Matthews-Geringer and Hough-Goldstein 1988). Partial deterrency of oviposition by cabbage maggots was achieved using turpentine-soaked stakes (Havukkala 1982), and 3,5-dimethoxy-4-hydroxycinnamic acid, produced in the frass of a lepidopteran pest of cabbage (Jones *et al.* 1988). My objective was to evaluate the efficacy of the ternary mixture as a potential oviposition deterrent for the cabbage maggot.

5.1 Material and Methods

The two-choice bioassay described in section 2.1.1 was modified by replacing filter papers with sand (0.5 cm deep) in the petri dish. The oviposition stations contained a 5 g freshly cut slice of rutabaga root placed 0.2 cm deep in moist sand inside an inverted glass beaker. Otherwise all bioassay procedures were identical to those for onion maggots.

For bioassays, gravid female cabbage maggots, 15 days old, were obtained from cultures maintained in SFU insectory. For counting, eggs were recovered from the sand in the oviposition stations by floating. Both two-choice and no-choice experiments were run, in which the ternary mixture was released from 50 μL capillary tubes taped inside the inverted glass beaker.

The data were analysed by t-tests as in previous sections.

5.2 Result and Discussion

Oviposition by cabbage maggots was partially deterred by the ternary mixture in the two-choice bioassay, but deterrency was only slight in the no-choice bioassay (Table 5.1). This

Table 5.0 Deterrency of the ternary mixture to oviposition by female *Delia radicum* to cut pieces of rutabaga.

Treatment	Number of replicates	Number of eggs laid per female ($\bar{X} \pm SE$)		t-test probability, stimulus vs control	Perecent deterrency of ovipostion compared to expected 50:50 distribution ($\bar{X} \pm SE$)[a]
		Stimulus	Control		
Choice-bioassay Rutabagas (5g)	7	1.87 ± 0.40	5.58 ± 0.79	0.0006	54.20 ± 7.05
No-choice Rutabagas (5g)	8	1.48 ± 0.37	2.14 ± 0.32	0.0080	26.26 ± 9.75

[a] Calculated as in section 2.1.3.

result suggests that monoterpenes may have potential as operational deterrents for cabbage maggots, and further suggest that they may be efficacious for other anthomyiids.

6.0 Concluding Discussion

As it becomes increasing difficult to control the onion maggot with conventional chemical insecticides due to resistance development (Howitt 1958; Harris *et al.* 1982), disruption of oviposition with naturally derived deterrents may become feasible, particularly when they are used in an integrated manner, e.g. with exclusion barriers (Vernon and Mackenzie 1993) or in a stimulo-deterrent tactic (Cowles and Miller 1990, 1992). The cheap supply of monoterpenes from various sources of pine oil together with their proven efficacy in this study suggest that further development of monoterpene-based deterrency is warranted.

Like other oviposition deterrents, the ternary mixture may have caused continuous oriented movement away from the deterrent source (Dethier *et al.* 1960). Davis (1985) hypothesized that deterrents may act either by blocking the perception of onion volatiles when they bind to and inhibit antennal receptors, or they may be perceived by specific receptors and interpreted by the central nervous system. The latter hypothesis appears to explain the mode of action on oviposition deterrents on onion maggots, since several compounds of varied chemical nature deter oviposition (Wiens *et al.* 1978; Cowles and Miller 1992; Cowles *et al* 1990; Alfaro *et al* 1981; Javer *et al.* 1987). This variability suggest that combinations of monoterpene-based deterrents with other plant-derived deterrents of different chemical natures may be used to present an overwhelming message of the prevalence of non-host plants. Such combinations would improve the efficacy of integrated pest management tactics involving deterrents.

My results lead to the following specific conclusions:

1. The oviposition deterrency of Norpine 65 for the onion maggot lies primarily in three monoterpenes, 3-carene, limonene and p-cymene.
2. A mixture of the three monoterpenes can provide more effective deterrency than the individual consitituents alone.
3. Oviposition deterrent properties occur in other related conifer monoterpenes.

4. The bioactive monoterpenes in Norpine 65 can also cause oviposition deterrency in the cabbage maggot, suggesting that monoterpenes may have widespread deterrent properties against other dipteran species.

A more general conclusion is that further research on testing combinations of deterrents and integrating their use with other pest management tactics against *D. antiqua* and other dipteran pests should proceed with dispatch so that effective integrated methods are available as alternatives to convectional chemical insecticides.

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