Effects of an nC24 agricultural mineral oil on tritrophic interactions between French bean (*Phaseolus vulgaris* L.), two-spotted mite (*Tetranychus urticae* Koch) and its predator, *Phytoseiulus persimilis* Athias-Henriot

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Statement of Authentication

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

Yingen Xue
15 March 2007
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Abstract

A comprehensive evaluation of the effects of an \( nC24 \) agricultural mineral oil (AMO) on tritrophic interactions between French bean \textit{Phaseolus vulgaris} cv. Redlands Pioneer [Fabales: Fabaceae], two-spotted mite (TSM) \textit{Tetranychus urticae} Koch [Acari: Tetranychidae] and the predatory mite \textit{Phytoseiulus persimilis} Athias-Henriot [Acari: Phytoseiidae] was conducted under laboratory conditions.

The relative topical and residue toxicity of the AMO to the egg, larva, protonymph and adult stages of TSM and \textit{P. persimilis} were evaluated by using a Potter spray tower to apply aqueous emulsions of the AMO to French bean leaf discs. The egg was the most susceptible stage of TSM sprayed with the oil and, conversely, the egg was the least susceptible stage of \textit{P. persimilis}. Photomicrographs taken by an environmental scanning electron microscope (ESEM) showed that the egg surface of TSM is usually well covered with fine silk that females use to attach their eggs to plant surfaces. All the motile stages of TSM tested were tolerant to the AMO. The most susceptible motile stage of \textit{P. persimilis} was the larva. The susceptibility of the protonymph and adult stages of both species was similar.

AMO deposits significantly impeded the development of immature TSM. No immature TSM could develop to adulthood on deposits of 0.30% w/w aqueous emulsions of the AMO, and the effect could last 7 d. Most immature TSM died in the egg or larval stages. In contrast, oil concentrations less than 0.50% deposits had relatively less impact on \textit{P. persimilis}. Negative effects of AMO on \textit{P. persimilis} decreased sharply after 3 d.

Deposits of the AMO also significantly suppressed feeding and oviposition by TSM and oviposition suppression was largely related to feeding deterrence. AMO deposits affected the searching efficiency and predation rate of \textit{P. persimilis}, with the impact of the oil increasing rapidly with increasing oil concentration. The most important factors were suppression of walking speed by oil deposits and the oil-contamination of mechanoreceptors and chemoreceptors on anterior tarsi and pedipalps.
Impact of sublethal 0.25% AMO deposits had significant effects on the functional and numerical responses of *P. persimilis* to TSM. Although *P. persimilis* adult females consumed fewer prey and produced fewer eggs at low prey densities (4 and 8 adult TSM females/disc) they consumed more prey at higher densities (16, 32, 64 adult TSM females/disc) on AMO deposits than they did at these prey densities on water-sprayed control. This outcome was attributed to the impact of deposits of the AMO on feeding by the prey, an impact that led to smaller-sized prey in the AMO treatments.

Volatile s from the AMO had no positive or negative effect on the preference of TSM or *P. persimilis* to volatiles induced by TSM feeding on French bean leaves. However, French bean plants treated with 0.50% and 1.00% aqueous AMO emulsions were more attractive to *P. persimilis* than distilled-water treated control plants, and plants treated with 0.25% AMO emulsions.

Chemical analysis of the volatiles from French bean plants infested with TSM and the bean sprayed with the AMO indicated that 0.50% and 1.00% AMO deposits elicited the production of volatile compounds, which included all the chemicals in TSM-induced volatiles: salicylate (MeSA), (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) 3-octen-1-ol, 2,3-hexanediol, (4E)-4-hexen-1-yl acetate, limonene, octamethyl cyclotetrasiloxane and (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT). The quantities of 2,3-hexanediol and DMNT were greater in the 1.00% AMO-induced volatiles than in the TSM-induced volatiles but quantities of MeSA and TMTT were higher in the TSM-induced volatiles than in 1.00% AMO-induced volatiles. The qualitative difference between volatiles from TSM-infested plants and AMO-sprayed plants was that four chemicals were only present among the chemicals released from the oil sprayed plants. These chemicals were cyclooctatetraene, decamethyl cyclopentasiloxane, dodecamethyl cyclohexasiloxane and caryophyllen-13-al.

The impact of the AMO on the quantities of volatile chemicals released by sprayed French bean plants was clearly related to increasing dose. Single spray application of
0.25 % AMO to plants did not induce the production of detectable quantities of MeSA and TMTT, but higher concentrations did.
Chapter 1. Introduction


Studies to date (Burnett 1979, van de Vrie 1985, Nicetic *et al.* 2001) indicate that, despite the effectiveness of *P. persimilis* for biological control of two-spotted mite in greenhouses, the predator alone appears unable to maintain two-spotted mite populations below an economic injury level for an extended period of time. Thus the practical implementation of spider mite control using *P. persimilis* in commercial greenhouses is complicated by the need for selective acaricides to adjust the prey/predator ratio and maintain adequate long-term control, and by the need to control other pests and diseases.

Petroleum-derived spray oils (PDSOs) have been used as crop protectants for over a hundred years. Although the advent of synthetic organic pesticides in the 1940s reduced interest in the use of PDSOs, concerns over environmental and health issues related to
the former have led to recent renewed interest in the potential for using PDSOs in integrated pest and disease management (IPDM) programs (Beattie & Smith 1997, Beattie et al. 2002b). Their potential for use in pest control has been greatly enhanced by production of high quality products, horticultural mineral oils (HMOs, formerly called narrow-range petroleum spray oils) and agricultural mineral oils (AMOs, formerly broad-range petroleum spray oils), since the 1960s (Agnello 2002). HMOs and AMOs are biorational products that are ideal for use in sustainable IPDM programs (Beattie et al. 2002b). They are less disruptive to natural enemies of pests, and generally much safer to use from environmental and human health perspectives than synthetic pesticides (French et al. 1976, Davidson et al. 1991, Johnson 1994, Beattie & Smith 1997). Historically, products have been used as dilute (< 2% v/v) aqueous spray emulsions to suffocate susceptible pests, mainly scale insects and mites (Herron et al. 1995, Beattie et al. 2002b). Behavioural effects of oil deposits on feeding and oviposition of a wider range of pests are now recognised as more important (Beattie et al. 2002b). The compatibility of HMOs and P. persimilis in integrated pest management (IPM) programs for TSM on greenhouse tomato and roses has been reported (French et al. 1976, Nicetic et al. 2001, Nicetic et al. 2002).

However, little study has been undertaken to determine why the predator is less susceptible to PDSOs than its prey. The mechanisms responsible for the behavioural effects are still poorly understood. The potential role of PDSOs in activating the direct and indirect defence systems of plants is unclear. There are still significant gaps in our knowledge about how to evaluate behavioural effects within the tritrophic context of plant-herbivore-carnivore, because interactions between herbivores and their host plants and between herbivores and their natural enemies can be only understood when considered within a tritrophic context (Price et al. 1980). In order to develop an integrated control program for pests and diseases of TSM-susceptible crops it is very important to understand the effect of PDSOs on the tritrophic interactions between host plant-TSM-P. persimilis.

I hypothesised that: (1) PDSOs have lower toxicity to P. persimilis than to TSM as contemporary HMOs and AMOs are considered to be biorational pesticides; (2) PDSOs
have significant influences on the feeding and oviposition behaviour of TSM and *P. persimilis*; (3) PDSOs oils can affect the prey-searching efficiency of *P. persimilis* by changing walking activity, walking speed, and walking pattern, or by contaminating the mechanoreceptors and chemoreceptors of the predator, thereby interfering with the predators’ response to physical and chemical stimulants produced by the prey; (4) PDSOs affect the functional and numerical responses of *P. persimilis* to TSM; (5) PDSOs, as a complex mixture of a large number of different hydrocarbon molecules, may directly enhance or mask the function of herbivore-induced volatiles in the host or prey location of *P. persimilis* to TSM; (6) PDSOs may activate the direct and indirect defence systems of plants, including production of plant volatiles in response to herbivore feeding, and thereby enhance location of prey by the predator.

The major objectives of my research were to test these hypotheses by:

- Comparing spray and deposit toxicity of AMO to TSM and *P. persimilis* and determining the most appropriate oil concentration for controlling the former without harming the latter;
- Comparing the effect of AMO on the development of TSM and *P. persimilis*.
- Investigating the effects of AMO on the feeding, oviposition and settlement behaviour of TSM.
- Determining the impact of AMO on the searching efficiency of *P. persimilis* and predation behaviour.
- Evaluating the influence of AMO on the functional and numerical response of *P. persimilis* to TSM.
- Determining the effects of AMO on the olfactory response of TSM and *P. persimilis* to herbivore-induced plant volatiles.
- Investigating whether AMO triggers emission of herbivore-induced plant volatiles and then comparing these volatiles to determine if they can be used by TSM and *P. persimilis* as foraging cues.
Chapter 2. Literature Review

The general biology and behaviour of two-spotted mite (*Tetranychus urticae* Koch [Acari: Tetranychidae])

**General biology of *Tetranychus urticae***

Two-spotted mite feeds on many species of plants and is a major pest of ornamentals, vegetables, cotton and fruit trees throughout the world (Hussey *et al.* 1969, Hely *et al.* 1982). The literature on TSM is voluminous. Much of the pertinent information has been summarised by Huffaker *et al.* (1969, 1970), van de Vrie *et al.* (1972), Jeppson *et al.* (1975), Hussey & Huffaker (1976) and Helle & Sabelis (1985).

The life cycle includes egg, larva, protonymph, deutonymph and adult stages. The larval, protonymphal and deutonymphal stages are further divided into feeding (active) and quiescent (resting) stages (Laing 1969, van de Vrie *et al.* 1972, Osborne *et al.* 1999). Females normally lay eggs on the undersides of leaves. Developmental times of TSM vary with conditions such as temperature, humidity, host plant, leaf age, and other factors. However, temperature is the most important factor that influences the rate at which mites develop (Osborne *et al.* 1999). Mites developed from egg to adult in an average of 7.6 d on lima bean (*Phaseolus lunatus* L. [Fabales: Fabaceae]) at 27°C and 90 le deutonym

association referred to as ‘guarding’. The male is attracted by the sex pheromone released by quiescent female deutonymphs and remains in the immediate vicinity of the quiescent deutonymph, ready to mate with the emergent female (Cone *et al.* 1971a, Cone *et al.* 1971b, Penman & Cone 1972, 1974). The life span of the adult female is divided into the preovipositional period and ovipositional period. The preovipositional period can last less than 0.5 d and as long as 3 d depending on temperature. The ovipositional
period can last from 10 d at 35ºC to 40 d at 15ºC (Sabelis 1981). The number of eggs produced per female per day and the total eggs laid per female in her life vary with temperature, host plant, relative humidity, and other factors (van de Vrie et al. 1972). Sabelis (1981) found that the peak oviposition was 161 eggs per female, which occurred at 25ºC, with the maximum rate of 12 eggs per female per day occurring 2 d after the first eggs were laid.

**General behaviour of *Tetranychus urticae***

As a *T. urticae* female settles on a host plant and begins to feed, it also commences to produce webbing. The eggs are deposited in or under the webbing. The emergent larvae and nymphs likewise produce webbing (Gerson 1985). The web can be used to protect the eggs, find a mate, and to disperse (Hussey & Parr 1963, Gerson 1985, Osborne et al. 1999).

As TSM is a polyphagous phytophagous spider mite, it may be difficult to find a specific biochemical entity that would elicit an attractant-type-of-response (Dabrowski & Rodriguez 1971). However, every plant species emits a specific mixture of volatiles, and thus phytophages may discriminate between host plants on the basis of these mixtures (Dicke 1986). Volatiles of strawberry have far-reaching (distant) effects on the behaviour of *T. urticae* (Dabrowski & Rodriguez 1971, Dabrowski et al. 1971, Rodriguez et al. 1971, Rodriguez et al. 1976, Hamilton-Kemp et al. 1988). Dicke (1986) also found that TSM were activated by plant volatiles and if they strayed out of the odour field they returned. These responses of TSM might be important in the location of suitable host plants.

**The general biology and behaviour of predatory mite *Phytoseiulus persimilis* Athias-Henriot**

**General biology of *Phytoseiulus persimilis***

The predacious mite *Phytoseiulus persimilis* specialises on *Tetranychus* spp. During the early 1960s, research on this species was conducted in Great Britain, Holland, Canada, and United States (Osborne et al. 1999). The life cycle of *P. persimilis*, which includes
The larva apparently does not feed and remains inactive unless disturbed. The protonymph and deutonymph feed on the egg, larva and protonymph of spider mites. The protonymph begins to search for food almost immediately after emerging, with an intermittent period of inactivity presumably due to satiation. The deutonymph feeds throughout most of its development. Adult mites begin to feed soon after moulting. All stages of spider mite are eaten by the adult female *P. persimilis* (Takafuji & Chant 1976, Osborne *et al.* 1999).

The number of prey consumed depends on the density and stage of prey and predator, temperature, and humidity (Shaw 1984, 1985). *P. persimilis* has a very low energy demand for maintenance processes and allocates most of its energy to egg production (Eveleigh & Chant 1981b, Sabelis 1985, Nwilene & Nachman 1996b).

*P. persimilis* has a relatively rapid development, 4-5 d from oviposition to adult (Huffaker *et al.* 1969). Sex ratio is approximately 4 females to one male (Laing 1968). The life span of the adult female is divided into the preovipositional period and ovipositional period. A female that has mated once can lay eggs throughout her life; whereas an unmated female will not reproduce (Laing 1968, Amano & Chant 1978a, b). The preovipositional period was determined to be 1.9 d at 20ºC by (Sabelis 1981). The rate of oviposition does not depend on the age of the female, but on the number of eggs previously laid. Eggs will be laid at a rate dependent on prevailing conditions until the maximum number is reached, or until the female dies from old age at about 50 d (Sabelis 1981). The most important conditions that influence the rate of oviposition are temperature, humidity and prey density (Osborne *et al.* 1999). The average number of eggs laid per female per day was 2.4 eggs at 20.3ºC (Laing 1968) and 5 at 30ºC (Sabelis 1981). Fecundity is also influenced by temperature. Maximum fecundity is 75 eggs per female, which occurs at approximately 26ºC with the optimal temperature range of 17-28ºC (McClanahan 1968, Sabelis 1981), but Takfuji and Chant (1976) reported that on average, *P. persimilis* produced 79 eggs per female.
General behaviour of *Phytoseiulus persimilis*

*P. persimilis* has high dispersal powers. Its distribution is highly correlated with that of its prey (Eveleigh & Chant 1982c, McMurtry 1982). The ability of *P. persimilis* to disperse and find new colonies of prey depends on the physical characteristics of the environment (Takafuji 1977), which include prey distribution and density, predator density, the duration of infestation or the amount of the spider-mite webbing present (Osborne *et al.* 1999). The dispersal of *P. persimilis* from leaf to leaf is hampered by trichomes on stems of tomato (van Haren *et al.* 1987).

Prey searching by phytoseiid predators can be divided into three phases: searching (1) spider-mite patches in the habitat, (2) spider-mite colonies within a patch and (3) spider mites within a colony (Sabelis & Dicke 1985, Dicke *et al.* 1998). They use herbivore-induced plant volatiles in long-range prey-habitat location and are arrested by these volatiles in a prey patch (Sabelis & Dicke 1985, Zemek & Nachman 1999, Pels & Sabelis 2000, van den Boom 2003, van den Boom *et al.* 2004, de Boer & Dicke 2005b). *P. persimilis* searches randomly within a prey patch until physical contact between predator and prey occurs (Jackson & Ford 1973, Sabelis 1981). The width of the searching path varies from 0.42 mm when mites are relatively stationary (moving their appendages but not walking) to 0.84 mm when actively walking (Sabelis 1981). The encounter rate with prey strongly depends on the walking speed and walking pattern (Sabelis 1981). Eggs of prey are apparently detected by the predator through contact with mechanoreceptors and chemoreceptors on its anterior tarsi. If the sensory input from the tarsi indicates the presence of a spider-mite prey, the predatory mite brings its mouthparts into contact with the egg, which it palpates with its pedipalps. Chemoreceptors on the pedipalps respond to a feeding stimulant on the outside of the spider mite egg. The predator then penetrates the shell of the egg with its chelicerae (Jackson & Ford 1973).

Searching behaviour comprises three parameters: (1) biological characteristics and abilities of species (Mori & Chant 1966b, Eveleigh & Chant 1981c, 1982c, Sabelis & Dicke 1985), (2) external environmental factors, such as prey density, humidity, and temperature (Mori & Chant 1966a, b, Eveleigh & Chant 1981c, 1982c), and (3) internal
physiological state, such as starvation (Mori & Chant 1966b, Sabelis & Dicke 1985, Bell 1990, van Rijn et al. 2005). The effects of leaf hairs on the searching efficiency and predation rate of adult female *P. persimilis* have been documented by Krips et al. (1999a). The webbing produced by TMS aids the searching predator in finding its prey. When webbing is contacted, the predator intensifies its search in the immediate area. The webbing appears to act as an arrestant for dispersing predators (Dong & Chant 1986, Osborne et al. 1999). Silk webbing of spider mites influences the walking activity, walking speed and walking pattern of predatory mites (Sabelis 1981, Eveleigh & Chant 1982c). Females were able to find prey twice as fast when webbing was present compared to when webbing was absent (Osborne et al. 1999). It is known that certain synthetic pesticides affect the development, survival (Ashihara et al. 1988), feeding behaviour (Jackson & Ford 1973), and oviposition and settling behaviour (Ashihara et al. 1988, Dong & Niu 1988, Dong 1990, Dong & Niu 1990, 1991, Dong et al. 1992) of *P. persimilis*.

**Petroleum-derived spray oils**

**Chemistry of petroleum-derived spray oils**

Contemporary use of PDSOs or distillates as agricultural crop protectants dates back to the 1800s, but it was not until the latter half of the 20th century that advances in petroleum chemistry allowed substantial modification and diversification in their commercial applicability (Agnello 2002). Contemporary HMOs and AMOs are refined from base oils in the lubricating oil fraction. They comprise of a mixture of isoalkanes (isoparaffins), cycloalkanes (cycloparaffins or naphthenes), and aromatic hydrocarbons (Agnello 2002, Kuhlmann & Jacques 2002). The pesticidal activity and risk of phytotoxicity are mostly related to the types of hydrocarbon molecules, carbon number, and the content of unsaturated molecules. Paraffinic molecules have higher pesticidal activity than naphthenic molecules (Pearce et al. 1948, Riehl & LaDue 1958, Jacques & Kuhlmann 2002). Unsaturated aromatic hydrocarbons pose a higher risk of phytotoxicity, particularly acute phytotoxicity, as they are more chemically reactive and easily photodegraded to toxic acids (Jacques & Kuhlmann 2002). The molecular weight and median equivalent *n*-paraffin carbon number (median *nCy*), which is related to the
median distillation temperature, are also critical for pesticidal activity and phototoxicity. Pesticidal efficacy increases as the median $nCy$ value increase to about $nC23$. On the other hand, chronic phytotoxicity increases with the median $nCy$ value, especially above a value of $nC27$ (Riehl & LaDue 1952, Jacques & Kuhlmann 2002). The effect of median $nCy$ on plant hazard and efficacy are shown in Figure 2.1.

![Figure 2.1. Effect of median $nCy$ values on plant hazard and efficacy (based on Jacques & Kuhlmann 2002).](image)

### Classification of petroleum-derived spray oils

Kuhlmann & Jacques (2002) proposed a three level classification for PDSOs: ‘mineral oil’ (MO), ‘agricultural mineral oil’ (AMO) and ‘horticultural mineral oil’ (HMO), in relation to increasing degrees of refinement. MOs have a higher level of refinement and safety than petroleum distillates, as they do not contain any toxic hydrocarbon molecules or other contaminants. The initial boiling point for MOs ($nC13$) is 232°C at atmospheric pressure (101.3 kPa), which constitutes a certain degree of crop safety and pesticidal efficacy by eliminating the very lightest oils that can cause acute phytotoxicity and offer only a very low level of pest control. Contemporary products that meet internationally recommended standards are known, as stated above, as AMOs and HMOs. All AMOs and HMOs must be paraffinic with % $C_P$ values $\geq$ 60% ($\geq$ 60% of carbon atoms must be in chains rather than in rings) and with unsulphonated residue (UR) values $\geq$ 92% ($\leq$ 8% unsaturated molecules). The difference between an HMO and AMO is that the range of equivalent $n$-paraffin carbon numbers between the 10% and 90% distillation points: for HMOs 5-6, and for AMOs is $> 6$. HMOs were, until 2002 (Beattie et al. 2002b; Kuhlmann & Jacques 2002), called narrow-range petroleum spray oils and AMOs were called broad-range petroleum spray oils. Common median $nCy$ values of HMOs are $nC21$ and $nC23$. For AMOs they are $nC23$, $nC24$ and $nC25$ (Beattie et al. 2002b).
Modes of action of petroleum-derived spray oils against arthropods

The most widely held theory of the mode of action of PDSOs against arthropods is that they act physically, by blocking the spiracles (Taverner 2002). However, there are, depending the characteristics of the oils, several theories.

The model of action of PDSOs was investigated from the early 1900s. Shafer (1911), Sen (1914) and Freeborn & Atsatt (1918) reported the fumigant action of kerosene. However, the theory applies to relatively volatile oils, such as kerosene, as the higher-molecular-weight fractions have lower volatilities and they show no apparent fumigant action (Moore & Graham 1918).

Moore & Graham (1918) observed penetration of kerosene emulsion into tracheae of red scale (Aonidiella aurantii (Maskell) [Hemiptera: Diapidae]). de Ong (1926) found that lubricating oil could enter the spiracles and penetrate into the tracheae of red scale and that this apparently resulted in suffocation. Many studies reported observations in consistent with anoxia (Ebeling 1945, Brown 1951, Davidson et al. 1991, Taverner 2002).

PDSOs may also cause nervous and cellular membrane disruption, which is usually associated with the unsaturated components of products or surfactants (Ebeling 1936, Brown 1951, Hassall 1982, Taverner 2002).

In addition to the suffocation, PDSOs can influence the behaviour of arthropods by preventing them from locating, feeding and ovipositing on hosts (Liu et al. 2006, Nguyen et al. 2007). The means by which oil deposits affect arthropod behaviours are poorly understood, as there has been limited research. For each species, impacts may involve one or more effects such as physical disruption of epicuticular lipids and the masking of oviposition stimulants (Eigenbrode & Espelie 1995), or the interference with host location due to the coating of olfactory receptor organs (Simons 1982, Nguyen 2004). Impacts of PDSOs on arthropod behaviour are now considered to be the most important mode of action (Beattie et al. 2002b).
Physiological effects of petroleum-derived spray oils on plants


The impact of sublethal doses of PDSOs on underlying plant physiology, particular membrane-dependent processes and plant growth regulators has been reviewed by Johnson et al. (2002). Both acute and chronic symptoms induced by PDSOs can be attributed to the stress response by the sprayed plants (Hodgkinson et al. 2002, Johnson et al. 2002, Tan 2004). Responses by plants to various stresses, for example, high temperatures, water deficiency, flooding, nitrogen deficiency, ozone and heavy metal are characterised by the temporary production of ameliorating proteins and enzymes, which are involved in defence, damage limitation, and recovery (Zhang & Davies 1989, Coutts et al. 1994, Faktor et al. 1997, Morange 1997, Johnson et al. 2002, Wu & Lin 2002, Vuorinen et al. 2004, Maksymiec et al. 2005). The phytotoxic factors, such as ozone, can activate various defence responses in plants. These include the expression of defence-related genes, the emission of ethylene and other plant volatiles, the biosynthesis of signalling molecules such as jasmonic acid, salicylic acid and ethylene (Kangasjarvi et al. 1994, Rao et al. 2000, Vuorinen et al. 2004). Accordingly, it has been suggested that the phytotoxic factors can potentially act as significant abiotic elicitors of direct and indirect defence systems of plants (Sandermann et al. 1998, Paolacci et al. 2001), which include induced emission of some of the same compounds induced by herbivore feeding (Vuorinen et al. 2004).
However, there is a general lack of data about the physiological effects of sublethal doses PDSOs on plants (Johnson et al. 2002), and subsequently on the tritrophic interaction between plant, herbivores and carnivores.

**Effects of petroleum spray oils on pests and natural enemies**

**Laboratory bioassay of the suffocant efficacy of petroleum spray oils**

Mortality caused through suffocation by PDSOs is the most direct effect on arthropods. Bioassay procedures and requirements were reviewed by Herron & Barchia (2002). There are usually two methods used to evaluate the toxicity of PDSOs in the laboratory. Dipping is a very convenient method to test the efficacy of oils. It is simple, requiring little, if any, sophisticated laboratory equipment (Herron & Barchia 2002). Several researchers have made direct comparisons between dipping, field efficacy and spraying. It was found that oils usually proved to be more efficacious when dipping was used than when sprays were applied. Dipping overestimates the efficacy of oils when extrapolated to practical field outcomes (Lawson & Weires 1991, Agnello et al. 1994, Liu & Stansly 1995b, c, Stansly & Liu 1997, Schuster & Stansly 2000, Stansly et al. 2002). Herron & Barchia (2002) suggested spraying rather than dipping should be the preferred method of evaluating HMOs and AMOs.

The Potter spray tower provides uniform spray coverage (Herron & Barchia 2002, Stansly et al. 2002). In the past decade, it has been used extensively for laboratory bioassay of HMOs and AMOs. Riedl et al (1995) used a Potter spray tower to evaluate three oils against eggs and larvae of codling moth (Cydia pomonella (L.) [Lepidoptera: Tortricidae]). It has been used to determine the efficacy of HMOs to citrus pests, green peach aphid (Myzus persicae (Sulzer) [Hemiptera: Aphididae]) (Herron et al. 1995, Herron et al. 1998a), silverleaf whitefly (Bemisia tabaci (Gennadius) [Hemiptera: Aleyrodidae]) (Liu & Stansly 1995b), broad mite (Polyphagotarsonemus latus (Banks) [Acari: Tarsonemidae]) (Herron et al. 1996), European red mite (Panonychus ulmi (Koch) [Acari: Tetranychidae]) (Agnello et al. 1994, Herron et al. 1998b), TSM (Herron et al. 1995, Herron et al. 1998b), tomato thrips (Frankliniella schultzei
(Trybom) [Thysanoptera: Thripidae]), tomato russet mite (Aceria lycopersici (Wolffenstein) [Acari: Eriophyidae]), greenhouse whitefly adults (Trialeurodes vaporariorum (Westwood) [Hemiptera: Aleyrodidae]), and common brown leafhopper (Orosius argentatus (Evans) [Hemiptera: Cicadellidae]) nymphs (Kallianpur et al. 2002) and cyclamen mite (Phytomenus pallidus (Banks) [Acari: Tarsonemidae]) (Spooner-Hart & Herron 2003).

Although PDSOs can control a wide range of pests, they also are toxic to beneficial organisms, particularly from direct contact with sprays (Davidson et al. 1991). Adult parasitoids such as Aphytis holoxanthus De Bach [Hymenoptera: Aphelinidae] and Encarsia pergandiella (Howard) [Hymenoptera: Encyrtidae] can become trapped and killed in fresh oil residues (Rosen 1967, Stansly & Liu 1997). For coccinellids (Nephaspis oculatus (Blatchley) [Coleoptera: Coccinellidae]) and the lacewings, Ceraeochrysa cubuna (Hagen) and Chrysoperla rufilabris (Burmeister) [Neuroptera: Chrysopidae], eggs appear to be the most sensitive stage (Liu & Stansly 1996, Stansly et al. 2002). In these respects though, mineral oils have important advantages of selectivity over synthetic pesticides (Stansly et al. 2002).

Herron & Barchia (2002) suggested that large objects that may obstruct the spray tube should not be placed on the Potter spray tower spray stage, and that to increase the aqueous spray deposit a coarse spray nozzle should be used in preference to a fine spray nozzle when using the Potter spray tower with oils. The optimum amount of diluent to be used in a Potter spray tower should be at least 5 mL and applied at a low pressure of about 45 kPa (Herron & Barchia 2002). The susceptibility of TSM to HMOs was significantly lower than that of Panonychus ulmi and P. citri (McGregor). The difference in susceptibility may be related to the variation in the sizes and distribution of the dorsal setae and the structure and function of their respiratory system (Herron et al. 1995, Herron et al. 1998b).

High heterogeneity is a consistent and significant problem in mineral oil bioassay data (Barchia et al. 2003). There were a number of instances where excessive variance of heterogeneity was evident in the probit analysis of PDSO efficacy data (Riehl & LaDue...
From the late 1940s, many studies (Pearce et al. 1948, Riehl & LaDue 1958, Trammel 1965, Herron et al. 1995, Herron et al. 1998b) compared the efficacy at the LD$_{95}$ level. However, because variance at LD$_{95}$ is higher than at LD$_{50}$, a 50% level estimate is usually more precise than those at extreme percentage levels (Finney 1971, Copenhaver & Mielke 1977). Experimental designs with doses symmetrically spaced around the 50% response level are not suitable for precise estimation of the extreme percentage level (Robertson et al. 1984).

**Behavioural effects of petroleum-derived spray oils on pests**

The physical repellency, antifeeding and oviposition deterrence of PDSOs are important in reducing infestation. Behavioural effects were first noticed in the 1910s when crude oil was used with nicotine sulphate for control of citrus leafminer (*Phyllocnistis citrella* Stainton [Lepidoptera: Gracillariidae]) (Beattie et al. 1995). Despite subsequent reports of behavioural efforts on citrus leafminer, red scale, and aphids (Dammerman 1929, Ebeling 1936, 1950) the potential for using mineral oils to alter the behaviour of a broad range of pests was overlooked for decades as focus shifted to the use of synthetic organic toxins to kill pests. In the early 1990s, behavioural effects of oil deposits on citrus leafminer were re-discovered (Beattie & Smith 1997).

From the late 1970s, behaviour effects, especially repellency and oviposition deterrence of PDSOs on a wide range of pests were reported, including weevils such as citrus root weevil (*Diaprepes abbreviatus* (L.) [Coleoptera: Curculionidae]) (Schroeder 1977, Schroeder & Green 1983), pear psylla (*Cacopsylla pyricola* Foerster [Hemiptera: Psyllidae]) (Zwick & Westigard 1978), citrus psylla (*Diaphorina citri* Kuwayama
Hemiptera: Psyllidae]) (Rae et al. 1997), whiteflies (Larew 1988, Larew & Locke 1990, Liu & Stansly 1995a, c, Liang & Liu 2002, Wu et al. 2002, Xue et al. 2002a), citrus leafminer (Beattie et al. 1995, Rae et al. 1996), codling moth (Riedl et al. 1995), Helicoverpa sp. [Lepidoptera: Noctuidae] (Mensah et al. 1995), white apple leafhopper (Typhlocyba pomaria McAtee [Homoptera: Cicadellidae]) (Fernandez et al. 2001), tomato thrips (Xue et al. 2002b), greenhouse thrips (Heliothrips haemorrhoidalis (Bouché) [Thysanoptera: Thripidae]) (Liu et al. 2002b), TSM (Liu & Beattie 2002), citrus red mite (Cen et al. 2002), Queensland fruit fly (Bactrocera tryoni (Froggatt) [Diptera: Tephritidae]) (Liu et al. 2002a), asiatic apple leafminer (Phyllonorycter ringoniella (Matsumura) [Lepidoptera: Gracillariidae]) (Sun 2002). Anti-feeding also was recorded in greenhouse thrips (Liu et al. 2002b) and citrus red mite (Cen et al. 2002). Behavioural effects of AMO and HMO deposits on feeding and oviposition of a wider range of pests are now recognised as more important for the control of pests than mortality caused by anoxia (Beattie et al. 2002b). In most cases research suggests that oil deposits interfere with the oviposition site selection by adult female pests through repellent or deterrent effects.

PDSOs can also be used for control of plant diseases indirectly through control of vectors. They can reduce virus transmission from aphids by lowering the chance for acquisition of aphids or deterring aphids from probing or feeding (Bradley et al. 1963, Zschiegner et al. 1972, Lehmann et al. 1975). Electronic monitoring of probing and feeding by adult green peach aphid has shown that mineral oils delay stylet penetration and reduce virus transmission (Simons et al. 1977, Powell 1992). Video-monitored stylet penetration of green peach aphid has also shown that applications of oil to PYV-infected leaves significantly delayed the initiation of stylet penetration by aphids (Powell et al. 1998). Mineral oil interferes with retention of virions on aphid vector stylets (Wang & Pirone 1996). They can be used to reduce transmission of tospovirus- and phytoplasma-associated diseases by affecting the behaviour of vector species, such as the western flower thrips and leafhopper (Clift et al. 2002).

However, there is little research for the behavioural mechanism of PDSOs. Liu et al. (2001) reported the relationship between the oviposition by citrus leafminer and PDSO
fractions. An \( nC25 \) fraction was more effective than \( nC17 \) or \( nC22 \) fractions (Liu et al. 2001). In other studies, Liu et al. (2002a) reported that an \( nC20-22 \) fraction from an \( nC23 \) horticultural mineral oil significantly reduced oviposition by Queensland fruit fly. They speculated that this may have been related to the presence of molecules comprising a single 5- or 6- membered di- or tri-substituted ring and isoparaffinic side-chains totalling 16 carbon atoms in length.

There are several methods for evaluating feeding behaviour of piercing and sucking arthropod pests. Feeding scars that develop on the death of cells are the most direct evidence of feeding by spider mites. Leaf damage indexes have been developed to quantify the amount of feeding in field trials (Hussey & Parr 1963, French et al. 1976). Feeding scars have been used to evaluate feeding behaviour under laboratory conditions (Hall & Thacker 1993).

The probing behaviour of aphids can be monitored by use of an electronic monitoring system (McLean & Weigt 1968, Hardie et al. 1992, Reese et al. 1994). For this procedure, each adult apterous aphid was attached to a gold wire, with colloidal silver as adhesive on its dorsal side. The tethered aphid was then placed in the centre of the adaxial surface of the expanded leaf and left to feed. Current was conducted to the soil by a long copper rod inserted near the root base and attached to the output wire of the monitor. When the aphid inserted its mouthparts, the circuit was completed, and the waveforms produced were recorded and analysed (Webster et al. 1993, Brewer & Webster 2001). Tiny pests such as mites are probably too small for such methods to be used.

Video cameras have been successfully used to record the penetration of host plant surfaces by aphid stylets (Hardie et al. 1992, Powell et al. 1998). Antennal and body movements can be used as clues to indicate if an aphid has initiated and completed a stylet penetration (Hardie et al. 1992, Powell et al. 1993). The feeding behaviour of adult females of \( T. urticae \) also can be observed with a video camera and TV monitor (Bancroft & Margolies 1996, Maeda et al. 2000a). Feeding and non-feeding durations can be recorded. Feeding can be distinguished from non-feeding by observation of the
movement of fluids through the curtile of the mite (Bancroft & Margolies 1996, Maeda et al. 2000a).

**Behavioural effects of petroleum-derived spray oils on natural enemies**

Although there are many reports of behavioural effects of synthetic pesticides on pests (Haynes 1988), there is only very limited knowledge relating to the effects of PDSOs on beneficial arthropods. Jackson & Ford (1973) found that some pesticides had marked repellent effects on *P. persimilis*. The residues of pesticides on the eggs of prey affected acceptance by the predator. Pesticides also affected oviposition and settlement behaviour of predators (Dong & Niu 1988, Dong 1990, Dong & Niu 1990, 1991, Dong et al. 1992, Blackwood et al. 2001). Understanding the behavioural effects of PDSOs on natural enemies and the behavioural mechanism would be important.

Searching behaviour of parasitoids and predators are important research subjects for understanding prey-carnivore interactions. Traditionally, the behaviour of carnivores was recorded in either a manual or a semi-automated way (Jackson & Ford 1973, Eveleigh & Chant 1981c, 1982c, Bancroft & Margolies 1996, Momen 1999). With advances in computer technology, versatile automated tracking systems have been developed for behavioural experiments (Varley et al. 1994, Noldus et al. 2001). They have been used to study the searching behaviour of natural enemies (Sutterlin & van Lenteren 1997, Krips et al. 1999a, Rott & Ponsonby 2000, Choudhury & Copland 2003).

**Functional and numerical responses of predator to prey**

Understanding the predator-prey interactions is essential for the successful development of biological control programs. Many of the critical aspects of predator and prey interactions can be discerned by examining the relationship between predator feeding behaviour and the density of their prey (Livdahl & Stiven 1983). If the components of this relationship can be estimated, the parameters of the resulting equation can be applied to predictions about the dynamics of predator-prey associations (Hassell 1978), and should provide a succinct summary of mutual adaptations of predators and their prey, and as such useful tools in comparative studies of coevolution (Livdahl 1979).
Solomon (1949) separated the responses of consumers to the density of their resources into functional and numerical responses, with the former describing how the consumption rate of individual consumers changes with respect to resource density and the latter how the per-capita reproductive rate changes with resource density. The term *behavioural response* may be more appropriate than functional response because it describes the hunting and attack behaviour of the consumer, in contrast to the reproductive (numerical) response of the population (Berryman 1998).

**Functional responses of predator to prey**

Holling (1959a) identified three basic types of functional responses to prey density in general: Type I (linear) functional response in which the attack rate (attack rate = number killed/prey density) of the individual consumer increases linearly with prey density but then suddenly reaches a constant value when the consumer is satiated (Figure 2.2). This model is often found to be biologically unrealistic, yet it remains fundamental to predator-prey co-evolutionary theory (Tully *et al.* 2005).

Type II (cyrtoid) functional response in which the attack rate increases at a decreasing rate with prey density until it becomes constant at satiation (Figure 2.3). The type II responses are typical of specialist predators.

Type III (sigmoid) functional response in which the attack rate accelerates at first and then decelerates towards satiation (Figure 2.4). Type III functional responses are typical of generalist natural enemies which readily switch from one food species to another and/or which concentrate their feeding in areas where certain resources are most abundant (Berryman 1998).
Figure 2.2. Holling’s type I functional response (A: relationship between prey consumed per prey and prey density; B: relationship between attack rate and prey density).
Figure 2.3. Holling’s type II functional response (A: relationship between prey consumed per prey and prey density; B: relationship between attack rate and prey density).
Although arthropods have type I and III responses, the type II response is observed most frequently (Begon *et al.* 1996). Holling (1959b) derived a mathematical model for the type II functional response from experiments in which blindfolded people acted as predators by searching a table top with their finger tips for sandpaper disk prey, the so-called ‘disk equation’. This model illustrates the principal of time budget in behavioural
ecology. It assumes that a predator spends its time in two kinds of activities: searching for prey and prey handling, which includes chasing, killing, eating and digesting. The equation is given by:

\[ N_a = aTNP/(1 + aT_hN) \]

The number of prey attacked \((N_a)\) is a function of prey density \((N)\), the total available searching time \((T)\), predator density \((P, \text{ usually one in experiment})\), and instantaneous rate of discovery or attack coefficient \((a)\), and the handling time per prey item \((T_h)\). If \(P = 1\) in experiment and \(T = 1\) day the equation would be:

\[ N_a = aN/(1 + aT_hN) \]

To provide better parameter estimate the equation was transformed by Dowd & Riggs (1965) to:

\[ N/N_a = 1/a + T_hN \]

the so called Woolf transformation.

Livdahl & Stiven (1983) used the following transformation:

\[ 1/N_a = T_h + 1/(aN) \]

However, the comparison studies on the linear transformation and non-linear regression above indicated that the random predator equation overestimates the value of \(a\) and \(T_h\), whereas Holling’s disc equation and the Livdahl & Stiven (1983) model underestimated the parameters (Badii et al. 1999). When error variances are proportional to the number of prey attacked, the Woolf transformation not only linearises Holling’s disc equation, but also reduces the variance heterogeneity, thus providing the most reliable parameter among the transformations based on the disc equation. When error variance is constant, non-linear regression provides the best estimates (Fan & Petitt 1994, Badii et al. 1999).

Royama (1971) and Rogers (1972) noted that the disc equation is an instantaneous equation and is unsatisfactory for experimental data when an appreciable amount of time is involved in the experiment. Integrating over the rate of change of prey density,
they adapted the disc equation for the description of experimental data (Livdahl & Stiven 1983):

$$N_a = N \{1 - \exp[-a(1 - T_h N_a)] \}$$

This model is more appropriate than Holling’s disc equation, where dead prey are not replaced during trials and trail duration is relatively long leading to a significant reduction in prey density over time (Houck & Strauss 1985, Watson et al. 2000). This model has become known as the ‘random predator equation’.

However, Fan & Petitt (1994, 1997) found that Holling’s disc equation has been used most commonly to describe the type II functional response, although criticised by Royama (1971) and Rogers (1972). Even Rogers (1972) acknowledged that it is possible to use the two equations to describe the same experimental results, although when this is done different estimates of the attack coefficient and handling time are obtained. This is because the disc equation assumes that, for example, the predator searched systematically for its prey and did not waste any effort in re-searching part of the area, whilst the random predator equation assumes that the predator searched at random. Thus the instantaneous rate of discovery ($a$) calculated from the random predator equation is greater than those calculated from the disc equation.

William & Juliano (1996) suggested that the use of the mean in a regression can severely underestimated the confidence intervals of the estimates. Fan & Petitt (1994, 1997) argued that as the heterogeneity is typical in functional response data, the mean values should be used in parameterisation of the equation.

**Numerical responses of predator to prey**

For insect parasites, the rate of increase of the parasite population can usually be regarded as a linear function of the number of hosts attacked, the powerful and apparently very general conclusions rest on the simplest of assumptions namely that each host attacked gives rise to a constant number of adult parasites in the next generation (Hassell & May 1973). So the numerical response of insect parasitoids is generally analysed by plotting the percentage of parasitism versus host density. With
predators, it may be evaluated by regressing log density against log density of its prey. The regression slope expressed the ‘strength’ of the response by a given predator (Izraylevick et al. 1996, Cedola et al. 2001).

Reproduction rate of predators naturally depends on their predation rate. The more prey consumed, the more energy the predator can allocate for reproduction. Beddington et al. (1976) described an equation to model the relationship between food ingested and fecundity:

\[ F = \left( \frac{\lambda}{e} \right) (N_a - c) \]

Where \( F \) is fecundity; \( \lambda \) and \( c \) are constants; \( e \) is the mean biomass per egg; \( N_a \) is the prey consumed.

This equation can be rewritten:

\[ F = E_1 N_a - E_0 \]

Where \( E_1 \) is constant related to the predator’s efficiency in converting prey into predator eggs, biomass per egg and biomass per prey consumed. \( E_0 \) is the metabolic cost per time unit measured in terms of reduced fecundity.

By combining the equation with the disc equation, an expression for the predator reproductive rate in terms of prey density can be defined as:

\[ F = E_1 aN/(1 + aT_h N) - E_0 \]

**Functional and numerical responses of phytoseiid predators to prey**

Phytoseiid predators need little food for maintenance processes and allocate most of their energy to egg production. Consequently, the rate of food intake appears to be related to ovipositional rate in a remarkably linear pattern, except at very low prey densities when food intake does not meet the demands for maintenance energy. Numerical response curves thus reflect in a straightforward way the functional response curves (Sabelis 1985).

The functional and numerical responses of phytoseiid mites to the density of tetranychid mites has been investigated by several authors (Chant 1961, Mori & Chant 1966b,

Mori & Chant (1966a) and Takafuji & Chant (1976) reported a dome-shaped curve (type IV) in the functional response of *P. persimilis* to adult female *T. urticae* and of *Iphiseius degenerans* (Berlese) (Acari: Phytoseiidae) to adult female *Tetranychus pacificus* McGregor, respectively. They attributed the decrease in consumption rate at high prey densities to the disturbance of other moving prey colliding with the predator. Type II response was obtained for the same species as above by several authors (Laing & Osborn 1974, Fernando & Hassell 1980, Eveleigh & Chant 1981a, 1982a, Ryoo 1996).

The role of herbivore-induced plant volatiles in tritrophic interaction of plant-spider mite-predatory mite

Arthropods live in a chemical world. Food webs are overlaid with infochemical webs that mediate direct and indirect interactions between the plant-herbivore-carnivores (Dicke & Loon 2000, Dicke & Grostal 2001). Foraging herbivorous and carnivorous arthropods employ chemical information both prior to and after physical contact with their food (Vet & Dicke 1992, Dicke & Loon 2000). Predators of herbivores function within a multitrophic context, their physiology and behaviour are influenced by elements from other trophic levels such as their herbivore prey (second trophic level) and its plant food (first trophic level) (Price et al. 1980). Natural enemies base their foraging decisions on information from these different trophic levels, in which the chemical information plays an important role (Vet & Dicke 1992). The importance of herbivore-induced volatiles as foraging cues for natural enemies has been reported in many systems consisting of plants, herbivores, and carnivorous natural enemies (Dicke 1986, Dicke et al. 1990a, c, Lewis & Martin 1990, Vet & Dicke 1992, Pels & Sabelis 2000, Dicke & Grostal 2001, van de Boom et al. 2004, de Boer & Dicke 2005b).

The response of host-plants to herbivore damage

Plants respond to herbivore feeding damage by emitting volatiles that attract natural enemies that in turn attack the herbivore (de Moraes et al. 1998, Pare & Tumlinson 1999, Kessler & Baldwin 2001, van den Boom 2003, de Boer & Dicke 2005b), a process that is called indirect defence (Agrawal et al. 2002, Dicke et al. 2003b). When plants were infested by herbivores, they activate their indirect defences (volatile production) to complement the direct defence response against herbivores (Kant et al. 2004). Spider mite-induced synthase activity has been reported (Bouwmeester et al. 1999). Genes involved in spider mite-induced volatile formation have been discovered in some plant species (Arimura et al. 2000, Ament et al. 2004, Arimura et al. 2004, Kant et al. 2004, Mercke et al. 2004). Transgenic plants that emit some components of the volatiles have been developed and these genetic modified plants attracted ‘bodyguards’ in the form of carnivorous predatory mites (Kappers et al. 2005). The
influence of plants on the interactions between herbivores and natural enemies has been reviewed by Price et al. (1980).

The production of predator-attracting info-chemicals was thought to occur systemically throughout the spider mite-infested plant (Bruin & Sabelis 1989, Dicke et al. 1990a, Dicke et al. 1993b, Dicke 1994). The spider mite-infested plants may interact with undamaged neighbouring intraspecific plants (Bruin & Sabelis 1989, Bruin et al. 1992, Horiuchi et al. 2003a) or interspecific plants (Bruin & Sabelis 2001) through chemical information exchanged in the air or in the soil (Dicke & Dijkman 2001). The plants exposed to herbivore-induced conspecific plant volatiles can also produce similar volatiles and attract carnivores (Horiuchi et al. 2003a). Choh et al. (2004) found that the responses of neighbour plants have passive adsorption and active production. The mechanism involved in such interactions has been studied at the molecular level (Arimura et al. 2000).

The composition of herbivore-induced volatiles varies among different plant species or cultivars. The plants are more important than the herbivore in affecting the composition of the volatile blends (Takabayashi et al. 1991, Takabayashi et al. 1994a). Plant species differ in their response to herbivores and the bouquets emitted differ qualitatively in several to many compounds (Dicke et al. 1990a, Takabayashi et al. 1991, van den Boom 2003, van de Boom et al. 2004). Predatory mites discriminate between the volatile mixtures associated with different plant species infested by the same herbivore (Dicke et al. 1991). However, plants that have a strong direct defence do not emit induced volatiles that attract predators. This infers that the combination of direct and indirect defences is to some extent compatible in plant species (van den Boom et al. 2002). Furthermore, the mixture emitted can vary with plant genotype (Dicke et al. 1991, Takabayashi et al. 1991, Krips et al. 2001, Llusia & Penuelas 2001). Variation between genotypes seems to be mostly quantitative (Takabayashi et al. 1991). These differences can also lead to differences in the degree of attraction of phytoseiid mites. Thus herbivore-induced plant volatiles contain information about the plant species or genotype that is damaged (Dicke et al. 1998). To date, volatiles released upon T.
urticae-infestation from at least 16 plant species from 8 plant families have been analysed (de Boer & Dicke 2005b).

The emission of herbivore-induced volatiles may also vary depending on leaf age (Takabayashi et al. 1994b, Gnanvossou et al. 2003), the herbivore species (Dicke et al. 1998, Takabayashi et al. 2000), and abiotic factors, such as, light, time of year and water stress (Takabayashi et al. 1994a, Maeda et al. 2000a).

The application of jasmonic acid (JA) induces a volatile blend that is similar, but not identical, to that emitted by spider mite-infested plants (Dicke et al. 1999, Gols et al. 1999, Horiuchi et al. 2001, Gols et al. 2003). Ozone exposure triggered emission of herbivore-induced plant volatiles in lima beans, but it did not disturb tritrophic signalling (Vuorinen et al. 2004). This suggested that plant defence against phytotoxic ozone and the production of herbivore-induced volatiles for attraction of natural enemies may have been related to adaptive coevolution (Vuorinen et al. 2004).

**Composition of spider mite-induced volatiles**

Chemical analyses of the headspace composition of uninfested and spider mite-infested plants showed qualitative and quantitative differences. Spider-mite infested lima bean leaves emit relatively large amounts of terpenoids, (E)-β–ocimene, (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), linalool and phenolic compound, methyl salicylate (MeSA) (Figure 2.5), but uninfested and mechanically damaged plants do not. Four of the compounds, (E)-β–ocimene, linalool, DMNT and methyl salicylate were found to be preferred by predatory mites (Dicke et al. 1990a). In addition, the oximes 2-methylbutanal-O-methoxyoxime, 3-methylbutanal-O-methoxyoxime and an unknown oxime were detected in the headspace of both old and young infested cucumber (Cucumis sativus L. [Violales: Cucurbitaceae]) leaves. 3-methyl-butanal-O-methoxyoxime and the unknown oxime were much more abundant in the headspace of infested old cucumber leaves. They may mask the predators attractants emitted by old cucumber leaves (Takabayashi et al. 1994b).
The composition of volatiles varies qualitatively and quantitatively depending on the different plant-herbivore combination and abiotic factors (Takabayashi et al. 1991, Takabayashi et al. 1994b, Takabayashi et al. 2000, Sun & Yin 2002, Dicke et al. 2003a). Although the composition of volatiles was affected by both the herbivore and plant, the plants were more important than the herbivore in affecting the composition of the volatile blends (Takabayashi et al. 1991).

JA is a key regulator of spider mite-induced volatiles (Ament et al. 2004). Application of JA induces a volatile blend that is similar to that emitted by spider mite-infested plants (Dicke et al. 1999, Gols et al. 2003). The volatile blends induced by JA are MeSA-free. Although the volatile blend attracts *P. persimilis*, adding synthetic MeSA to MeSA-free blend significantly increased the mites' choice for this odour (de Boer & Dicke 2004b).

*P. persimilis* uses MeSA and TMTT to discriminate between volatile blends from lima bean induced by *T. urticae* or the nonprey caterpillar *Spodoptera exigua* (Hübner)
(Lepidoptera: Noctuidae) (de Boer et al. 2004). MeSA has been identified as an attractant for predatory arthropods, such as, anthocorid bugs (Hemiptera: Anthocoridae), predatory mites, and a green lacewing (Dicke et al. 1990a, Scutareanu et al. 1997, Shimoda et al. 2002, James 2003, de Boer & Dicke 2004b).

The indirect defences of plants can be manipulated in agricultural systems (Kessler & Baldwin 2001). MeSA can be a useful tool to manipulate the behaviour of herbivore natural enemies as a method of biological control against herbivorous pests in agroecosystems (Shimoda et al. 2002). Field evaluation of MeSA for enhancing the effectiveness of carnivorous natural enemies has been carried out (James 2003, James & Price 2004, James & Castle 2005). The use of controlled-release MeSA in field crops can increase the recruitment and residency of populations of certain beneficial arthropods. This phenomenon has the potential to enhance the efficacy and reliability of conservation biological control in crop pest management (James & Price 2004, James & Castle 2005).

**Response of predatory mites to herbivore-induced plant volatiles**

Damage by herbivorous spider mites induces plants to produce volatiles that attract predatory mites that consume the spider mite. A clear attraction of *P. persimilis* to volatiles from lima bean infested with *T. urticae* has been consistently reported for over 20 years (Dicke 1986, Takabayashi & Dicke 1992, Garms et al. 1998, Arimura et al. 2000, Ozawa et al. 2000, Choh et al. 2004, de Boer & Dicke 2004b, de Boer et al. 2005, Shimoda et al. 2005). Phytoseiid mites use herbivore-induced plant volatiles in long-range prey-habitat location (Sabelis & van de Baan 1983) and are arrested by these volatiles in a prey patch (Margolies et al. 1997). The responses of predatory mites to these volatiles are considered to be an important factor in the local extermination of prey populations by phytoseiids (Dicke et al. 1998).

The response of the predators to herbivore-induced plant volatiles from a specific plant-herbivore combination varies with genetic variation of predatory mite populations (Margolies et al. 1997, Maeda et al. 1999, 2001). Different species of predatory mites respond to odours differently and the response to the volatiles is correlated to the
predator's ability to control populations of the prey species. Janssen et al. (1990) tried to preselect predatory mites with the aid of olfactometer experiments, and hoped that this would increase the probability of finding an efficient natural enemy.

Causal factors such as starvation (Sabelis & van de Baan 1983, Dong & Chant 1986, Dicke 1988), nutrient deficiency (Dicke 1988), pathogen infestation (Dicke et al. 2000), experience (Dicke et al. 1990b, Takabayashi & Dicke 1992, Takabayashi et al. 1994a, Krips et al. 1999b, Drukker et al. 2000) and feeding history (Takabayashi & Dicke 1992, Koveos & Broufas 1999, Maeda et al. 2000b, de Boer & Dicke 2004a) also contribute to variations in the response of predators to volatiles. Usually predators reared on a specific plant species prefer volatiles from this species. However, the ability *P. persimilis* to learn allows them to respond to the volatiles from different plants species in an environment where their prey can feed on different plant species (de Boer et al. 2005).

The presence of competitors (Janssen et al. 1997, Dicke et al. 1998, Janssen et al. 1998, 1999) and volatiles from the feeding activity of non-prey also affect olfactory responses of *P. persimilis* (Shimoda & Dicke 1999, 2000, Horiuchi et al. 2003b). Janssen et al. (1997) found that *P. persimilis* can release an odour that elicits prey to produce another odour, which subsequently causes an avoidance response by the predator to a prey patch containing conspecifics.

**Response of TSM to herbivore-induced plant volatiles**

It appears that not only do the potential natural enemies of primary herbivore pests take advantage of plant-released volatiles, but that secondary herbivores may be using these cues as well. *T. urticae* showed an avoidance response to *T. urticae*-infested bean leaves in a vertical airflow olfactometer (Dicke 1986, Dicke et al. 1991). Bean plants infested with *T. urticae* produce a terpinoid, linalool, which is thought to cause the dispersal of *T. urticae* (Dicke et al. 1993a). *T. urticae* probably recognises infochemicals (kairomones) from its predators or cues from other injured spider mites and consequently avoids feeding or ovipositing in areas exposed to these cues (Groстал & Dicke 1999).
However, Y-tube olfactometer experiments showed that *T. urticae* is slightly attracted by the odour of cucumber infested with conspecifics and more mites were found on plants infested with conspecifics than on clean plants in greenhouse release experiments (Pallini et al. 1997). Similar positive olfactory responses of *T. urticae* to slightly infested lima bean leaves has recently been reported by Horiuchi et al. (2003b). The lima bean leaves exposed to *T. urticae*-induced conspecific plant volatiles attracted *T. urticae* in addition to the predator (Horiuchi et al. 2003a). Gols et al. (2003) found that the presence of herbivore-induced chemicals and/or spider mite products enhanced the settlement of additional spider mites. Horiuchi et al. (2003a,b) assumed that volatiles from slightly infested leaves might be a signal for *T. urticae*, indicating available food resources and a patch where predators were not present as the slightly infested leaves did not attract the predators.

Odour mediated the response of phytophagous mites to heterospecific competitors in addition to conspecifics. *T. urticae* strongly avoided the plants with western flower thrips (*Frankliniella occidentalis* (Pergande) [Thysanoptera: Thripidae]) (Pallini et al. 1997). TSM avoids plants with predators (Pallini et al. 1999). However, the possible source of the odour has not been determined, which may be from the predator or alerted conspecific (the alarm pheromone) or both of them (Pallini et al. 1997, Pallini et al. 1999).

**Bioassay techniques for the volatiles from herbivore-induced plants**

**Y-tube olfactometer:** Y-tube olfactometers are extensively used for evaluating the attraction or repellence of chemicals in pesticide sciences and chemical ecology. The Y-tube was developed for comparing the attraction response of predatory mites to spider mite-induced plant volatiles in 1983 (Figure 2.6) (Sabelis & van de Baan 1983). It was modified by adding an air filter of activated charcoal (Figure 2.7) (Takabayashi & Dicke 1992). It has been extensively used for the herbivore-induced plant volatiles (Janssen et al. 1990, Dicke et al. 1993a, Koveos & Broufas 1999, Shimoda & Dicke 2000, Maeda & Takabayashi 2001, van den Boom et al. 2002, de Boer & Dicke 2004b, Shimoda et al. 2005).
**Figure 2.5.** Y-tube olfactometer (Sabelis and van de Baan 1983) (picture from (Jervis 2005)).

**Figure 2.6.** Modified version of Y-tube olfactometer (Takabayashi & Dicke 1992).

**Vertical airflow olfactometer:** The vertical airflow olfactometer can be used to study the orientation behaviour of predatory mites to steep gradients of a volatile and to wind direction. Orientation to wind direction is eliminated by using an olfactometer in which an air stream approaching the predator from below a gauze screen, upon which the
A steep gradient of odour is obtained by putting a cylinder filled with prey-infested leaves vertically below the screen (Sabelis et al. 1984).

**Wind tunnel:** Wind tunnels are used to study the flying behaviour and orientation to wind direction of insects. Sabelis & van der Weel (1993) found that well-fed *P. persimilis* moved upwind in the presence of *T. urticae*-induced volatiles. Starved predators always moved upwind (Sabelis & van der Weel 1993). Wind tunnels are also used to test the plant-plant information transfer (Bruin & Sabelis 2001).

**Greenhouse release-recapture (multi-plant set-up):** Tests are conducted in an open system with whole plants. Odour mixing in this apparatus is not as controlled as in the closed-system olfactometer, and is based on diffusion from neighbouring plants rather than from odours from infested plants. The method can be considered as a semi-field procedure (Pallini et al. 1997, Janssen et al. 1999, Zemek & Nachman 1999, Agrawal et al. 2002, Dicke et al. 2003a).

**Collection and identification of herbivore-induced volatiles**

Historically, volatiles have been extracted from plants by solvent extraction or steam distillation. These methods have the advantage in that they provide relatively large quantities of material to work with, and the extraction is relatively complete. However they have a number of major disadvantages. For instance, extracts are complex mixtures of volatile and non-volatile compounds and numerous fractionation steps may be required to isolate pure compounds; the profile of volatiles obtained is often not representative of blend released by the intact living organism (Millar & Sims 1998).

Because of these drawbacks, most recent work has focused on collection of volatiles emitted into the airspace around a living organism (Takabayashi et al. 1991, Dicke et al. 1999, Krips et al. 2001, Agrawal et al. 2002, Mercke et al. 2004, Kappers et al. 2005). A number of methods have been designed for this purpose.

Direct headspace sampling is the simplest sampling method. But this method is practical only for compounds whose concentration and vapour pressure are sufficient to provide at least nanogram quantities in the sampling aliquot (Millar & Sims 1998).
Thermal desorbing is often used for the collection of spider mite-induced volatiles (Takabayashi et al. 1994b, Dicke et al. 1999, Agrawal et al. 2002, Choh et al. 2004). Volatiles are absorbed by thermal adsorbents such as Tenax, Porapak Q or activated charcoal, and then released by thermo-desorption with a carrier gas and subsequently cryofocused in a thermal desorption cold trap injector. The cold trap injector is heated and the volatiles are transferred onto the column of a gas chromatograph (GC) or a gas chromatograph-mass spectrometer (GC-MS) (Takabayashi et al. 1991, Arimura et al. 2004, van den Boom et al. 2004, Vuorinen et al. 2004, Shimoda et al. 2005).

The volatiles absorbed by adsorbents also can be eluted with solvent, and then injected into the injection port of GC or GC-MS. However, this method requires internal standards, as sampling losses can occur during the sample preparation steps prior to analysis (Arimura et al. 2000, Horiuchi et al. 2003b, Ament et al. 2004, Choh et al. 2004, Kant et al. 2004).

Solid phase microextraction (SPME) is a rapid, solvent-free sample preparation technique developed for extracting organic pollutants from water bodies (Louch et al. 1992, Schäfer et al. 1995, Zabaras 2003). The sampling technique involves trapping volatiles on absorbent-coated fibres followed by thermal desorption of the volatiles by insertion of the fibre directly into a GC injector. Since its commercial release, SPME has become a very popular technique employed by scientists (Yang & Peppard 1994, Pawliszyn 1997, Millar & Sims 1998, Zabaras et al. 1999, Maeda & Takabayashi 2001, Zabaras 2003). However the absorbent SPME reportedly does not reflect the original blend ratio of volatiles exactly (Agelopoulos & Pickett 1998).

Methods for data analysis

In conventional PDSO anoxia bioassays we are concerned with how much PDSO is required to kill 50% of a pest population. Probit analysis is commonly used to express the relationship between the mortality and the PDSO dose (Finney 1971, Robertson 1992, Herron et al. 1995, Herron et al. 1998b).

Analysis of variance (ANOVA) is used to determine significance differences between three or more treatments. When there are data from two treatments that are related or matched in some way, paired $t$-test is used. When there are data from two different samples or treatments that receive different levels of an independent variable, the independent-samples $t$-test can be used. When there are data from three or more different samples or treatments that receive different levels of an independent variable, one-way ANOVA can be used. A significant overall ($F$) for three or more different samples indicates there is a difference somewhere among the treatments. In order to find out exactly which treatments differ from which other treatments post hoc comparisons must be conducted (Horiuchi et al. 2003b, Kant et al. 2004, Hills 2005).

ANOVA is one of the parametric statistics, which depend on the assumption that scores of the dependent variable are normally distributed in the population. When sample size is large (30 or more), the parametric statistics are robust. Violation of assumption of normality has little effect on its accuracy (Hills 2005).

However, in animal behaviour research, it often occurs that the assumption of normality is not met and the samples in each treatment are small (< 30). In these cases, alternative nonparametric tests can be performed instead. The accuracy of the nonparametric test is not dependent on the satisfaction of population assumptions. However, they tend to be less powerful than parametric tests. The nonparametric alternative for ANOVA above is Wilcoxon signed-rank $T$ test, Mann-Whitney $U$ test and Kruskal-Wallis test respectively (Siegel & Castellan 1988, Krips et al. 1999a, James & Price 2004, Hills 2005).

When data is from the experiment that has two more independent variables and each independent variable has two or more levels, factorial ANOVA should be used. The
simplest factorial ANOVA is a two-way factorial ANOVA. The experiment for the predation rate of a predatory mite on different leaf hair densities, in which each type of leaf has different prey density, is a two-way factory design (Krips et al. 1999a).

Chi-square ($\chi^2$) is used when there is frequency (nominal) data. There are two types of chi-square, the chi-square for goodness of fit test and the chi-square for contingency table analysis. Chi-square for goodness of fit test is used to analyse differences between observed frequencies and expected frequencies, for example, the frequencies of a fruit fly making the choice in a four-arm olfactometer or the frequencies of predatory mite making the choice in a two-arm Y-tube olfactometer (Horiuchi et al. 2003b). When there are only two response categories for frequencies, like the frequencies of a predatory mite to make the choice between the two-arms in Y-tube olfactometer, a binomial test can also be used under the null hypothesis that the distribution over the two response categories is 50:50 (Choh et al. 2004, de Boer & Dicke 2004b). Contingency table analysis is used to test if the frequencies differ significantly between two or more groups. For example, the response frequencies collected for different predatory mite populations in a Y-tube. A significant overall response indicates that there is a difference somewhere among the groups. In order to find out exactly which groups differ from which other groups a 2
For the comparative study of functional responses, analysis of covariance (ANCOVA) can be used to test the overall differences between two or more functional response curves, in which the prey density was used as the covariate (Houck & Strauss 1985, Lester et al. 2000, Castagnoli et al. 2001). However, this test is subject to the usual assumption of normality. When the distribution of residuals is nonnormal, the nonparametric rank-sum test can be used (Holander & Wolfe 1973, Gibbons 1976, Houck & Strauss 1985).

All the analyses above can be performed using computer based statistical programs such as SPSS (SPSS. 2001, Hills 2005) or SAS (Der & Everitt 2002).
Chapter 3. Toxicity of topically applied nC24 agricultural mineral oil to *Tetranychus urticae* and *Phytoseiulus persimilis*, and the ultrastructure of their eggs

Introduction

Use of pesticides in IPM-systems requires *a priori* assessments of their possible side-effects on beneficial arthropods (Blumel *et al.* 1993). The term ‘biorational’ is used to denote any type of natural or synthetic material active against pest populations but relatively innocuous to non-target organisms, and therefore non-disruptive to biological control (Stansly & Liu 1995, Stansly *et al.* 1996). It is a relative term: no pesticide is totally selective or non-selective (Stansly *et al.* 2002). For each case it is necessary to evaluate the margin of safety that exists between effective control of the target pest and compatibility with the key natural enemies operating within the agro-ecosystem (Stansly *et al.* 2002). Although MOs can control a wide range of pests, they also are toxic to beneficial organisms particularly from direct contact with sprays (Rosen 1967, Stansly *et al.* 2002). The selectivity of MOs between whiteflies and their important natural enemies, including the parasitoid *Encarsia pergandiella*, the coccinellid predator *Nephaspis oculatus* and the lacewings *Ceraeochrysa cubnna* and *Chrysoperla rufilabris* has been investigated (Liu & Stansly 1996, Stansly & Liu 1997, Schuster & Stansly 2000, Stansly *et al.* 2002).

The spray toxicity of MOs against adult female TSM was reported by Herron *et al.* (1995, 1998b) and their use with *P. persimilis* in IPM programs for TSM on greenhouse tomato (*Solanum lycopersicon* L. [Solanales: Solanaceae]) and roses (*Rosa* spp. [Rosales: Rosaceae]) has been reported by French *et al.* (1976) and Nicetic *et al.* (2001, 2002). Although there are many reports of the toxicity of synthetic pesticides to *P. persimilis* (Zhang & Sanderson 1990, Malezieux *et al.* 1992, Blumel *et al.* 1993), only limited research has been conducted on the toxicity of MOs to *P. persimilis*. The toxicity of MOs to immature stages of TSM and the safety margins for effective control of TSM and compatibility with *P. persimilis* are still unknown.
In the research reported in this chapter, I sought to investigate those gaps stated above and provide some basic information for combining MOs and *P. persimilis* in IPM systems for TSM. Part of the study involved environmental scanning electron microscopy (ESEM) to explain possible influences of egg ultrastructure on the relative susceptibility of TSM and *P. persimilis* eggs to MOs.

**Materials and methods**

**Plants and Mites**

*P. persimilis* and TSM were obtained from the Beneficial Bug Company (Richmond NSW, Australia). French bean (*Phaseolus vulgaris* L. cv. Redlands Pioneer) plants required for the study were grown in a climate-controlled room at 25°C ± 2°C, 16:8 L:D photoperiod until they were 18-20 cm tall and with two developed primary leaves. They were then used to culture TSM and *P. persimilis* at the same conditions as above and to cut leaf discs.

**Agricultural mineral oil**

The AMO used in the experiments was nC24 SK Enspray 99 (Oilblend Pty Ltd, Sefton, NSW, Australia). Its chemical and physical properties are listed in Appendix 2.

**General procedures of bioassays**

A range of serially diluted emulsions of AMO in water (% w/w), chosen to give responses between 20-90% mortality, was used in all bioassays. A Potter spray tower was used to spray the abaxial surface of bean leaf discs on a flat PVC plate. Five mL aliquots of the oil emulsion or distilled water were applied with a Potter spray tower operated at at 55 kPa, 25°C
et al. 1995, Herron et al. 1998b). After application of spray, the leaf discs were placed on flat pads of moist cotton wool in 90 mm Petri dishes and held in a plant growth chamber at 25° ± 2°C and 65% RH with a 16:8 L:D photoperiod. Mortality was assessed after 24 h. Mites were counted as dead if they did not move their legs when gently touched with a small fine camel-hair brush.

**The relationship between serial dilution and AMO deposit**

The relationship between serial dilution and spray deposit produced with the Potter spray tower using 25 mm bean leaf discs is given in Figure 3.1.

![Potter spray tower calibration curve using AMO](image)

**Figure 3.1.** Potter spray tower calibration curve using AMO.

**Special procedures of bioassays for different development stages of mites**

**Eggs of TSM:** Five female adults were transferred with a fine camel-hair brush from infested plants to each 25 mm leaf disc and allowed to lay eggs for 24 h with adults and any eggs in excess of 50 removed. The discs were sprayed and kept in the plant growth chamber as described above. The hatched eggs were counted and larvae removed from the third day until no more eggs hatched.
**Larva and protonymph of TSM:** Twenty-five larvae or protonymphs were transferred, sprayed and kept in the plant growth chamber as described above. To eliminate the influence of the quiescent stage, live mites were recorded and removed from the disc every 24 h for 3 consecutive days, and mortality calculated.

**Adult TSM:** Twenty-five female adults were transferred, sprayed and kept in the plant growth chamber as above. Mortality was assessed after 24 h.

**Egg of *P. persimilis***: Twenty female adult TSM and five female adult *P. persimilis* were transferred to 34 mm bean leaf discs and left for 24 h, when adult predators and eggs in excess of 15 were removed. The discs were then sprayed and kept in the plant growth chamber as above. Hatched eggs were recorded and larvae removed for 3 consecutive days.

**Larva, protonymph and adult of *P. persimilis***: Twenty TSM female adults were transferred to 34 mm bean leaf discs for 24h. Then 20 larvae, 15 protonymphs or 15 adults were transferred to each arena. The discs were sprayed and kept in the plant growth chamber as above. Mortality was assessed after 24 h.

**Analysis of data**

Data were analysed using the probit analysis feature of SPSS (SPSS 2001) to calculate probit regressions, fiducial limits of dose and heterogeneity of the regressions. Relative toxicity comparisons were made at the LC$_{50}$ level (Finney 1971, Copenhaver & Mielke 1977, Robertson *et al.* 1984) by comparing the calculated 95% fiducial limit.

**Ultrastructure of TSM and *P. persimilis* eggs**

Eggs were obtained by transferring 20 female TSM adults and four female *P. persimilis* adults from infested plants to 20 mm bean leaf discs. After 24 h, the adults were removed from the discs. Eggs were then observed under a Philips FEI XL30 environmental scanning electron microscope (ESEM).
Results

Dose-response of TSM and *P. persimilis* to AMO

The egg of TSM was significantly more susceptible to AMO than the other stages of either TSM or *P. persimilis*, to topical applications of sprays of aqueous emulsions of nC24 SK Enspray 99 (Figure 3.2; Table 3.1). The egg of *P. persimilis* was the least susceptible stage: its LC$_{50}$ was significantly higher than all other stages tested of the two mite species (Figure 3.2; Table 3.1).

For TSM, the LC$_{50}$ for adult females was significantly lower than for the larvae. However, there was no significant difference for LC$_{50}$s of the larva and the protonymph, and between adult and protonymph. For *P. persimilis*, LC$_{50}$s for the larva, protonymph and adult female were similar.

Comparison of TSM and *P. persimilis* revealed that the LC$_{50}$ for the larva of TSM was significantly higher than that for the larva of *P. persimilis*. However, the differences for the protonymph and adult stages of the two species were not significant.

Pearson goodness-of-fit results indicated that no heterogeneity factor was required to be used in the regression of all stages for the two species (Table 3.2). Parallelism tests showed that the regression for the protonymph of TSM had a significantly lower slope than that for the protonymph of *P. persimilis*. The regression for the egg of TSM had a significantly higher slope than that for the egg of *P. persimilis*, but there were no differences between the two species for larvae and adults (Table 3.2).
Log of deposit (µg/cm²)

Probit of mortality

*T. urticae*
\[ y = -3.4623 + 2.7826x \]

*P. persimilis*
\[ y = -4.7387 + 1.7892x \]
Log of AMO deposit (µg/cm²)

Probit of mortality

\( P. \ persimilis \)
\[ y = -3.9105 + 2.4160x \]

\( T. \ urticae \)
\[ y = -2.9928 + 1.6196x \]
Table 3.1. LC₅₀ and 95% confidence limits for aqueous AMO emulsions applied as sprays to *T. urticae* and *P. persimilis*.

<table>
<thead>
<tr>
<th>Stage of mite</th>
<th><em>T. urticae</em></th>
<th><em>P. persimilis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC₅₀ (µg/cm²)</td>
<td>95% CL (µg/cm²)</td>
</tr>
<tr>
<td>egg</td>
<td>17.55</td>
<td>15.53 - 19.46</td>
</tr>
<tr>
<td>larva</td>
<td>93.86</td>
<td>76.71 - 110.33</td>
</tr>
<tr>
<td>protonymph</td>
<td>70.44</td>
<td>24.62 - 112.32</td>
</tr>
<tr>
<td>adult</td>
<td>63.89</td>
<td>52.74 - 74.36</td>
</tr>
</tbody>
</table>

Table 3.2. Goodness-of-fit and parallelism test for aqueous AMO emulsions applied as sprays to *T. urticae* and *P. persimilis*.

<table>
<thead>
<tr>
<th>Stage of mite</th>
<th>Goodness-of-fit</th>
<th>parallelism test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>T. urticae</em></td>
<td><em>P. persimilis</em></td>
</tr>
<tr>
<td></td>
<td>χ²</td>
<td>p</td>
</tr>
<tr>
<td>egg</td>
<td>14.437</td>
<td>0.274</td>
</tr>
<tr>
<td>larva</td>
<td>9.775</td>
<td>0.636</td>
</tr>
<tr>
<td>protonymph</td>
<td>8.357</td>
<td>0.757</td>
</tr>
<tr>
<td>adult</td>
<td>9.376</td>
<td>0.671</td>
</tr>
</tbody>
</table>
Ultrastructure of eggs of TSM and *P. persimilis* by ESEM

The micrographs showed that the surface of the egg of *T. urticae* is smooth and usually largely covered by silk spun from adult females to allow the egg to be attached to the leaf surface (Figure 3.6, a-d). The surface of the egg of *P. persimilis* is smooth with many discreet ‘bubble-like’ raised circular areas of variable diameter (Figure 3.6, e and f).
Discussion and Conclusions

The egg was the most susceptible stage of TSM sprayed with aqueous emulsions of the nC24 AMO, and, conversely, the egg of *P. persimilis* was the least susceptible stage of the predator. This could be due to one of the following factors:

- **Egg size.** The mode of action of MOs as ovicides is to physically inhibit gaseous exchange across the egg surface (Smith & Pearce 1948, Taverner 2002). Smaller eggs may therefore be more susceptible than larger eggs. The egg of TSM is about 0.14 mm in diameter (Osborne *et al.* 1999). The volume of a TSM egg is $1.44 \times 10^{-3} \text{ mm}^3$ compared to $1.20 \times 10^{-2} \text{ mm}^3$ for *P. persimilis* (Toyoshima & Amano 1998). The egg of *P. persimilis* is therefore some 8.35 times larger than that of TSM.

- **Egg incubation period.** Smith & Pearce (1948) found that the duration of exposure of eggs of European red mite, citrus red mite and European corn borer (*Ostrinia nubilalis* (Hübner) [Lepidoptera: Pyralidae]) to PDSO was critical to high mortality. I found that at 25°C, the incubation period for a TSM egg was about 4 d compared to 1 d for *P. persimilis*.

- **Egg surface structure.** The egg surface of TSM is usually well covered with fine silk that females use to attach their eggs to plant surfaces. This might trap oil on the surface more than would occur if the egg was not covered with silk. The ultrastructure of the egg of TSM by scanning electron microscopy (SEM) was reported by (Crooker 1985). During preparation of samples for SEM some structures may be dissolved (Dittrich 1971). The emergence of ESEM provided a useful means to investigate the surface structure of eggs without destroying surface structures of samples. In an ESEM, all specimens, wet/dry, conductive/insulating, can be examined in their natural state without the need for specimen preparation.

- **Special respiratory apparatus.** Maturing TSM eggs (eggs older than 68 h, 28°C) have a special respiratory system. If embryonic development is sufficiently advanced, the respiratory system cones project above the egg surface (Dittrich 1971). This might influence the impact of oil applied topically in sprays.
Herron et al. (1998b) reported that the LC50s for nC23 Ampol D-C-Tron NR and nC21 Caltex Lovis against adult TSM were 48.81 (40.21 - 60.53) µg/cm² and 72.76 (55.16 - 100) µg/cm² respectively. The result for our nC24 AMO against similar adult TSM is 63.89 (52.74-74.36) µg/cm² indicating no significantly difference in result between the two studies. I recorded an LD50 of 63.89 (52.74 - 74.36) µg/cm² for nC24 SK EnSpray 99. I anticipated a lower value: that the nC24 oils would be more effective than the nC21 and nC23 oils.

Herron et al. (1995, 1998b) found adult TSM more tolerant to HMOs than other pests for which PDSOs have traditionally been used. My results support this view. I also found that all the motile stages tested were tolerant to AMO. LC95 was 259.82 (216.94 - 330.59) µg/cm² for adult, 730.12 (503.27 - 1605.49) µg/cm² for protonymph and 527.96 (406.95 - 769.05) µg/cm² for the larva, respectively. The results suggest that scope is limited for TSM control directly in management programs based on the use of 1-2% MO sprays. Such a limitation has been demonstrated in the field evaluation of mite control in apples (Thwaite et al. 2002). However, Cooper et al. (2002) showed that HMOs can be used weekly at 0.3% or fortnightly at 0.5% to control TSM and rose powdery mildew (Sphaerotheca pannosa var. rosae (Wallr. et Fr.) Lév. [Erysiphales: Erysiphaceae]) simultaneously. The results suggest that TSM control with HMO is more complex than the acute toxicity gleaned from bioassay type studies.

For TSM the most susceptible stage to AMO is the egg, in contrast to the larva stage for P. persimilis. The susceptibility for protonymph and adult stages was similar for both species. Therefore, doses that kill most of the motile stages of TSM are also harmful to P. persimilis and the most appropriate doses for use of MOs in IPM with P. persimilis would be low, not high. For instance, 0.2-0.3% (w/w) SK EnSpray 99, which can kill 75-85% of TSM eggs will also kill 35-55% larvae and protynymphs and 30-45% adults of P. persimilis. This dose should be relatively safe for use with P. persimilis in IPM programs for TSM if sprays are strategically applied to upper canopies only as was done, for example, by Nicetic et al. (2001).
Heterogeneity in MOs probit regressions have been reported by several authors (Riehl & LaDue 1958, Trammel 1965, Agnello et al. 1994, Herron et al. 1995, Stansly & Liu 1995, Herron et al. 1996, Herron et al. 1998b, Barchia et al. 2003, Spooner-Hart & Herron 2003). However, I did not find heterogeneity in my regression analysis. Such a heterogeneous response can be compensated by using more than the minimum of 120 individuals (Robertson et al. 1984, Herron et al. 1998b). Use of the Potter spray tower, which gives highly uniformity and reproducible spray deposits, can reduce the variance (Herron et al. 1998b). Also, in my experiments, two leaf discs were used for each replicate, and the mean percentage mortality was used for regression analysis. This may have reduced heterogeneity.
Chapter 4. Toxicity of $n$C24 agricultural mineral oil deposits to *Tetranychus urticae* and *Phytoseiulus persimilis*

**Introduction**

Although most arthropod PDSO-induced mortality is directly related to wet deposits shortly after spray application, dried deposits may also cause significant mortality (Ebeling 1936). Both wet and dry deposits of MOs can cause high mortality of adult silverleaf whitefly (Liu & Stansly 1995c, d) for up to 5 d after treatment (Liu & Stansly 1995d). Deposits of sprays applied at concentrations as low as 0.00005% and aged for 1 d have significant larvicidal activity against European red mite (Agnello *et al.* 1994).

Aged oil deposits are also known to be toxic to the adult armoured scale parasitoid *Aphytis holoxanthus* (Rosen 1967). Adults of *Encarsia pergandiella*, an endoparasitoid of *B. tabaci* can become trapped and killed in fresh oil deposits. However, oil residues are much less toxic to adults in bioassays if applied as a spray to leaf surfaces rather than as a dip to a leaf (Stansly & Liu 1997, Stansly *et al.* 2002). The residues of MOs are moderately toxic to larvae and adults of green lacewing *Chrysoperla rufilabris* and *Ceraeochrysa cubana* (Stansly *et al.* 2002).

In Chapter 3, I showed that adult, protonymph and larval stages of TSM were tolerant to the $n$C24 AMO SK EnSpray 99. The tolerance of adult TSM to topically applied HMOs sprays was reported earlier by Herron *et al.* (1995, 1998b). The results suggest limited scope for killing TSM through direct contact with aqueous deposits ranging from 1-2% MO. This limitation in efficacy demonstrated in laboratory bioassay has also been demonstrated in field evaluations in apples (Thwaite *et al.* 2002). However, Cooper *et al.* (2002) showed that HMOs can be used weekly at 0.3% v/v or fortnightly at 0.5% v/v to control TSM and rose powdery mildew simultaneously. The results suggest that factors other than anoxia (resulting from temporary immersion of eggs and motile stages in wet spray deposits) possibly contributed to control of the mite.
I hypothesised that dry MOs deposits may affect survival of immature stages of TSM. I tested this hypothesis in this chapter by determining the impact of AMO deposits on the mortality of TSM eggs and motile stages and its effects on the survival of immature stages. I also determined the impact of deposits on the eggs and motile stages of *P. persimilis*.

**Materials and methods**

**Plants and Mites**

TSM and *P. persimilis* were obtained and maintained as described in Chapter 3.

**Agricultural mineral oil**

The AMO used in the experiments was SK Enspray 99. See Chapter 3 and Appendix 2 for further details.

**General procedures for obtaining the deposits and maintaining treated mites**

A Potter spray tower was used to apply 0.25, 0.50, 1.00 and 1.50% AMO emulsions to the abaxial surface of bean leaf discs (see Chapter 3). After spraying, the leaf discs were removed and placed on wet cotton wool in 90 mm Petri dishes. The deposits were allowed to dry for 2 h before eggs or motile stages of the mites were laid or transferred to the discs. The Petri dishes were kept in a plant growth chamber maintained at 25° ± 2°C, 65% RH, and with a 16:8 L:D photoperiod, until mortality was assessed. Mites were counted as dead if they did not move their legs when gently touched with a small fine camel-hair brush.

**Mortality caused by fresh dry oil deposits**

**TSM eggs:** To obtain about 50 undisturbed eggs per 25 mm-diameter bean leaf disc, 15, 20, 30, 40, and 40 adult female TSM were transferred respectively to discs prepared for the control (distilled water) and 0.25%, 0.50%, 1.00% and 1.50% of the aqueous oil emulsion treatment. The adults were removed 5 h later. The discs were kept in a plant
growth chamber (see Chapter 3). Hatched eggs were counted, and larvae were removed each day from the third day until hatching ceased. Each treatment was replicated 4 times.

**TSM larvae and protonymphs:** Twenty larvae or protonymphs were transferred to 25 mm treated bean leaf discs. The discs were kept in a plant growth chamber as above. The larval or protonymphal duration is approximately 2 d at 25°C. To eliminate the influence of the quiescent stage of the larva and of the protonymph, live mites were recorded and removed from the discs daily for 3 consecutive days. Mite mortality was thus calculated. Each treatment was replicated 4 times.

**TSM adult females:** Twenty female adults were transferred to 25 mm treated bean leaf discs. The discs were kept in a plant growth chamber as above. Mortality was assessed after 24 h. Each treatment was replicated 4 times.

**P. persimilis eggs:** Twenty eggs of *P. persimilis* were transferred to 34 mm treated bean leaf discs. The discs were kept in a plant growth chamber as above. Hatched eggs were recorded daily, and larvae were removed daily, for 3 consecutive days. Each treatment was replicated 6 times.

**P. persimilis larvae, protonymphs and adult females:** Twenty female adults of TSM were transferred to 34 mm treated bean leaf discs. Then, 20 larvae or 10 protonymphs or 10 female adult *P. persimilis* were transferred to each arena. The discs were kept in a plant growth chamber as above. Mortality was assessed after 24 h. Each treatment was replicated 6 times.

**Effects of fresh dry deposits of AMO on the development of immature mites**

**TSM:** Distilled water and 0.10%, 0.20%, and 0.40% aqueous AMO emulsions were applied to 25 mm bean leaf discs. To obtain about 50 undisturbed eggs per disc in each treatment, 15, 20, 30, or 40 female adults of TSM were transferred to the discs for the water control and deposits of 0.10%, 0.20%, and 0.40% aqueous oil emulsions respectively. The adults were removed 5 h later. The discs were kept in a plant growth chamber as above. The survival of different stages was evaluated daily from the third
day after the treatment until no more adults moulted. Each treatment was replicated 4 times.

**P. persimilis.** Distilled water and 0.10%, 0.20%, and 0.40% aqueous AMO emulsions were applied to 34 mm bean leaf discs. Ten adult TSM and ten eggs of *P. persimilis* were transfer to each disc. The discs were kept in a plant growth chamber as described above. Survival of different stages of *P. persimilis* was evaluated daily from the day after treatment until no more nymphs of the predator moulted to become adults. Fresh TSM were added to each disc daily to ensure an abundance of food for the predator. Each treatment was replicated 6 times.

**Effects of aged AMO deposits on the development of immature mites**

Potted bean plants, 12-15 cm tall with two developed primary leaves, were sprayed with an 0.30% aqueous AMO emulsion, or distilled water, to ‘run-off’, using a hand-held sprayer. They were kept in the controlled environment room at 25°C with a 16:8 L:D photoperiod and 4,110 lux fluorescent lights. The leaf discs were cut from the primary leaves of the plants 2 h, 1 d, 3 d, 5 d and 7 d after spraying and used for subsequent bioassay.

For TSM, 15 or 30 TSM adults were transferred to the 25 mm control or AMO treated disc respectively, to lay eggs. After 5 h the adult TSM were removed: at this point there were about 50 eggs per disc. The discs were kept in a plant growth chamber as above. The survival of different stages was evaluated from the third day after the treatment until no more adults moulted. Each treatment was replicated 6 times

For *P. persimilis*, 10 adult TSM and 10 eggs of *P. persimilis* were transferred to 34 mm bean leaf discs. The discs were kept in a plant growth chamber as above. The survival of different stages was evaluated from the next day after treatment until no more adults moulted. Fresh TSM were added to each disc daily to ensure an abundance of food. Each treatment was replicated 6 times
Statistical analysis

Data were subjected to one-way ANOVA. Duncan’s multiple comparisons or, if variances were heterogenous, the Games-Howell test was used to determine the differences for mortality among treatments when the ANOVA was significant (Day & Quinn 1989, SPSS. 2001). All analyses were performed using SPSS Version 11.0 (SPSS. 2001).

Results

Mortality caused by oil deposits

TSM egg mortality was significantly different among the treatments (F = 14.127, df = 3, p < 0.001), and increased from 38.14% to 81.55% as the concentration of AMO increased from 0.25% to 1.50% (Figure 4.1). Mortalities in the 1.00% and 1.50% treatments were significantly higher than in the 0.25% and 0.50% treatments, but there was no significant difference between the 1.00% and 1.50% treatments, and mortality in the 0.50% treatment was significantly higher than in the 0.25% treatment. The AMO deposits had no impact on *P. persimilis* eggs: corrected mortality in all treatments was zero (Figure 4.1).
Figure 4.1. Mortality *P. persimilis* (PP) and *T. urticae* (TSM) eggs caused by deposits of the nC24 AMO SK EnSpray 99. Bars of same colour with different letters differ significantly for the comparisons between the AMO concentrations (*p* < 0.05).

TSM larval mortality differed significantly among the treatments (*F* = 5.436, df = 3, *p* = 0.014). Mortalities in the 0.50% and 1.00% treatments were significantly higher than in the 0.25% and 1.50% treatments. However, there were no differences between the 0.25% and 1.50% treatments or between the 0.50% and 1.00% treatments. Average mortality for the 0.25% and 1.50% treatments was 20.63%. It was 45.63% for the 0.50% and 1.00% treatments.

*P. persimilis* larval mortality increased from 8.33% to 77.50% as AMO concentration increased from 0.25% to 1.50%, and differences between treatments were significant (*F* = 52.788, df = 3, *p* < 0.001) (Figure 4.2). Mortality in 1.50% treatment was about 9× greater than in the 0.25% treatment.
Figure 4.2. Mortality of larvae of *P. persimilis* (PP) and *T. urticae* (TSM) caused by deposits of the nC24 AMO SK EnSpray 99. Bars of the same colour with different letters differ significantly for the comparisons between the AMO concentrations (p < 0.05).

TSM protonymph mortality differed significantly between treatments (F = 4.324, df = 3, p = 0.028) (Figure 4.3). Mortality (57.50%) in the 1.00% AMO treatment was significantly higher than in the other treatments but there were no significant differences among the 0.25%, 0.50% and 1.50% treatments.

*P. persimilis* protonymph mortality differed significantly among the treatments (F = 15.412, df = 3, p < 0.001) (Figure 4.3). Mortality in the 1.00% and 1.50% treatments was 10× higher than in the 0.25% treatment and 2× higher than in the 0.50% treatment. Mortality in the 0.50% treatment was about 5× higher than in the 0.25% treatment. However there was no significant difference between the 1.00% and 1.50% treatments, for which average mortality was 51.53%.
TSM adult female mortality differed significantly between treatments ($F = 24.347$, df = 3, $p < 0.001$). Mortalities in the 1.00% and 1.50% treatments were significantly higher than in the lower concentration treatments of 0.25% and 0.50%. However there was no significantly difference between the 1.00% and 1.50% treatments, in which mortality averaged 57.50%. Mortality (6.25%) in the 0.25% treatment was much lower than that in the 0.50% treatment.

*P. persimilis* adult female mortality did not differ significantly between treatments ($F = 2.385$, df = 3, $p = 0.099$) and averaged 9.58% (Figure 4.4) although, there was a trend to increased mortality with increasing concentration.
Figure 4.4. Mortality of adults of *P. persimilis* (PP) and *T. urticae* (TSM) caused by deposits of the nC24 AMO SK EnSpray 99. Bars of same colour with different letters differ significantly for the comparisons between the AMO concentrations (p < 0.05).

**Effects of fresh dry AMO deposits on the development of immature mites**

For TSM, mortalities of its immature stages increased with increasing AMO concentrations in the aqueous emulsions applied to the bean leaf discs (Figure 4.5). Most TSM died in the egg or larval stages. Although eggs hatched in the 0.40% treatment, no mites survived to the nymphal stage or adulthood in this treatment.
The proportion (percentage) of eggs that developed to adults differed significantly in the 0%, 0.10% and 0.20% oil treatments ($F = 119.658$, $df = 2$, $p < 0.001$), with 89.81%, 26.45% and 8.00% reaching adulthood in the three treatments, respectively (Figure 4.6). As no mites developed to adulthood in 0.4% AMO treatment, it was not included in the analysis.

Figure 4.5. Mortality of immature stages of *T. urticae* on fresh dry deposits of the *n*C24 AMO SK EnSpray 99.

Figure 4.6. The percentage of *T. urticae* eggs that developed to adulthood on fresh dry deposits of the *n*C24 AMO SK EnSpray 99. Bars with different letters differ significantly ($p < 0.05$).
For *P. persimilis*, mortalities of its immature stages differed significantly among treatments (F = 50.566, df = 3, p < 0.001). Mortalities were 3.33%, 6.67%, 33.04%, 57.78% respectively, for the water control and the 0.10%, 0.20%, 0.40% oil treatments (Figure 4.7). The control and 0.10% oil treatment did not differ significantly. Most *P. persimilis* died as nymphs in the AMO treatments tested.

![Figure 4.7. Mortality of immature stages of *P. persimilis* on fresh dry deposits of the nC24 AMO SK EnSpray 99. Bars with different letters differ significantly for total mortality (p < 0.05).](image)

**Effects of aged AMO deposits on the development of immature mites**

For TSM, eggs laid on 2 h, and 1 d, 3 d, 5 d and 7 d-old AMO deposits did not develop to adulthood. In contrast, in the control more than 90% developed in the 2 h, and 1 d, 3 d, 5 d treatments, and 86.67% developed in the 7 d-old treatment (Table 4.1; Figure 4.8). Most of the TSM died in the egg or larval stage (Figure 4.8).
Table 4.1. Mortality (%) of immature stages of *T. urticae* and the proportion survival to adulthood on aged deposits of 0.30% aqueous emulsions of the nC24 AMO SK EnSpray 99.

<table>
<thead>
<tr>
<th>Deposit age</th>
<th>Treatment</th>
<th>Egg</th>
<th>Larva</th>
<th>Nymph</th>
<th>Survival to adulthood</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 h</td>
<td>control</td>
<td>1.33</td>
<td>3.00</td>
<td>3.33</td>
<td>92.33</td>
</tr>
<tr>
<td></td>
<td>0.30% AMO</td>
<td>71.00</td>
<td>29.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>1 d</td>
<td>control</td>
<td>1.33</td>
<td>3.67</td>
<td>5.33</td>
<td>90.33</td>
</tr>
<tr>
<td></td>
<td>0.30% AMO</td>
<td>72.67</td>
<td>27.33</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>3 d</td>
<td>control</td>
<td>1.33</td>
<td>2.67</td>
<td>4.33</td>
<td>91.67</td>
</tr>
<tr>
<td></td>
<td>0.30% AMO</td>
<td>52.33</td>
<td>47.33</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>5 d</td>
<td>control</td>
<td>1.67</td>
<td>2.67</td>
<td>3.00</td>
<td>93.00</td>
</tr>
<tr>
<td></td>
<td>0.30% AMO</td>
<td>43.12</td>
<td>53.44</td>
<td>3.44</td>
<td>0.00</td>
</tr>
<tr>
<td>7 d</td>
<td>control</td>
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<td>8.33</td>
<td>1.67</td>
<td>86.67</td>
</tr>
<tr>
<td></td>
<td>0.30% AMO</td>
<td>45.00</td>
<td>54.00</td>
<td>1.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Figure 4.8. Mortality (%) of immature stages of *T. urticae* during development from egg to adult on aged deposits of 0.30% aqueous emulsions of the nC24 AMO SK EnSpray 99.

For *P. persimilis*, 50.00%, 65.00%, 71.67%, 73.33% and 75.00% of eggs successfully developed to adulthood on the 2 h, 1 d, 3 d, 5 d and 7 d-old deposits of 0.30% oil, respectively (Table 4.2). The highest mortalities were for nymphs (Figure 4.9). Total
mortalities of the immature stages differed in relation to the age of the deposits \( (F = 3.450, \text{ df } = 3, \ p = 0.022) \) (Figure 4.9). Total mortality in the 2 h-old deposit was significantly higher than total mortality in the 3 d, 5 d and 7 d-old treatments. There were no significant differences between the 1 d, 3 d, 5 d, and 7 d-old treatments, and the average total mortality for these treatments was 22.89\%, about half of that in the 2 h-old deposit.

**Table 4.2.** Mortality of immature stages of *P. persimilis* and the proportion of mites that developed to adults on aged deposits of 0.30% aqueous emulsions of the nC24 AMO SK EnSpray 99.

<table>
<thead>
<tr>
<th>Deposit age</th>
<th>Treatment</th>
<th>Egg</th>
<th>Larva</th>
<th>Nymph</th>
<th>Survival to adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 h</td>
<td>control</td>
<td>1.67</td>
<td>1.67</td>
<td>0.00</td>
<td>96.67</td>
</tr>
<tr>
<td></td>
<td>0.30% AMO</td>
<td>1.67</td>
<td>10.00</td>
<td>38.33</td>
<td>50.00</td>
</tr>
<tr>
<td>1 d</td>
<td>control</td>
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<td>0.00</td>
<td>1.67</td>
<td>96.67</td>
</tr>
<tr>
<td></td>
<td>0.30% AMO</td>
<td>1.67</td>
<td>6.67</td>
<td>26.67</td>
<td>65.00</td>
</tr>
<tr>
<td>3 d</td>
<td>control</td>
<td>0.00</td>
<td>1.67</td>
<td>3.33</td>
<td>95.00</td>
</tr>
<tr>
<td></td>
<td>0.30% AMO</td>
<td>0.00</td>
<td>6.67</td>
<td>21.67</td>
<td>71.67</td>
</tr>
<tr>
<td>5 d</td>
<td>control</td>
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<td>0.00</td>
<td>0.00</td>
<td>96.67</td>
</tr>
<tr>
<td></td>
<td>0.30% AMO</td>
<td>1.67</td>
<td>5.00</td>
<td>20.00</td>
<td>73.33</td>
</tr>
<tr>
<td>7 d</td>
<td>control</td>
<td>0.00</td>
<td>0.33</td>
<td>0.17</td>
<td>95.00</td>
</tr>
<tr>
<td></td>
<td>0.30% AMO</td>
<td>0.00</td>
<td>5.00</td>
<td>20.00</td>
<td>75.00</td>
</tr>
</tbody>
</table>
Figure 4.9. Mortality of immature stages of *P. persimilis* during development from egg to adult on aged deposits of 0.30% aqueous emulsions of the nC24 AMO SK EnSpray 99. Bars with different letters differ significantly for total mortality (*p* < 0.05).

**Discussion and Conclusions**

TSM eggs were very susceptible to oil deposits but the larval, protonymph and adult female stages were less susceptible. The lower susceptibility of TSM larvae and protonymphs to deposits at 1.50% than at 1.00% AMO is puzzling. The only explanation I can offer is that the higher deposit may have influenced the behaviour of the mites in a manner that made them less susceptible to the oil deposits. In contrast, the eggs of *P. persimilis* were not affected by AMO deposits, but larvae and nymphs were more susceptible, especially on deposits of higher AMO concentrations.

AMO deposits significantly impeded development of immature TSM. No immature TSM could develop to adulthood on 0.3% AMO deposits, and the effect could last 7 d. Most immature TSM died in the egg or larval stages. In contrast, the lower concentration AMO deposits had relatively less impact on *P. persimilis*, and the greatest impact was on the nymphal stage. The negative effects of AMO on *P. persimilis* decreased sharply after 3 d.

My experiments showed that impacts of AMO deposits on TSM mortality were greater than its impacts on its predator, *P. persimilis*. They also indicated that use of AMOs is
compatible with IPM programs where *P. persimilis* is released to control TSM. In such programs I consider the optimal concentration for SK Enspray 99 oil to be 0.3% w/w (approximately 0.35% v/v).
Chapter 5. Impacts of nC24 agricultural mineral oil deposits on the behaviour of Tetranychus urticae

Introduction

For almost a century after PDSOs were first used to control phytophagous arthropods, they were primarily used to suffocate small, generally sedentary pests such as scales and mites. Recently though, emphasis has focused on behavioural effects of oil deposits on arthropods, particularly on feeding and oviposition behaviour (Beattie et al. 2002b). Although behavioural effects of oil on arthropods were first noticed in the 1910s when crude oil was used with nicotine sulphate for control of citrus leafminer, they were overlooked for decades as focus shifted to use of synthetic toxins to kill pests (Beattie et al. 1995). In the early 1990s, behavioural effects on citrus leafminer were re-discovered (Beattie et al. 1995). Impacts have now been recorded for a wide range of taxa. In most cases research suggests that oil deposits interfere with oviposition site selection by adult female pests through repellent or deterrent effects (Schroeder et al. 1977, Larew & Locke 1990, Liu & Stansly 1995c, Mensah et al. 1995, Riedl et al. 1995, Rae et al. 1997, Fernandez et al. 2001, Cen et al. 2002, Liu et al. 2002a, Liu et al. 2002b, Sun 2002, Xue et al. 2002b, Liu et al. 2006, Nguyen et al. 2007). Behavioural effects of AMO and HMO deposits on feeding and oviposition of a wider range of pests are now recognised as more important than mortality induced by anoxia (Beattie et al. 2002b).

The most notable outcome of studies on behavioural effects appears to be that, in most instances, desirable levels of control can be achieved with significantly lower concentrations of oil in sprays (0.25% - 0.50%) than historically recommended concentrations (1.00% - 2.00%) used to kill pests by suffocation (Beattie et al. 1995, Beattie et al. 2002a, Cen et al. 2002).

Liu & Beattie (2002) found that HMO deposits suppressed the oviposition of TSM. Given that TSM is difficult to suffocate with MOs (Herron et al. 1995), they suggested that reported instances of successful control for TSM with MOs could be largely related

In this chapter I extended the studies reported by Liu & Beattie (2002) and assessed in impacts of SK Enspray 99 deposits on TSM behaviour. I undertook experiments on repellency, antifeeding and oviposition deterrency of SK Enspray 99, and examined the impact of aged deposits.

**Materials and methods**

**Plants and Mite**

TSM were obtained and maintained as described in Chapter 3. In this instance mites in each culture were the same age to within 1 d (i.e., eggs were laid on the same day). Adult females were kept in the cultures for 4 d after moulting peaked to ensure that females used in the experiments were gravid.

**Agricultural mineral oil**

The AMO used in the experiments was SK Enspray 99. See Chapter 3 and Appendix 2 for further details.

**Effects of fresh dry AMO deposits on the feeding and oviposition**

Five mL 0.25%, 0.50%, 1.00%, 1.50% SK Enspray 99 aqueous emulsions or distilled water were applied to the abaxial surface of 25 mm bean leaf discs with a Potter spray tower operating at 55 kPa with a 3 s settling time. Sprayed discs were placed onto wet cotton wool in 90 mm Petri dishes and allowed to dry for 2 h before 10 adult female TSM were transferred to each disc. They were kept in a plant growth chamber maintained at 25° ± 2°C and 65% RH, and with a 16:8 L:D photoperiod. The numbers of feeding scars and eggs were assessed after 24 h. Each treatment was replicated 8 times.
Effects of aged AMO deposits on feeding and oviposition

Potted bean plants, 12-15 cm tall with two developed primary leaves, were sprayed with 0.30% aqueous AMO emulsion or distilled water to ‘run-off’ using a hand-held sprayer. They were then kept in a controlled environment room at 25°C with a 16:8 L:D photoperiod and 4110 lux fluorescent lights. The 25 mm diameter leaf discs were cut from the primary leaves at 2 h, 1 d, 3 d, 5 d and 7 d after the initial AMO application and were then placed abaxial surface uppermost on wet cotton wool in 90 mm Petri dishes. Ten adult female TSM were transferred to each distilled-water (control) or AMO sprayed disc. Mite infested discs were kept in a plant growth chamber maintained at 25° ± 2°C and 65% RH, and with a 16:8 L:D photoperiod. The number of feeding scars and eggs were assessed after 24 h. Each treatment was replicated 6 times.

Choice response to AMO and water deposits

Leaf discs were punched from bean leaves so that the midrib of each 25 mm disc bisected into two equal halves. Each disc was placed upside down on wet filter paper on a PVC plate. Parafilm® (Alcan Packaging) was then used to cover one half of each disc, with the edge of the Parafilm aligned with the midrib. The abaxial surface of the exposed half was sprayed with distilled water by Potter spray tower at 55 kPa with a 3 s settling time. After the spray fully dried, the other half of the same surface was sprayed with aqueous AMO emulsion using the same method as above. The AMO emulsion concentrations were 0.125%, 0.25%, 0.50%, 1.00% and 1.50% (w/w). The leaf discs, with one half of each treated with distilled water and the other with AMO emulsion, were placed upside down (i.e., adaxial surface down) on wet cotton wool in 90 mm Petri dishes. Ten female adult TSM were transferred onto each midrib. The infested discs were kept in a plant growth chamber maintained at 25° ± 2°C, 65% RH, and with a 16:8 L:D photoperiod. The number of mites on each half of each disc was assessed after 4 h. Mites on midribs were excluded from the statistical analysis. Each treatment was replicated 8 times.
Statistical analyses

All statistical analyses were performed using SPSS 11.0 for Windows (SPSS, 2001). Feeding and oviposition differences between treatments were tested using the Kruskal-Wallis nonparametric test, followed by multiple comparisons using the Bonferroni correction when a significant difference was found (Siegel & Castellan 1988). Data of TSM preference between distilled water and AMO deposits were subjected to chi-square tests. The null hypothesis was that the TSM had a 50:50 distribution over the two half surfaces of each leaf disc. Furthermore, the contingency table analysis with a chi-square test was used to assess the repellency difference among the different concentrations. When there was a significant difference among the treatments, each combination of two treatments was tested with a \( 2 \times 2 \) contingency table analysis using the Bonferroni method to adjust the p-values (\( p = 0.05/10 = 0.005 \)) (Siegel & Castellan 1988, SPSS, 2001).

Results

Effects of fresh dry AMO deposits on feeding and oviposition

Numbers of feeding scars were significantly different between treatments and decreased with increasing concentration of AMO applied to the leaf discs (\( \chi^2 = 36.015, \text{ df} = 4, p < 0.001 \)) (Figure 5.1). Feeding scars in all AMO treatments were significantly less than that in the control: scarring in the 0.25% AMO treatment was approximately 30% of that in the control. There were no significant differences between the 0.50%, 1.00% and 1.50% AMO treatments and the scarring in these treatments was only 2.8% of that observed in the control.

Numbers of eggs differed significantly between treatments and decreased with increasing concentrations of AMO applied to the discs (\( \chi^2 = 35.312, \text{ df} = 4, p < 0.001 \)) (Figure 5.2). Egg numbers in AMO treatments were significantly less than that in the control: the mean number in the 0.25% AMO treatment was 23.82% of the mean number in the control (> 75% less). There were no significant differences between
the 0.50%, 1.00% and 1.50% AMO treatments and the average number of eggs in these treatments was 5.92, only 3.1% of the average in the control.

![Figure 5.1](image1.png)

**Figure 5.1.** Extent of feeding damage by *T. urticae* on deposits of the nC24 AMO SK EnSpray 99. Bars with different letters differ significantly (*p* < 0.05/10 = 0.005).

![Figure 5.2](image2.png)

**Figure 5.2.** Impact of deposits of the nC24 AMO SK EnSpray 99 on eggs laid by *T. urticae* females. Bars with different letters differ significantly (*p* < 0.05/10 = 0.005).

### Effects of aged AMO deposits on the feeding and oviposition

Deposits of 0.30% AMO in all aged treatments significantly reduced numbers of feeding scars by at least 75% in comparison with the water-sprayed control (Figure 5.3). However, there were significant differences, determined by multiple comparisons,
between deposits of different ages, which ranged from 2 h to 7 d. \( \chi^2 = 18.601, p = 0.001 \) (Figure 5.4). Percent reduction in feeding (antifeeding rate) on the third day (73.22%) was significantly lower than for the other times, but the other treatments did not differ significantly. The average reduction in feeding in the 2 h, 1 d, 5 d and 7 d treatments was 86.29%.

**Figure 5.3.** Impact of deposits of the \( n \)C24 AMO SK EnSpray 99 on feeding (number of feeding scars per leaf disc) by *T. urticae* females. ** indicates that the difference between control and AMO treatment was significant (p < 0.01).

**Figure 5.4.** Percent reduction in feeding (antifeeding effect) by *T. urticae* adult females on aged deposits of 0.30% aqueous emulsions of the \( n \)C24 AMO SK EnSpray 99. Bars with different letters differ significantly (p < 0.05/10 = 0.005)
Deposits of 0.30% SK Enspray 99 in all aged treatments significantly suppressed TSM oviposition by 50% in comparison with the water-sprayed control (Figure 5.5). The extent of suppression differed significantly between treatments ($\chi^2 = 23.101, p < 0.001$) (Figure 5.6). Rates of suppression in the 3 d, 5 d and 7 d treatments were significantly lower than in the 2 h and 1 d treatments. There was no significant difference between the 2 h and 1 d treatments, and rates of suppression in these two treatments averaged 80.15%. Rates of suppression were similar in 3 d, 5 d, and 7 d treatments, and averaged 55.19%.

**Figure 5.5.** Impact of deposits of the $\#C24$ AMO SK EnSpray 99 on oviposition (numbers of eggs laid) by T. urticae females. ** indicates that the difference between control and AMO treatment was significant ($p < 0.01$).
Choice response to AMO and water deposits

In this experiment, adult TSM females showed a significant negative preference for AMO treated halves of leaf discs in comparison to their preference for settling on adjacent water-sprayed control halves of the discs (Figure 5.7). However, the extent of repellency treatments did not differ significantly between treatments (0.125%, 0.25%, 0.05%, 1.00% and 1.50% AMO) ($\chi^2 = 7.206$, df = 4, $p = 0.125$) and the average repellency rate was 74.75%.
**Discussion and Conclusions**

The number of feeding scars decreased with increasing concentrations of aqueous AMO emulsions applied to leaf discs on which adult TSM females were placed. A similar relationship was found for numbers of eggs laid on sprayed leaf discs. These results suggest that oviposition suppression was largely related to feeding deterrence. I hypothesise that feeding deterrence results in oviposition suppression, as the mites cannot obtain enough nutrition to produce eggs.

Liu & Beattie (2002) reported that 50 µg/cm² of an nC24 HMO did not significantly suppress oviposition by TSM females. However, I found that 0.25% (39.69 µg/cm²) of the nC24 AMO I evaluated reduced the number of eggs laid by 76.18% compared to the control. The reason needs to be investigated further.

Cen et al. (2002) determined that repellency and antifeeding effects of the 0.25% concentration of an nC24 HMO on citrus red mite lasted at least 3 d. My studies showed the behavioural effects of AMO on TSM at 7 d after the treatment were still significant.
A similar response was observed in studies reported in Chapter 4 on the toxicity of the AMO to TSM.

From the results in this chapter and in Chapters 3 and 4, I conclude that low concentrations (0.30 - 0.50%) of SK Enspray 99 AMO can effectively control TSM through their combined effects on mite mortality and behaviour. In my studies, 0.30% \( nC24 \) AMO sprays, killed 85% of eggs and no mites that hatched from eggs laid up to 7 d after the application of sprays reach adulthood. Deposits of this dose also suppressed feeding and oviposition by adult females by 70% and 55%, respectively, for the 7 d-old deposits. This dose is also safe to use in TSM IPM programs with \( P. \ persimilis \). This conclusion is supported by the successful control of TSM using 0.3% to 0.5% v/v \( nC24 \) HMO as reported by Cooper et al. (2002).
Chapter 6. Impact of nC24 agricultural mineral oil deposits on the searching efficiency of *Phytoseiulus persimilis*

**Introduction**

Searching behaviours of predatory mites are determined by their biological characteristics, their internal physiological state and external environmental factors. There are many reports on behaviour parameters related to biological factors and the internal physiological state (Mori & Chant 1966b, Eveleigh & Chant 1981c, 1982c, Sabelis & Dicke 1985, van Rijn et al. 2005). Influences of prey density and distribution, humidity, temperature have also been documented (Mori & Chant 1966b, a, Eveleigh & Chant 1981c, 1982c, Friese & Gilstrap 1982, Ryoo 1996).

*P. persimilis* searches randomly within a prey patch until physical contact with its prey occurs (Jackson & Ford 1973, Sabelis 1981). The width of the searching path of *P. persimilis* can be up to 0.84 mm when walking (Sabelis 1981). The encounter rate with prey strongly depends on the walking speed and walking pattern (Sabelis 1981).

Surface structures of host plant substrates, such as the presence of leaf hairs, can significantly influence the searching efficiency of natural enemies (Keller 1987, van Lenteren *et al.* 1995, Dicke 1996, Sutterlin & van Lenteren 1997, Krips *et al.* 1999a). For instance, walking speeds of *P. persimilis* are inversely related to trichome density, and this affects the host encounter rate. Consequently, the time until first predation increases with the leaf hair density. Predation rates decrease as hair density increases (Krips *et al.* 1999a, Stavrinides & Skirvin 2003). The trichomes on stems of tomato plants hamper the dispersal of *P. persimilis* (van Haren *et al.* 1987).

It is known that certain synthetic pesticides affect the settling behaviour of *P. persimilis* (Ashihara *et al.* 1988, Dong & Niu 1988, Dong 1990, Dong & Niu 1990, 1991, Dong *et al.* 1992, Blackwood *et al.* 2001). Some pesticides have marked repellency effects on *P. persimilis* and residues of pesticides on the eggs of its prey affect acceptance by the
predator (Jackson & Ford 1973). However, there are no substantial reports on impacts of MOs on *P. persimilis* searching behaviour and predation although it is known that adult parasitoids can become trapped and killed in fresh MO residues (Rosen 1967, Stansly & Liu 1997).

In the study reported in this chapter, I used an automated video tracking system to determine the influence of AMO deposits on the walking activity, walking straightness, and walking speed of *P. persimilis*, and on its searching efficiency and rate of predation.

**Materials and methods**

**Plants and Mites**

TSM and *P. persimilis* were obtained and maintained as described in Chapter 3. In this instance, to obtain uniform female adults of the predator, they were subsequently reared in 90 mm Petri dishes containing TSM infested bean leaves on moistened cotton wool. The Petri dishes were kept in a plant growth chamber maintained at 25° ± 2°C, 65% RH, and with a 16:8 L:D photoperiod. Predators in each culture were the same age to within 1 d. Adult females were kept in the cultures for 4-7 d after reaching the last instar to ensure that all those used in the experiments were gravid (Laing 1968, Jackson & Ford 1973).

**Agricultural mineral oil**

The AMO used in the experiments was SK Enspray 99. See Chapter 3 and Appendix 2 for further details.

**Walking behaviour**

The AMO was applied with a Potter spray tower. Five mL aliquots of water or oil emulsion were applied at 55 kPa with a 3 s settling time. The procedure was based on Herron *et al.* (1995) (see Chapter 3), and resulted in 39.69 µg, 79.37 µg and 158.74 µg/cm² deposits respectively for 0.25%, 0.50% and 1.00% w/w aqueous oil emulsions. All emulsions were thoroughly agitated immediately before spraying. Distilled water
was used as the control. Each disc was allowed to dry for 2 h upside down on wet filter paper and then placed upside down on a layer of wet cotton wool in a 90 mm Petri dish. Single adult *P. persimilis* females, each starved for 24 h, were then transferred onto each leaf disc. Each mite was allowed to settle for 5 min after it was placed on the disc. The wet cotton wool discouraged movement of mites from the leaf discs. Walking behaviour was recorded with a Sony DXC-151AP video camera mounted on a Leica M8 stereo microscope under a 5,930 lux cold light source. Responses of females were recorded for 10 min. The images from the camera were digitised by a computer and analysed by using an automated video tracking package EthoVision 3.1.16 (Noldus Information Technology 2005). Twenty replicates (replicate = one mite per leaf disc) were conducted for each oil concentration. All experiments were conducted in the laboratory at 25° ± 2°C.

Setting the correct sampling rate for tracking is very important. If the rate is too high, the noise caused by small movements of the predator will be picked up, leading to overestimation of parameters such as distance moved and velocity. If the sample rate is too low, loss of data leads to underestimation of the above parameters. Step-down sampling is used to establish the optimal sample rate (Noldus Information Technology 2005). This was done by sampling at a maximum rate of 25 samples/s and calculating the total distance moved; then re-running the analysis a number of times by reducing the sampling rate in set steps (down sampling steps: DSS). The next step was to plot the calculated values for total distance moved against the DSS. The point of inflexion on the x-axis indicates the optimal DSS. Dividing the original sample rate by the optimal DSS gave the optimal sample rate, I determined an optimal DSS of 8-13, and an optimal sampling rate for *P. persimilis* of 1.923 - 3.125 samples/s. I used a rate of 3.125 samples/s in the image analysis (Figure 6.1).

Walking activity was defined as the proportion of time spent walking. Walking speed was calculated as the distance covered per unit of walking time. Walking straightness was determined by (a) placing the traced walking tracks of individual mites over a sheet of paper divided into 1 × 1 mm cells (grids), (b) counting the number of cells crossed, (c) recording the number of times the track crossed cells, and (d) calculating straightness as
a percentage of (b) over (c), the sum of the number of times tracks crossed all cells. The method was based partially on Sutterlin & van Lenteren (1997). Higher values of walking straightness indicate a straighter walking path and lower incidences of criss-crossing of tracks. Searching efficiency was calculated as the number of cells crossed per minute.

Figure 6.1. Diagramatic representation of procedures used for determining the optimal sample rate

**P. persimilis predation on AMO-contaminated TSM eggs on sprayed leaf discs**

For this experiment, 25 mm diameter leaf discs were placed singly and upside down on moist cotton wool in 90 mm Petri dishes. Each was then infested with 50 TSM eggs. This was achieved by placing six adult females on each disc for 24 h. Surplus eggs, and the adult females, were then removed. A Potter spray tower was then used to apply 0.25%, 0.50%, 1.00% aqueous emulsions of the AMO to the TSM egg-infested discs. Distilled water was used as the control. The deposits were allowed to dry for 2 h.
A single adult *P. persimilis* female, starved for 24 h, was then placed on each TSM egg-infested leaf disc. Each disc was then observed in the laboratory for 1 h, under fluorescent lights (650-700 lux), and the number of *P. persimilis* females that consumed one or more eggs in the first hour recorded. Each dish was then placed in a plant growth chamber with a 16:8 L:D photoperiod at 25° ± 2°C and 65% RH for 24 h after which the number of eggs consumed by each female during this interval was recorded. Forty adult female predatory mites (= replicates) were tested for each treatment. Replication and observations for all treatments were evenly spaced over time.

**P. persimilis predation on AMO-contaminated TSM eggs on unsprayed leaf discs**

To obtain adequate numbers of TSM eggs, 15 adult females were placed on 25 mm mite-free leaf discs that had been placed upside down on wet cotton wool in 90 mm Petri dishes to lay eggs for 24 h. The adult females were then removed and eggs were sprayed, using a Potter spray tower, with 0.25%, 0.50% and 1.00% (w/w) aqueous AMO emulsions and distilled water (control). The deposits were allowed to dry for 2 h. Fifty sprayed eggs were then transferred with a fine brush from the sprayed discs to fresh mite-free leaf discs. In each instance, the 50 eggs were distributed evenly over the abaxial surface of each disc. A single starved (24 h) *P. persimilis* female was then placed on each leaf disc. Observation procedures and conditions for maintaining the discs were the same as described above.

**Statistical analyses**

All statistical analyses were performed using SPSS 11.0 for Windows (SPSS 2001). For walking behaviour and predation over 24 h, differences between treatments were tested using the Kruskal-Wallis nonparametric test, followed by multiple comparisons using the Bonferroni correction when a significant difference was found (Siegel & Castellan, 1988). Data for predation in the first hour were subjected to contingency table analysis with a $\chi^2$ test. When there was a significant difference among the treatments, each combination of two treatments was tested with a 2 × 2 contingency table analysis using the Bonferroni method to adjust the $p$-values ($p = 0.05/6 = 0.008$).
Results

Walking behaviour

Typical examples of the search paths in the 0.25%, 0.50% and 1.00% oil and control (distilled water) treatments are shown in Figure 6.2. The paths consisted of gentle right and left turns, circles and wide loops, which were often caused by avoidance of the edge of the arena. The predators repeatedly returned to the edge of the leaf disc or rib while walking in the arenas. Walking distances were clearly different among the treatments and shortest in the leaf discs treated with 1.00% AMO.

Figure 6.2. Typical examples of the search paths of adult *P. persimilis* females on bean leaf discs with deposits of the \( n \)-C24 AMO SK EnSpray 99: (a) distilled water control, (b) 0.25% AMO, (c) 0.50% AMO and (d) 1.00% AMO. ■ denotes the starting position
Walking activity

Walking activity did not differ significantly between the AMO treatments ($\chi^2 = 4.407$, df = 3, p = 0.221) (Figure 6.3). The average walking activity was $57.23 \pm 1.37\%$ for all treatments.

![Bar chart showing walking activity (%) vs. oil concentration (% w/w)](image)

**Figure 6.3.** Effect of deposits of the nC24 AMO SK EnSpray 99 on the walking speed of adult *P. persimilis* females. Vertical lines represent SE of means. Bars with same letters do not differ significantly for the comparisons between treatments (p > 0.05).

Walking speed

Walking speeds on leaf discs sprayed with distilled water and 0.25%, 0.50%, and 1.00% aqueous AMO emulsions differed significantly between treatments ($\chi^2 = 49.83$, df = 3, p < 0.001, Figure 6.4) and inversely in relation to oil concentration. Walking speeds in the three AMO treatments were significantly lower than in the control and the speed in the 1.00% AMO treatment was significantly lower than in the 0.25% and 0.50% AMO treatments. The speed in the control was $44.57 \pm 2.73$ mm/min., which was double that
in the 0.50% AMO treatment. The speed in 1.00% AMO treatment was less than half that of the control.

![Bar graph showing walking speed vs. oil concentration. Bars represent SE of means. Bars with different letters differ significantly (p < 0.05).]

**Figure 6.4.** Effect of deposits of the nC24 AMO SK EnSpray 99 on the walking speed of adult *P. persimilis* females. Vertical lines represent SE of means. Bars with different letters differ significantly for the comparisons between treatments (*p* < 0.05).

**Walking straightness**

Walking straightness differed significantly between treatments (*χ² = 25.700, df = 3, p < 0.001; Figure 6.5). Straightness in the 0.25%, 0.50% and 1.00% AMO treatments was significantly higher than in the distilled water control. However, there were no significant differences between the 0.25%, 0.5% and 1% AMO treatments. The average walking straightness for the three AMO treatments was 89.59 ± 1.04%, and 76.39 ± 2.17 for the control.
**Figure 6.5.** Effect of deposits of the nC24 AMO SK EnSpray 99 on walking straightness of adult *P. persimilis* females. Vertical lines represent SE of mean. Bars with different letters differ significantly for the comparisons between treatments (p < 0.05).

**Searching efficiency**

Searching efficiency on leaf discs treated with distilled water and 0.25%, 0.50% and 1.00% aqueous AMO emulsions were 22.27 ± 1.67 mm$^2$, 13.43 ± 1.29 mm$^2$, 10.76 ± 1.28 mm$^2$ and 7.31 ± 0.79 mm$^2$/min, respectively. The response was therefore inversely related to AMO concentration, and differences between treatments were significant ($\chi^2 = 35.746$, df = 3, p < 0.001; Figure 6.6). Searching efficiency in the AMO treatments was significantly lower than in the distilled water control and the impact of 1.00% AMO was significantly greater than that of 0.25% AMO.
Numbers of adult *P. persimilis* females that began preying on TSM eggs within 1 h of being placed on leaf discs with TSM eggs sprayed with distilled water or aqueous AMO emulsions differed significantly between treatments ($\chi^2 = 96.665$, df = 3, $p < 0.001$; Figure 6.7). All oil treatments significantly suppressed predation within the hour. There was no significant difference between the AMO treatments.

Predation rates of *P. persimilis* on oil-contaminated TSM eggs on oil sprayed leaf discs were significantly different from that observed in the control ($\chi^2 = 83.182$, df = 3, $p < 0.001$, Figure 6.8). The rate in the control was $61.75 \pm 2.55\%$ compared to an average of $13.15 \pm 1.31\%$ for the AMO treatments. There were no significant differences between the three oil treatments.
Figure 6.7. Number of adult *P. persimilis* females that began preying on TSM eggs within 1 h of being placed on unspayed leaf discs with TSM eggs sprayed with distilled water or aqueous emulsions on *n*C24 AMO SK EnSpray 99. Bars with different letters differ significantly for the comparisons between treatments (p < 0.05).

Figure 6.8. Predation rates of adult *P. persimilis* females on TSM eggs on bean leaf discs on which the eggs and leaf discs were sprayed with the *n*C24 AMO SK EnSpray. Vertical lines represent SE of means. Bars with different letters differ significantly for the comparisons between treatments (p < 0.05).
Predation of *P. persimilis* to contaminated TSM eggs on clean leaf discs

Numbers of adult *P. persimilis* females that began preying on TSM eggs within 1 h of being placed on unsprayed leaf discs with TSM eggs sprayed with distilled water or aqueous emulsions of nC24 AMO differed significantly between treatments ($\chi^2 = 18.917$, df = 3, $p < 0.001$; Figure 6.9). Predation was significantly lower in 0.50% and 1.00% oil treatments than that in control.

Figure 6.9. Number of adult *P. persimilis* females that began preying on TSM eggs within 1 h of being placed on unsprayed leaf discs with TSM eggs sprayed with distilled water or aqueous emulsions of nC24 AMO SK EnSpray 99. Bars with different letters differ significantly for the comparisons between treatments ($p < 0.05$).

Predation rates of *P. persimilis* on oil-contaminated TSM eggs on unsprayed leaf discs were not significantly different from that observed in the control ($\chi^2 = 3.064$, df = 3, $p = 0.382$, Figure 6.10). The average predation rate was 64.48 ± 1.21%.
Discussion and Conclusions

An important impediment to effective biological control of spider mites using *P. persimilis* in commercial crops is the need to simultaneously control a range of other pests and diseases. However, disruption to *P. persimilis* in the process of controlling other pests and diseases can be minimised by the use of softer or highly specific chemicals. MOs are generally considered as softer options and they have been effectively used in a program with *P. persimilis* for the control of *T. urticae* in greenhouse roses (Nicetic *et al.* 2001, 2002). However, this study demonstrated that AMO deposits can significantly reduce walking speeds and searching efficiency of *P. persimilis* in the laboratory.

The walking activity of *P. persimilis* was not significantly influenced by oil deposits and it was found to be between 55% and 61% on bean leaves. Similar *P. persimilis* walking activities of 68% on rose leaves and 70-90% on gerbera leaves were reported by Krips *et al.* (1999). Walking speed and searching efficiency both decreased with
increasing oil concentration. This similar pattern of response, and the absence of an
effect of oil on walking activity or straightness, indicates that the decline in searching
efficiency was mostly due to a suppression of walking speed.

The straightness of walking on oil deposits was greater than that in the distilled water
control. This may have been due to AMO deposits reducing the walking speed of the
predatory mite. With a limited area of leaf disc, a higher walking speed increases the
frequency of a mite crossing its own walking path in both the control and low
concentration AMO treatments.

The reaction of adult *P. persimilis* females to AMO-contaminated eggs on untreated leaf
discs suggests that oil deposits on the surfaces of eggs prolonged the time taken for
predation to occur by changing the physical and chemical properties of the egg surface.
However, it seems that *P. persimilis* females finally overcame the confusion by
‘learning’. The results of predation on eggs on oil sprayed and non-oil sprayed leaf discs
suggest that when the predatory mite walked on an oil deposit its mechanoreceptors and
chemoreceptors in its anterior tarsi and pedipalps were contaminated by oil, leading to
the mite to not respond normally to physical and chemical stimulants of its prey. My
research indicates that the important factors that affect searching efficiency and
predation rate are suppression of walking speed and the oil-contamination of
mechanoreceptors and chemoreceptors on anterior tarsi and pedipalps, with the impact
of the oil increasing rapidly with increasing oil concentration.

While it is clear that short-term negative impacts of oil on *P. persimilis* are much less
than that of synthetic pesticides, the significant reduction in walking speed and
searching efficiency on oil sprayed leaves indicates that longer term effects could
reduce the efficacy of predatory mites where exposure to oil is high. In the case of
continual exposure of *P. persimilis* to oil-sprayed plant surfaces, reduced searching
efficiency could lead to lower prey consumption, smaller size and reduced fecundity. In
the commercial situation these effects would be expected to be greatest when pest mite
populations were lower and inundative releases of *P. persimilis* were less frequent. To
reduce the likelihood of reduced efficacy of *P. persimilis* in a control program using
MO, I would recommend that a refuge from oil be provided. Nicetic et al. (2001, 2002) investigated a range of control options for TSM in greenhouse roses. He found a program of 0.50% v/v HMO sprays every 14 d to the upper production layer of the canopy combined with inundative releases of *P. persimilis* to the lower maintenance layer was highly effective in maintaining TSM populations below the economic threshold.

The experiments reported in this chapter were conducted with individual *P. persimilis* in a laboratory. While this experimental approach may result in a simplification of behaviour exhibited by *P. persimilis* in their normal habitat, it has the benefits of allowing clearer interpretation of the observed behaviour of individual mites. In the environment, food webs of plants-herbivores-carnivores are overlaid with infochemical webs that mediate direct and indirect interactions between plants, herbivores and carnivores (Dicke & Grostal, 2001). The utilisation of spider mite-induced plant volatiles by predatory mites for location of prey has been well documented (Sabelis & Baan, 1983; Zemek & Nachman, 1999; Pels & Sabelis, 2000; Dicke et al., 2003). Further research that considers the tritrophic interactions between the host plant, phytophagous and predatory mites is necessary to more fully understand the effects of HMOs and AMOs on the control efficacy of *P. persimilis*. 
Chapter 7. Impact of sublethal dose of nC24 agricultural mineral oil deposit on the functional and numerical response of predatory mite *Phytoseiulus persimilis* to *Tetranychus urticae*

Introduction

Since the early work of Solomon (1949) and Holling (1959a, b), functional and numerical responses have played a pivotal role in understanding prey-predator interactions and their ecological and evolutionary consequences on community dynamics (Tully *et al.* 2005). They can be used to infer basic mechanisms underlying the interactions of predator-prey behaviour, to clarify coevolutionary relationships, and to enhance practical predictive power for biological control (Houck & Strauss 1985, Lester & Harmsen 2002). When behavioural components of predator-prey interactions are interpreted with respect to some quantitative model, the resultant descriptive model can be applied to predictions about the dynamics of predator-prey associations (Hassell 1978).

Functional response describes how the consumption rate of an individual consumer changes with respect to resource density, and numerical response is how the per-capita reproductive rate changes with resources density (Solomon 1949).

Holling (1959a) identified three basic types of functional responses to prey density in general: Type I (linear) functional response in which the attack rate of the individual consumer increases linearly with prey density but then suddenly reaches a constant value when the consumer is satiated; Type II (cyrtoid) functional response in which the attack rate increases at a decreasing rate with prey density until it becomes constant at satiation; Type III (sigmoid) functional response in which the attack rate accelerates at first and then decelerates towards satiation.

The functional and numerical responses of phytoseiid mites to the density of tetranychid mites has been investigated by many authors (Chant 1961, Mori & Chant 1966b,
Functional and numerical responses of predatory mites to their prey can be modified by chemical and physical characteristics of host plants, for example, different species (Skirvin & de Courcy Williams 1999, Koveos & Broufas 2000, Skirvin & Fenlon 2001), and leaf hairiness density (Karban et al. 1995, Cedola et al. 2001, Stavrinides & Skirvin 2003). They are also influenced by long-term feeding history (Castagnoli & Simoni 1999, Lester et al. 2000) and diet shift (Castagnoli et al. 2001). Plant pollens (Wei & Walde 1997), alternative prey and other predators (Lester & Harmsen 2002), even the patch number (Lester et al. 2005) can affect responses. They can be modified by some abiotic factors, for instance, temperature (Everson 1980) and pesticides (Perera 1982, Kehrli & Wyss 2001, Claver et al. 2003, Wang et al. 2005).

Nevertheless, there is very little information on the impact of mineral oils on functional and numerical responses of predatory mites to their prey. Such an understanding of sublethal dosage of insecticides on predator-prey interactions would improve the potential for compatible utilisation of MOs and P. persimilis, and thereby provide opportunities to improve IPM programs for TSM. I determined these responses to 0.25% deposits of aqueous emulsion of nC24 SK EnSpray 99 in this chapter. I chose this oil concentration on the basis of research reported in earlier chapters that showed that it has a significant effect on TSM behaviour without causing high TSM or P. persimilis mortality.
Materials and methods

Plants and Mites

TSM and *P. persimilis* were obtained and maintained as described in Chapters 3 and 6. In this instance, adult TSM females used in the experiments had just moulted, and were in the pre-oviposition period.

Agricultural mineral oil

The AMO used in the experiments was SK Enspray 99. See Chapter 3 and Appendix 2 for further details.

Experimental procedure

The functional and numerical responses of *P. persimilis* to adult TSM females were measured on 34 mm bean leaf discs with 2 h old deposits of 0.25% AMO or distilled water applied to the discs with a Potter spray tower (see Chapter 3). Individual discs were placed upside down on a layer of moist cotton wool in a 90 mm Petri dish. The prey densities were 4, 8, 16, 32, or 64 newly moulted adult females per bean leaf disc. As their pre-oviposition period is 1-2 d at 25°C, TSM egg production was assumed to be negligible. Each leaf disc was infested with one female predator. A water barrier prevented mites from escaping from the leaf discs. The Petri dishes were kept in a plant growth chamber at 25°C.
Statistical analysis

The disc equation of Holling (1959b) was used to model the relationship between the number of prey killed ($N_a$) and initial prey density ($N$)

$$N_a = aN/(1 + aT_hN)$$

The random predator equation (Royama 1971, Rogers 1972) was also fitted to the data, and the better of the two fits was used.

$$N_a = N\{1–\exp[-a(1 – T_hN_a)]\}$$

Estimates of instantaneous rate of discovery or attack coefficient ($a$), and the handling time per prey item ($T_h$) were calculated using a nonlinear regression (NLR) procedure based on the Levenberg-Marquardt method (SPSS. 2001). The starting values of $a$ and $T_h$ required by the NLR procedure were found by linearly regressing $1/N_a$ against $1/N$ for each arena type (Figure 7.1; Figure 7.2). The Y-intercept is the initial estimate of $T_h$ and the reciprocal of the regression coefficient (slope) is an estimate of $a$ (Livdahl & Stiven 1983, Watson et al. 2000).

The starting values of $T_h$ and $a$ used were 0.172 and 1.522 for the control, respectively, and 0.092 and 0.652 for the AMO treatment, respectively (Figure 7.1; Figure 7.2). These initial estimates were then refined by NLR. Data did not fit the model if the asymptotic 95% confidence intervals (CIs) of $a$ and $T_h$ estimates included zero (i.e. the estimates did not differ significantly from zero) (Watson et al. 2000).
$y = 0.172 + 0.657x$

$r^2 = 0.994$
The numerical responses were modelled using the equation of Beddington et al (1976):

\[ F = \left( \frac{\lambda}{e} \right) (N_a - c) \]

Where \( F \) is fecundity; \( \lambda \) and \( c \) are constants; \( e \) is the mean biomass per egg; \( N_a \) is the prey consumed. This equation can be rewritten:

\[ F = E_1 N_a - E_0 \]

Where \( E_1 \) is a constant related to the predator’s efficiency in converting prey into predator eggs, biomass per egg and biomass per prey consumed. \( E_0 \) is the metabolic cost per time unit measured in terms of reduced fecundity. By combining the equation with the functional response equation an equation expressing predator reproductive rate in terms of prey density is obtained.

\[ F = \frac{E_1 aN}{(1 + aT_hN)} - E_0 \]

Differences between the two functional response curves in different treatments and the differences between the two numerical response curves in different treatments were analysed using a nonparametric rank sum test. To apply the test, a single functional response curve (or numerical response curve) is fitted to the pooled samples, and regression residuals are computed, keeping track of which residuals are from the first sample and which are from the second. Then they were tested by the Mann-Whitney \( U \) procedure, a nonparametric test for group difference (Holander & Wolfe 1973, Gibbons 1976, Houck & Strauss 1985).

The Mann-Whitney \( U \) test was used to determine differences for number of prey consumed, body width and length, and number of eggs produced between the same densities of different arena types. All analyses were performed using SPSS software (SPSS 2001).

**Results**

**Functional response**

Data in the control and AMO treatments fitted the disc equation better than the random predator equation (Table 7.1): the values of \( r^2 \) for the disc equation were greater than for the random predator equation. The asymptotic standard error of attack coefficient \( (a) \) for
the disc equation was smaller than that for the random predator equation in both control and AMO treatments. However, the asymptotic standard error of the handling time ($T_h$) for the disc equation was greater than that for the random predator equation in both control and AMO treatments. The values of $a$ and $T_h$ from the disc equation were smaller than those from the random predator equation.

Table 7.1. Comparison of fitness using the disc and random predator equations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Equation</th>
<th>$r^2$</th>
<th>$a$</th>
<th>Asymptotic standard error</th>
<th>$T_h$</th>
<th>Asymptotic standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>disc</td>
<td>0.9931</td>
<td>1.5573</td>
<td>0.0956</td>
<td>0.1734</td>
<td>0.0027</td>
</tr>
<tr>
<td></td>
<td>random predator</td>
<td>0.7662</td>
<td>2.0017</td>
<td>0.2369</td>
<td>0.1753</td>
<td>0.0015</td>
</tr>
<tr>
<td>AMO</td>
<td>disc</td>
<td>0.9863</td>
<td>0.6344</td>
<td>0.0743</td>
<td>0.0856</td>
<td>0.0061</td>
</tr>
<tr>
<td></td>
<td>random predator</td>
<td>0.9347</td>
<td>0.9305</td>
<td>0.1584</td>
<td>0.0946</td>
<td>0.0041</td>
</tr>
</tbody>
</table>

The functional responses of adult *P. persimilis* females to TSM adults on the two treatments fitted the disc equation (Figure 7.3). The functional response curves were significantly influenced by the AMO deposit ($z = -3.100$, $p = 0.002$). The attack coefficient ($a$) and handling time ($T_h$) were smaller for the AMO deposit than for the control (Table 7.2). Numbers of prey consumed by *P. persimilis* at densities of 4 and 8 prey in the control were significantly more than those in the AMO treatment, but reversed at densities of 16, 32 and 64 prey per disc (Table 7.3).
Figure 7.3. Functional responses of *P. persimilis* on adult *T. urticae* females in control and AMO treatments. Error bars are SE of means.

Table 7.2. Attack coefficient (*a*) and handling time (*T*<sub>h</sub>) with asymptotic 95% confidence interval in control and AMO treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>a</em></th>
<th>95% CI</th>
<th><em>T</em>&lt;sub&gt;h&lt;/sub&gt;</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.5573*</td>
<td>1.2532 – 1.8613</td>
<td>0.1734*</td>
<td>0.1646 – 0.1821</td>
</tr>
<tr>
<td>AMO</td>
<td>0.6344*</td>
<td>0.3979 – 0.8708</td>
<td>0.0856*</td>
<td>0.0663 – 0.1048</td>
</tr>
</tbody>
</table>

*indicates the difference between the control and AMO treatments is significant

Table 7.3. Mann-Whitney *U* test for the number of adult *T. urticae* females consumed by *P. persimilis* at different prey densities in the control and AMO treatments.

<table>
<thead>
<tr>
<th>Density</th>
<th>Mean of number consumed by predator</th>
<th><em>z</em></th>
<th><em>p</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>AMO</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.9472*</td>
<td>2.1167*</td>
<td>-2.223</td>
</tr>
<tr>
<td>8</td>
<td>4.0556*</td>
<td>3.1579*</td>
<td>-2.494</td>
</tr>
<tr>
<td>16</td>
<td>4.5790*</td>
<td>5.4500*</td>
<td>-2.241</td>
</tr>
<tr>
<td>32</td>
<td>5.2105**</td>
<td>7.8947**</td>
<td>-4.190</td>
</tr>
<tr>
<td>64</td>
<td>5.4500**</td>
<td>8.8000**</td>
<td>-4.751</td>
</tr>
</tbody>
</table>

*and** indicate that the differences between the control and AMO treatments were significant at* *p < 0.05 or** *p < 0.01.*
The body width and length of adult TSM

Both body length and body width of adult TSM females in the control were significantly greater than those in the AMO treatment (Table 7.4).

Table 7.4. Mann-Whitney U test for TSM body length and width in the control and AMO treatments

<table>
<thead>
<tr>
<th>TSM</th>
<th>Control</th>
<th>AMO</th>
<th>z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length (µm)</td>
<td>53.88**</td>
<td>46.06**</td>
<td>-7.392</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Width (µm)</td>
<td>28.75**</td>
<td>23.38**</td>
<td>-7.898</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

** indicates that the differences between the control and AMO treatments were significant (p < 0.01).

Numerical response

The relationships between numbers of prey consumed and eggs produced in the control and AMO treatments fitted the Beddington et al (1976) equation (Figure 7.4). $E_i$ in the control was significantly greater than that in the AMO treatment, demonstrating that the converting efficiency and/or the biomass of per prey in the control were greater than that in the AMO treatment. None of the estimates of $E_0$ were significantly different from zero, which indicates that *P. persimilis* females have a very low energy demand for maintenance of processes and that they allocate most of their energy to egg production (Table 7.5).
Figure 7.4. Relationship between the number of prey (adult TSM females) consumed and eggs produced by P. persimilis adult females.

Table 7.5. Parameter estimates of $E_1$ and $E_0$ with 95% confidence intervals in the control and AMO treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$E_1$</th>
<th>95% CI</th>
<th>$E_0$</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.682</td>
<td>0.651 – 0.713*</td>
<td>-0.101</td>
<td>-0.243 – 0.041</td>
</tr>
<tr>
<td>AMO</td>
<td>0.369</td>
<td>0.312 – 0.427*</td>
<td>0.022</td>
<td>-0.324 – 0.369</td>
</tr>
</tbody>
</table>
Figure 7.5. Numerical responses of adult *P. persimilis* females to adult *T. urticae* females in the control and AMO treatments. Error bars are SE of means.

Table 7.6. Mann-Whitney *U* test for the number of eggs produced by *P. persimilis* at different prey densities in the control and AMO treatments

<table>
<thead>
<tr>
<th>Density</th>
<th>Mean of eggs produced by predator</th>
<th>z</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control treatment</td>
<td>Oil treatment</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.9167**</td>
<td>0.8750**</td>
<td>-3.669</td>
</tr>
<tr>
<td>8</td>
<td>2.6389**</td>
<td>1.2188**</td>
<td>-4.589</td>
</tr>
<tr>
<td>16</td>
<td>3.0263**</td>
<td>1.8824**</td>
<td>-3.057</td>
</tr>
<tr>
<td>32</td>
<td>3.9444</td>
<td>2.9444</td>
<td>-1.615</td>
</tr>
<tr>
<td>64</td>
<td>3.9316</td>
<td>3.3421</td>
<td>-1.062</td>
</tr>
</tbody>
</table>

**indicates that the difference between the control and AMO treatments was significant (p < 0.01)

**Discussion and Conclusions**

In Chapter 3 I showed that only 8% of TSM eggs on deposits of oil sprays of 0.20% aqueous emulsions developed to adults, and that no eggs developed to adults on 0.30% deposits. It was therefore not feasible to use TSM eggs as prey, or the quiescent larva, protonymph or deutonymph as they are known to be unsuitable for derivation of functional and numerical responses (Laing & Osborn 1974). Adult *P. persimilis* were
chosen for the study because the larval stage does not feed, and the developmental times for both the protonymph and the deutonymph of *P. persimilis* are similar, about 1 d at 25°C, which is very short in comparison to length of time, about 50 d, over which female adults feed (Osborne *et al.* 1999).


*P. persimilis* adult females consumed fewer prey and produced fewer eggs at low prey densities (4 and 8 adult TSM females/disc) on AMO deposits than they did at these prey densities on water-sprayed discs. This result suggests that the AMO deposits suppressed the searching efficiency of the predator. The effects of leaf hairs on the searching efficiency and predation rate of adult female *P. persimilis* have been documented (Krips *et al.* 1999). My results show that AMO deposits significantly reduce the searching efficiency of *P. persimilis* by reducing its walking speed (see Chapter 5).

Although the predator consumed fewer prey and produced fewer eggs at low prey densities (4 and 8 adult TSM females/disc) it consumed more prey at higher densities (16, 32, 64 adult TSM females/disc) on AMO deposits than at these prey densities on water-sprayed control discs. My results suggest that this was due to the impact of the AMO on feeding by the prey (see Chapter 3) and this led to smaller-sized prey on AMO treated discs. Under these circumstances, the predator must consume more prey to be satiated. It seems that at the higher prey densities, the walking speed of *P. persimilis* was not as crucial for effective searching behaviour, as the predator has a higher chance of encountering prey as prey densities increase. This suggests that MO deposits can play an important role in regulating prey-predator interactions. On one hand, MO deposits reduce the feeding and oviposition by TSM; on the other hand, they enhance predation of *P. persimilis* at certain prey densities. The compatibility of MOs and *P. persimilis* in IPM programs for TSM on greenhouse tomato and roses has been reported (French *et al.* 1976, Nicetic *et al.* 2001, Nicetic *et al.* 2002). The impact of MO deposits on the
functional response of the predator to its prey is probably a major factor contributing to this compatibility.

My results indicate that *P. persimilis* has a very low energy demand for maintenance processes. The fecundity of *P. persimilis* increased linearly with increasing numbers of prey killed. Similar results also been reported by several researchers (Eveleigh & Chant 1981b, Sabelis 1985, Nwilene & Nachman 1996b). My results also showed that MOs and *P. persimilis* are compatible, especially when TSM populations are high.
Chapter 8. Impact of nC24 agricultural mineral oil on olfactory responses of *Phytoseiulus persimilis* and *Tetranychus urticae* to the herbivore-induced plant volatiles

**Introduction**

Ecosystems are overlaid with infochemical webs that mediate direct and indirect interactions between the plants, herbivores and carnivores (Polis & Strong 1996, Dicke & Loon 2000, Dicke & Grostal 2001). Foraging herbivorous and carnivorous arthropods assess chemical information both prior to and after physical contact with their food (Turlings et al. 1990, Vet & Dicke 1992, Dicke & Loon 2000). Plants respond to herbivore feeding damage by emitting volatiles that attract natural enemies to attack the herbivore (de Moraes et al. 1998, Pare & Tumlinson 1999, Kessler & Baldwin 2001, van den Boom 2003, de Boer & Dicke 2005b): this is known as an indirect plant defence mechanism (Agrawal et al. 2002, Dicke et al. 2003b).

The importance of TSM-induced volatiles as foraging cues for *P. persimilis* has been reported for at least 16 plant species in 8 plant families (de Boer & Dicke 2005b). Clear attraction of *P. persimilis* to volatiles from TSM-infested lima beans has been consistently reported during the past 20 years (Dicke 1986, Takabayashi & Dicke 1992, Garms et al. 1998, Arimura et al. 2000, Ozawa et al. 2000, Choh et al. 2004, de Boer & Dicke 2004b, de Boer et al. 2005, Shimoda et al. 2005). Phytoseiid mites use herbivore-induced plant volatiles in long-range prey-habitat location (Sabelis & van de Baan 1983) and are arrested by these volatiles on entering a prey patch (Margolies et al. 1997). Responses of predatory mites to these volatiles are considered to be an important factor in the local extermination of prey populations by phytoseiids (Dicke et al. 1998).

Plant volatiles are also important cues for foraging TSM. Uninfested lima bean leaves attract TSM (Dicke 1986) and volatiles of strawberry (*Fragaria virginiana* Duschesne [Rosales: Rosaceae]) have long-distance effects on the behaviour of the mite

Herbivores can also use herbivore-induced plant volatiles as cues for host location (Dicke 1986, Dicke et al. 1991). TSM has been reported to be slightly attracted by the odours of cucumber (Cucumis sativus L. [Violales: Cucurbitaceae]) infested with conspecifics, and more mites were found on greenhouse plants infested with conspecifics than on clean plants (Pallini et al. 1997). Similar positive olfactory responses of TSM to slightly infested lima bean leaves were recently reported by Horiuchi et al. (2003b). Gols et al. (2003) found that the presence of herbivore induced chemicals and/or spider mite products enhanced the settlement of the spider mites.

Responses by plants to various stresses, such as heat shock, water deficiency, ozone levels, and heavy metals are characterised by the expression of defence-related genes, the emission of ethylene and other plant volatiles, the biosynthesis of signalling molecules such as jasmonic acid, salicylic acid and ethylene (Kangasjarvi et al. 1994, Rao et al. 2000, Vuorinen et al. 2004). Accordingly, it has been suggested that these factors can activate direct and indirect defence systems of plants (Sandermann et al. 1998, Paolacci et al. 2001), including emission of plant volatiles induced by herbivore feeding (Vuorinen et al. 2004). MOs, as complex mixtures of a large number of hydrocarbons, may (a) directly stimulate feeding by TSM and predation by P. persimilis or (b) indirectly enhance host plant detection by TSM and prey detection by P. persimilis by inducing the release of plant volatiles or by masking their detection.

As there is limited knowledge about the physiological effects of MOs on plants, and therefore, on tritrophic interactions between host plant, herbivores and predators I sought, in this chapter, to determine if such interactions occur for P. persimilis and TSM.

Materials and methods

Plants and Mites

TSM and P. persimilis were obtained and maintained as described in Chapters 3 and 6.
Agricultural mineral oil

The AMO used in the experiments was SK Enspray 99. See Chapter 3 and Appendix 2 for further details.

Experiment I: Olfactory responses of P. persimilis and TSM to plants infested with different densities of TSM

Bean plants, each 18-20 cm tall and with two fully expanded primary leaves, were cut just above the potting mix in which they were growing. The stems of the excised plants were placed in bottles containing 40 mL of distilled water. Three excised plants, with a total of six leaves, were placed in each bottle. Each set of six leaves was infested with either 25, 50, 100 or 200 TSM adult females/leaf. The plants then placed in a climate-controlled room at 25 ± 2°C, 16:8 L:D photoperiod and 4110 lux fluorescent lights conditions for 48 h before the olfactometer responses of P. persimilis and T. urticae were tested in a Y-tube. Uninfested plants were used as control.

Experiment II: Olfactory responses of P. persimilis and TSM to blends of TSM-induced plant volatiles and AMO volatiles

TSM-induced plant volatile source: Bean plants were cut and infested with 200 TSM adult females/leaf, and then maintained as above. After 48 h, the plants were used as a T. urticae-induced plant volatile source.

AMO odour source: For each replicate, a piece 90 mm filter paper (Qualitative, Toyo Roshi Kaisha Ltd) was saturated with a 1 mL droplet of a freshly prepared 3.0% aqueous AMO emulsion. The quantity of AMO applied to the filter paper was approximately equal to the amount of AMO deposited on three plants sprayed with a 1.00% emulsion to run-off. Each filter paper was allowed to dry (2 h at 25° ± 2°C) and then used as an AMO odour source.

Olfactory responses of P. persimilis and TSM to (a) the AMO odour source vs distilled water treated filter paper and (b) the blends of TSM-induced plant volatiles + AMO
odour source vs uninfested plants + filter paper treated with distilled water, were tested in a Y-tube.

**Experiment III: Olfactory responses of *P. persimilis* and TSM to plants sprayed with different concentrations of AMO**

Bean plants with two fully expanded primary leaves were sprayed with 0.25%, 0.50% or 1.00% aqueous AMO emulsion or distilled water (control) to run-off using a hand-held sprayer. Each plant was allowed to dry for 2 h. It was then cut at the base of the stem and maintained as above. Olfactory responses of *P. persimilis* and TSM to the plants were tested 2 d, 3 d and 5 d after the application of the sprays.

**Y-tube olfactometer bioassays**

The olfactory response of *P. persimilis* and TSM were examined using a Y-tube olfactometer (Sabelis & van de Baan 1983, Takabayashi & Dicke 1992) (Figure 2.6). This equipment consisted of a Y-shaped glass tube with a metal wire in the centre. Pressurised air was filtered over activated charcoal. The airflow was divided into two and each subflow was led through a 2-L glass vessel, which contained an equal number of variously treated plants as an odour source. Subsequently the two odour flows were led through the two arms of the Y-tube olfactometer. The airflow through each olfactometer arm was 4 L/min, as measured with an airflow meter. The tests were undertaken in a fume cupboard (Figure 8.1). This prevented room odours affecting the experiment.
To enhance *P. persimilis* responses to volatiles, adult *P. persimilis* females were starved individually in Eppendorf tubes for 24 h prior to their release in the olfactometer. For each test, an individual female predator was placed at the ‘starting point’ of an iron Y-shaped wire fixed in the centre of the Y-tube. When the mite reached the distal end of either arm, its choice was recorded and it was then discarded. Predators that did not reach the end of an arm within 5 min of release were excluded from the statistical analysis. To correct for unforeseen asymmetry in the set-up of the apparatus, odour sources were interchanged after testing five mites. The system was purged for 5 min prior to release of the first predatory mite and after the interchange of odour sources. A total of 20 predators was used in an experiment. Each experiment was replicated four times on different days with a new set of odour sources and predatory mites. All bioassays were performed at 25° ± 2°C and 700 lux fluorescent light conditions.

For TSM, leaves heavily infested with adult females were placed in a plastic cup, which was placed on wet cotton wool, 2 h before use in experiments. Females readily started dispersing from the leaves and began walking along the side of the cup. The dispersing mites were introduced individually to the starting point of the iron Y-shaped wire. The method was the same as for *P. persimilis*. In addition, the Y-shaped wire was cleaned.
with a brush after each mite was tested. This ensured removal of any spider-mite web left on the wire (Horiuchi et al. 2003b).

**Data analysis**

The data were analysed with a $\chi^2$ test to test the null hypothesis that the mites had an equal chance to choose between the two odour sources. For the comparison of different treatments, a contingency table analysis with a chi-square test was used to test the overall differences among the treatments. When there was a significant difference among the treatments, a treatment-treatment combination was tested with a $2 \times 2$ contingency table analysis, using the Bonferroni approach to adjust the p-value (Siegel & Castellan, 1988, Horiuchi et al 2003b).

**Results**

**Experiment I. Olfactory responses of P. persimilis and TSM to plants infested with different densities of TSM**

Significantly more adult *P. persimilis* females moved toward the volatiles from the bean plant infested with 50 ($\chi^2 = 4.154, p = 0.042$), 100 ($\chi^2 = 7.911, p = 0.005$), and 200 ($\chi^2 = 7.200, p = 0.007$) TSM females per leaf than those from uninfested plants (Figure 8.2). There was no significant difference in the choice between the volatiles of 25 TSM per leaf and those of the control ($\chi^2 = 1.025, p = 0.311$). However among the different density treatments, the difference for the attraction of *P. persimilis* was not significant ($\chi^2 = 2.136, \text{df} = 3, p = 0.545$)
Figure 8.2. Y-tube olfactometer olfactory responses of adult *P. persimilis* females to bean plants infested with different densities of adult TSM females. Numbers in the bars represent individual mites that moved toward the volatiles (*p < 0.05; **p < 0.01).

Significantly more adult TSM females moved toward the volatiles from bean plants infested with 200 conspecifics than toward uninfested plants ($\chi^2 = 6.541$, $p = 0.011$). (Figure 8.3). However, there were no significant differences in the choice between the volatiles of 25 TSM per leaf vs the control ($\chi^2 = 0.114$, $p = 0.736$), 50 TSM per leaf vs the control ($\chi^2 = 0.462$, $p = 0.497$) and 100 TSM per leaf vs the control ($\chi^2 = 3.658$, $p = 0.056$).
Figure 8.3. Y-tube olfactometer olfactory responses of adult TSM females to bean plants infested with different densities of conspecifics. Numbers in the bars represent individual mites that moved toward the volatiles (*p < 0.05).

**Experiment II: Olfactory responses of *P. persimilis* and TSM to blends of TSM-induced plant volatiles and AMO volatiles**

There were no significant differences for the responses of adult *P. persimilis* females ($\chi^2 = 0.205$, $p = 0.651$) and adult TSM females ($\chi^2 = 0.200$, $p = 0.655$) between an AMO odour source and distilled-water treated control (Figure 8.4).

Figure 8.4. Y-tube olfactometer olfactory responses of adult *P. persimilis* females and adult TSM females to AMO volatiles. Numbers in the bars represent individual mites that moved toward the volatiles.
Significantly more *P. persimilis* females ($\chi^2 = 11.538, p = 0.001$) and TSM females ($\chi^2 = 4.263, p = 0.039$) moved toward the blends of TSM-induced plant volatiles and AMO volatiles than towards the distilled-water treated control (Figure 8.5). In comparison with the experiment for 200 TSM per leaf vs control (Experiment I), there was no significant difference between the response of *P. persimilis* to the blend vs the control and to 200 TSM-induced plant volatiles vs control ($\chi^2 = 0.320, df = 1, p = 0.572$). Similarly, there was no significant difference between the response of TSM to the blend vs the control and to 200 TSM-induced plant volatiles vs the control ($\chi^2 = 0.148, df = 1, p = 0.701$).

![Figure 8.5](image-url)  
*Figure 8.5.* Y-tube olfactometer olfactory responses of adult *P. persimilis* females and adult TSM females to blends of TSM-induced plant volatiles and AMO volatiles. Numbers in the bars represent individual mites that moved toward the volatiles (*$p < 0.05$; **$p < 0.01$).

**Experiment III: Olfactory responses of *P. persimilis* and TSM to plants sprayed with different concentrations of AMO**

Adult *P. persimilis* females exhibited no significant preference for volatiles from bean plants 2 d, 3 d and 5 d after the plants were sprayed with 0.25% AMO emulsions (Figure 8.6A), or to volatiles from bean plants 5 d after plants were sprayed with 0.50% or 1.00% AMO emulsions (Figure 8.6 B) by comparison with those from the plants treated with distilled water. However, significantly more adult *P. persimilis* females moved toward the volatiles from AMO-treated bean plants 2 d and 3 d after the plants...
were sprayed with 0.50% and 1.00% aqueous AMO emulsions, than toward volatiles from control plants (Figure 8.6 B, C).

Figure 8.6. Y-tube olfactometer olfactory responses of adult *P. persimilis* females to the bean plants treated with different concentrations of aqueous AMO emulsions. A. 0.25% AMO vs control; B. 0.50% AMO vs control; C. 1.00% AMO vs control. Number in bars represent individual mites that moved toward the volatiles (*p < 0.05; **p < 0.01).
For adult TSM females, there were no significant differences in choices between the volatiles from plants treated with 0.25%, 0.50% and 1.00% aqueous AMO emulsions and volatiles from the distilled-water treated control plants, 2 d, 3 d and 5 d after the plants were sprayed (Figure 8.7 A, B, C).

\[\text{Figure 8.7. Y-tube olfactometer olfactory responses of adult TSM females to the bean plants treated with different concentrations of aqueous AMO emulsions. A. 0.25% AMO vs control; B. 0.25% AMO vs control; C. 1.00% AMO vs control. Numbers in the bars represent individual mites that moved toward the volatiles.}\]
**Discussion and Conclusions**

Positive responses of adult *P. persimilis* females to volatiles from bean plants infested with adult TSM females were significant for all but the lowest (25 prey per leaf) of four prey densities tested and most significant for the two highest prey densities tested (100 and 200 prey per leaf). Similar results have been reported by other authors (Maeda & Takabayashi 2001, Horiuchi *et al.* 2003b).

High densities (200 mites per leaf) of adult TSM females elicited significant positive olfactory responses by conspecifics but responses to lower densities (25, 50 and 100 mites per leaf) were not significant. This result differs from that reported by Horiuchi *et al.* (2003b) who found that volatiles from slightly infested plants (20 TSM adult females/leaf) attracted conspecifics whereas volatiles from heavy infestations (300 TSM adult females/leaf) repelled the TSM. Horiuchi *et al.* (2003b) suggested that volatiles from slightly infested leaves may indicate an available food resource and a patch where predators are not present. They also suggested that leaves heavily infested with conspecifics were probably not a suitable food resource for foraging TSM, as a heavily infested plant was likely to die rapidly. Furthermore, they noted that such heavily infested plants attract *P. persimilis*, and that other predators also respond to TSM-induced plant volatiles (Horiuchi *et al.* 2003b). They hypothesised that the effects they observed, avoidance of heavily infested patches by conspecifics, were due to increasing release of the volatile, linalool, from leaves with increasing TSM densities, as linalool is one of the compounds responsible for TSM dispersal. The differences between my results for the impact of TSM densities and attraction of conspecifics to infested patches and those reported by (Horiuchi *et al.* 2003b) may be related to the TSM host plant used in the studies. I used French bean (*Phaseolus vulgaris*) whereas they used lima bean (*Phaseolus lunatus*). In studies reported in Chapter 9, I did not detect release of linalool from TSM infested French bean plants.

Pallini *et al.* (1997) reported that TSM was attracted to cucumber plants infested with conspecifics in olfactometer tests and greenhouse experiments. They suggested that the intrinsic growth rate of TSM also increased with TSM density, as it does for the
phytophagous passionvine mite, *Brevipalpus phoenicis* (Geijskes) [Acari: Tenuipalpidae] (Pallini *et al.* 1997). If so, *T. urticae* would be better off selecting infested rather than uninfested plants. In some olfactory experiments, Pallini *et al.* (1997) found significant preferences for infested plants, even when plants with more than 6000 adult TSM per plant were used as an odour source. They suggested that the preference of *T. urticae* for plants infested with conspecifics may be related the extent of TSM webbing on infested plants and consequent protection from some species of predators. Since it takes some time to produce a web that is sufficiently dense enough to offer such protection, it may be beneficial for TSM to move to plants where this web is already present (Pallini *et al.* 1997).

In the first experiment reported in this chapter, volatiles from nC24 AMO-impregnated filter papers had no positive or negative effect on the function of herbivore-induced volatiles in prey detection by *P. persimilis*, or of host plant location by TSM, when the oil was tested. However, bean plants treated with 0.50% and 1.00% aqueous AMO emulsions were more attractive to *P. persimilis* than distilled-water treated control plants, and plants treated with 0.25% AMO emulsions. These results suggest that the AMO may have, by directly or indirectly activating plant defences, induced emission of some of the same volatile chemicals released by plants in response to feeding by TSM. Many abiotic stress factors, such as heavy metals and ozone are known to induce the accumulation of secondary metabolites or the production of volatile compounds, which are typically induced during pathogen or herbivore attack. However, my results indicate that if volatiles were induced by the AMO, they must have been qualitatively or quantitatively different to TSM-induced volatiles, as volatiles from bean plants treated with the AMO had no significant effect on the olfactory responses of adult TSM females.
Chapter 9. Chemical analysis of volatiles from bean plants infested with *Tetranychus urticae*, and bean plants sprayed with nC24 agricultural mineral oil

Introduction

Many plant species are known to emit herbivore-induced volatiles in response to herbivore damage (van den Boom *et al.* 2004). *T. urticae*-infested lima bean leaves emit relatively large amounts of five compounds, (E)-β–ocimene, (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), linalool and methyl salicylate (MeSA). Four of these compounds, (E)-β–ocimene, linalool, DMNT and MeSA, attract *P. persimilis* (Dicke *et al.* 1990a).

The composition of volatiles varies qualitatively and quantitatively depending on the different plant-herbivore combination(s) and abiotic factors (Takabayashi *et al.* 1991, Takabayashi *et al.* 1994b, Takabayashi *et al.* 2000, Sun & Yin 2002, Dicke *et al.* 2003a). Plant types were more important than herbivore species in affecting the composition of the volatile blends (Takabayashi *et al.* 1991). The volatiles released upon *T. urticae*-infestation from at least 16 plant species from 8 different families have previously been analysed (de Boer & Dicke 2005b).

The production of volatile compounds by plants is often regarded as specifically directed against biotic threats. However, abiotic stress factors such as ozone and heavy metal ions can elicit similar responses. They can activate various defence responses in plants, including the emission of plant volatiles (Zhang & Davies 1989, Coutts *et al.* 1994, Faktor *et al.* 1997, Morange 1997, Johnson *et al.* 2002, Wu & Lin 2002, Vuorinen *et al.* 2004, Maksymiec *et al.* 2005, Schulze 2005).

Results of experiments reported in Chapter 8 suggested that deposits of AMO on bean leaves stimulated release of plant volatiles that were attractive to *P. persimilis*. The results also suggested that feeding by adult TSM females leads to the release of plant
volatiles that attract the predator. In this chapter I sought to confirm release of volatiles after application of AMO and after feeding by TSM, and to identify the volatiles.

**Materials and methods**

**Plants and Mites**

TSM and *P. persimilis* were obtained and maintained as described in Chapters 3 and 6.

**Agricultural mineral oil**

The AMO used in the experiments was SK Enspray 99. See Chapter 3 and Appendix 2 for further details.

**The preparation of odour sources**

For TSM-induced volatiles, stems of 18-20 cm tall bean plants, each with two fully expanded primary leaves, were cut just above potting-mix height. Sets of three such excised plants, a total of 6 leaves, were then placed, stem first, in bottles filled with 40 mL distilled water. Each leaf was then infested with 200 adult TSM females. The plants then placed in a climate-controlled room at 25° ± 2°C, 16:8 L:D photoperiod and 4110 lux fluorescent lights conditions for 48 h. After 48 h, the plants were used to collect headspace volatiles. Uninfested plants were used as the control.

For the AMO-induced volatiles, bean plants with two fully expanded primary leaves were sprayed with distilled water and 0.25%, 0.50% and 1.00% (w/w) aqueous AMO emulsions to run-off using hand-held sprayer. Each plant was allowed to dry for 2 h before it was cut at the base of the stem and maintained as above. Headspace volatiles were collected at 2 d, 3 d and 5 d after application of spray.

For detection of pure oil volatiles, 1 mL of a 3.00% aqueous AMO emulsion and 1 mL of distilled water placed on separate pieces of 90 mm filter paper (Qualitative, Toyo Roshi Kaisha Ltd), and the latter allowed to dry for 2 h.
Chemical analysis of the volatile compounds

The sets of three plants for each odour source were placed in separate airtight 2 L glass bottles. Headspace volatiles were collected by using 90 mg of Tenax TA (20/35 mesh, Alltech, Nicholasville, Kentucky, U.S.A) packed in a glass tube (3 mm i.d., 150 mm length) for 3 h at a flow rate of 250 mL/min of clean air. Tenax TA was heated at 230°C for 4 h before use. The air was cleaned through silica gel, molecular sieves 5A, activated charcoal and Tenax TA. Before the collection of volatiles, purified air was passed through the system for 30 min to purge the system (Takabayashi et al 1991, Horiuchi et al 2003b, De Boer et al 2004). Volatiles were collected at 25° ± 2°C. Sampling was repeated on four different days.

The adsorbed compounds were eluted with 2 mL of diethyl ether, and 0.5 µg of n-eicosane (the internal standard) was then added to the eluate. The eluate was concentrated to about 0.2 mL with a stream of gaseous N₂. The injection volume was 1 µL. Split ratio was 1:5. Injection port temperature was 250°C. The gas chromatograph (GC) used was a Varian Saturn 2000R equipped with a 30 m length, 0.32 mm i.d. and 0.25µm film thickness column (Alltech SE 30). The mass spectrometer (MS) used was a Varian Saturn 2000R. The GC was programmed at an initial temperature of 40°C for 5 min, then increased to 250°C at 15°C/min. The carrier gas was H₂ with a column flow of 1.6 mL/min. Compounds were identified by comparison of mass spectra with those in the NIST 98 library and Palisade Mass Spectral library, and by checking the retention indices. The peak area of each compound was measured by relative comparison with the internal standard.

The data for multiple comparisons were analysed using a one-way ANOVA followed by Duncan’s post hoc test (if the variances were equal) or Games-Howell test (if the variances were not equal). For the paired comparisons between two treatments, the Mann-Whitney U test was used (SPSS. 2001, Hills 2005).
Results

Chemical composition of volatiles from TSM-infested bean plants

The uninfested bean plants emitted small amounts of 2,3-hexanediol, (4E)-4-hexen-1-yl acetate, limonene, octamethyl cyclotetrasiloxane and (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) (Figure 9.1 B). The infested bean plants emitted large amounts of methyl salicylate (MeSA), (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) and a small amount of 3-octen-1-ol, in addition to the compounds mentioned above (Figure 9.1 A, Figure 9.2).

The total signal value (peak area) from volatiles produced by infested plants was 6.6 times more than that of the control plants. The peak areas of the four compounds, MeSA, DMNT, TMTT and (4E)-4-hexen-1-yl acetate, made up 91.58% of the total peak area in volatiles from infested plants (Figure 9.3)

![figure 9.1](image-url)

**Figure 9.1.** Gas chromatograph of volatiles from French bean. A, infested with TSM for 48 h; B, uninfested. (1) 2,3-hexanediol; (2) 3-octen-1-ol; (3) (4E)-4-hexen-1-yl acetate; (4) limonene; (5) octamethyl cyclotetrasiloxane; (6) DMNT; (7) MeSA; (8) TMTT; (IS) internal standard; Crossed peaks and unlabelled peaks are contaminants from the solvent or machinery.
**Figure 9.2.** Chemical structures of volatiles from TSM-infested bean plants.

**Figure 9.3.** Relative peak areas of gas chromatographs for volatile chemicals from TSM-infested bean plants and uninfested bean plants. (1) 2,3-hexanediol; (2) 3-octen-1-ol; (3) (4E)-4-hexen-1-yl acetate; (4) limonene; (5) octamethyl cyclotetrasiloxane; (6) DMNT; (7) MeSA; (8) TMTT. For each compound different letters above bars indicate that difference between uninfested and infested leaves were significant (p < 0.05). nd: not detected; nc: the peak area is very small and was not calculated.
Chemical composition of volatiles from bean plants treated with AMO

Fifteen volatile compounds were detected from bean plants 2 d after the plants were treated with 0.50% and 1.00% aqueous AMO emulsions (Figure 9.4). Three of the compounds, hexadecahydropyrene and two unidentified compounds, were also detected as volatiles from AMO impregnated filter paper 2 d after the papers were treated with oil (Figure 9.7). Five compounds, 2,3-hexanediol, (4E)-4-hexen-1-yl acetate, limonene, octamethyl cyclotetrasiloxane and DMNT, were also emitted by bean plants treated with distilled water but at much reduced levels. The seven other compounds emitted by plants treated with 0.50% and 1.00% aqueous AMO emulsion were cyclooctatetraene, 3-octen-1-ol, MeSA, decamethyl cyclopentasiloxane, dodecamethyl cyclohexasiloxane, caryophyllen-13-al and TMTT (Figures 9.4-9.8).

Figure 9.4 Gas chromatograph of volatiles from French bean plants 2 d after the plants were sprayed with different concentrations (w/w) of nC24 AMO SK EnSpray 99 in aqueous emulsions. A, 1.00%; B, 0.50%; C, 0.25%; D, distilled water; (1) 2,3-hexanediol; (2) cyclooctatetraene, (3) 3-octen-1-ol; (4) (4E)-4-hexen-1-yl acetate; (5) limonene; (6) octamethyl cyclotetrasiloxane; (7) DMNT; (8) MeSA; (9) decamethyl cyclopentasiloxane; (10) dodecamethyl cyclohexasiloxane; (11) caryophyllen-13-al; (12) TMTT (13) hexadecahydropyrene; (14) unknown; (15) unknown; (IS) internal standard; Crossed peaks and unlabeled peaks are contaminants from the solvent or machinery.
Figure 9.5. Gas chromatograph of volatiles from French bean plants 3 d after the plants were sprayed with different concentrations (w/w) of nC24 AMO SK EnSpray 99 in aqueous emulsions. A, 1.00%; B, 0.50%; C, 0.25%; D, distilled water. Labels correspond to those in Figure 9.4.

Figure 9.6. Gas chromatograph of volatiles from French bean plants 5 d after the plants were sprayed with different concentrations (w/w) of nC24 AMO SK EnSpray 99 in aqueous emulsions. A, 1.00%; B, 0.50%; C, 0.25%; D, distilled water. Labels correspond to those in Figure 9.4.
Figure 9.7. Gas chromatograph of volatiles from 2 h-old-dry deposits of \( n \)C24 AMO SK EnSpray 99 on filter paper; A, filter paper treated with AMO; B, filter paper treated with distilled water. Labels correspond to those in Figure 9.4.
Figure 9.8. Chemical structures of volatiles from nC24 AMO-treated bean plants (none of these molecules occur in AMOs).

The total signal values of plant volatiles released from the plants treated with distilled water and 0.25% aqueous AMO emulsions were similar 2 d, 3 d and 5 d after the application of sprays, but significantly less than the total signal values for plants treated with 0.50% and 1.00% AMO on the same days after treatment (Figure 9.9). However, a rapid reduction in the amounts of those compounds for plants treated with 1.00% AMO was observed 3 d after the application of sprays, and after 5 d for plants treated with 0.50% AMO (Figure 9.10).
The total peak area of volatiles from plants treated with 1.00% AMO was significantly more than that from plants treated with 0.50% at 2 d (Figure 9.9). After comparing the amounts of individual compounds, it was clear that only MeSA was significantly more prevalent in volatiles from plants treated with 1.00% AMO than in volatiles from plants treated with 0.50% AMO (Figure 9.11).

The peak areas of the six compounds, MeSA, 2,3-hexanediol, (4E)-4-hexen-1-yl acetate, DMNT, cyclooctatetraene and DMNT made up 78.50% of the blends emitted by bean plants treated with 1.00% AMO and 71.62% of the blends emitted by plants treated with 0.50% AMO at 2 d (Figure 9.11).

Comparison of volatiles from TSM-infested plants and volatiles from plants treated with 1.00% aqueous AMO emulsion.

Application of 1.00% aqueous AMO emulsions to bean plants resulted in the release of all of the volatile chemicals detected in chemicals released from TSM-infested plants. A very noticeable difference between chemicals released from TSM-infested and those released from AMO-treated plants was that four chemicals were only released from the latter. These were cyclooctatetraene, decamethyl cyclopentasiloxane, dodecamethyl cyclohexasiloxane and caryophyllen-13-al (chemicals 2, 9, 10 and 11 respectively on the x-axis in Figure 9.12). The quantity of 2,3-hexanediol (chemical 1) was greater in the AMO-induced volatiles than in the TSM-induced volatiles but the quantities of MeSA (chemical 8) and TMTT (chemical 12) were higher in the TSM-induced volatiles (Figure 9.12).
Figure 9.9. Total peak areas of gas chromatographs for volatiles of French bean plants after the plants were treated with different concentrations of nC24 AMO SK EnSpray 99. A, 2 d; B, 3 d; C, 5 d. Different letters on bars indicate significant differences between the treatments (p < 0.05).
Figure 9.10. Total peak areas of gas chromatographs for volatiles of French bean plants treated with 0.50% and 1.00% aqueous emulsions of nC24 AMO SK EnSpray 99. Different letters on bars indicate significant differences between the treatments (p < 0.05).

Figure 9.11. Relative peak areas of gas chromatographs for volatile chemicals from plants treated with 0.50% and 1.00% aqueous emulsions of nC24 AMO SK EnSpray 99. *indicates that difference of the same compound between the treatments is significant (p < 0.05). nc: the peak area is very small and was not calculated. There is no significant difference for unlabelled bars. Numbers of peaks correspond to those in Figure 9.4.
Figure 9.12. Comparison of volatiles from TSM-infested plants and volatiles from plants treated with 1.00% aqueous emulsion of nC24 AMO SK EnSpray 99. A, TSM infested; B, 1.00% AMO treated at 2d. * indicates that difference of the same compound between the treatments is significant (p < 0.05). nd: not detected. nc: the peak area is very small and was not calculated. There is no significant difference for unlabelled bars. Numbers of peaks correspond to those in Figure 9.4.

Discussion and Conclusions

A number of studies have reported that TSM-infested lima bean leaves emit relatively large amounts of five compounds, (E)-β-ocimene, DMNT, TMTT, linalool and MeSA (Dicke et al. 1990a, Choh et al. 2004, de Boer & Dicke 2005a). (Z)-3-hexen-1-olacetate,
one of the ‘green leaf’ volatile compounds has been reported to be emitted in relatively large quantity from lima bean leaves (de Boer et al. 2004). However, in my study, \((E)\)-\(\beta\)-ocimene and linalool were not detected in the volatiles from TSM-infested French bean plants, nor was \((Z)\)-3-hexen-1-ol acetate. MeSA, DMNT, TMTT and \((4E)\)-4-hexen-1-yl acetate comprised 91.58% of the total peak area of chemicals released from infested plants. These differences are probably related to intrinsic differences between the bean species.

My results indicate that \(n\)C24 SK EnSpray 99 deposits elicited a significant stress response by French bean plants and this response included production of volatile compounds related to indirect and direct defence responses of the plants. The results showed that deposits of 0.50% and 1.00% aqueous SK EnSpray 99 emulsions, when applied to run-off, induced release of all of the chemicals reported to be released in response to TSM infestations.

One striking difference between volatiles from TSM-infested plants and AMO-sprayed plants was that four chemicals were only present among the volatiles released from the oil sprayed plants. These chemicals were cyclooctatetraene, decamethyl cyclopentasiloxane, dodecamethyl cyclohexasiloxane and caryophyllen-13-al. Their function, biosynthesis and role in tri-trophic interactions between plants, phytophagous pests and their natural enemies could warrant further research. The impact of the AMO on the quantities of volatile chemicals released by sprayed bean plants was clearly related to increasing dose (\(\mu\)g oil cm\(^{-2}\)). Application of 0.25 % AMO to bean plants did not induce the production of detectable quantities of MeSA and TMTT, but higher concentrations did. Further research is required to determine if multiple applications of, for example, four 0.25% sprays at fortnightly intervals, would have the same effect as single 0.50% and 1.00% sprays.

The modes of action of MOs against phytophagous arthropods include suffocation and impacts on feeding and oviposition behaviour. Impacts on arthropod behaviour are now considered to be the most important (Beattie et al. 2002b). MOs also have curative and preventative effects on susceptible plant pathogens (Childers 2002, Nicetic et al 2002).
results suggest that elicitation of plant defence mechanisms by MOs might be a possible reason for some of these effects.
Chapter 10. General discussion and conclusions

The modes of action of MOs against phytophagous arthropods include suffocation and impacts on feeding and oviposition behaviour. Impacts on behaviour are now considered to be the most important mode of action (Beattie et al. 2002b). My research focused on determining the impacts of an nC24 agricultural mineral oil (AMO) on the interactions between French bean, two-spotted mite (TSM) and the predatory mite Phytoseiulus persimilis. This was achieved by comparing the relative toxicity of topically applied AMO and of AMO deposit to TSM and P. persimilis, investigating the behavioural effects of AMO on TSM and P. persimilis, evaluating the impact of AMO on the functional and numerical responses of P. persimilis to TSM, and determining the impact of AMO on production and function of herbivore-induced plant volatiles.

Relative toxicity of nC24 agricultural mineral oil to TSM and P. persimilis

My results indicate that for topical and residual toxicity of AMO to the mites, the egg of TSM was the most susceptible stage, and, conversely, the egg of P. persimilis was the least susceptible stage. Photographs taken with an environmental scanning electron microscope (ESEM) showed that the surface of a TSM egg is usually well covered with fine silk that females use to attach their eggs to plant surfaces. This might trap oil on the surface and, in addition to egg size, incubation period (Smith & Pearce 1948) and the special respiratory apparatus (Dittrich 1971), contribute to the susceptibility of eggs to mineral oils.

Herron et al. (1995) and Herron et al. (1998) found that adult TSM females were less susceptible to MOs than several citrus pests for which MOs have traditionally been used for their control. I also found that all the motile stages of TSM tested were tolerant to AMO. The results suggested that a limited scope for killing TSM directly in management programs based on the use of 1-2% MOs. This has proved to be a practical limitation in the field control of TSM in apples (Thwaite et al. 2002). I found that the
most susceptible stage of *P. persimilis* to the nC24 AMO was the larva. The susceptibility of the protonymph and adult stage was similar for TSM and *P. persimilis*, and doses that would kill most of the motile stages of TSM would also be harmful to *P. persimilis*. Therefore, the most appropriate doses for use of MOs on beans in IPM with *P. persimilis* would be low, not high. For example, 0.2-0.3% (w/w) sprays of SK EnSpray 99 which can kill 75-85% of TSM eggs will also kill 35-55% of larvae and protonymphs of *P. persimilis*, and 30-45% of predator adults on beans.

Spray deposits of aqueous emulsions of nC24 AMO significantly impeded the development of immature TSM. No immature TSM could develop to adulthood on 0.30% AMO deposits, and the effect could last 7 d. Most immature TSM died in the egg or larval stages. In contrast, 0.30% AMO deposits had relatively less impact on *P. persimilis*. Fifty percent of its immature stages developed to adulthood on fresh AMO deposits and the negative effects of the deposits decreased sharply after 3 d.

**The impact of nC24 agricultural mineral oil deposits on the behaviour of TSM and *P. persimilis***

Deposit of 0.25% (w/w) aqueous emulsion of the nC24 AMO had highly significant effects on the behaviour of adult TSM. The impact was largely related to feeding deterrence. This result indicates that control of TSM with MOs should be strategically aimed at suppressing feeding by the pest rather than focusing on killing any or all stages in the mite’s life cycle. I hypothesize that feeding deterrence results in oviposition suppression, as the mites cannot obtain enough nutrition to produce eggs.

My research demonstrated that the most important factors contributing to the impact of MO deposits on searching efficiency and predation rates of *P. persimilis* are suppression of walking speed and contamination of mechanoreceptors and chemoreceptors on anterior tarsi and pedipalps. These effects increased rapidly with increasing oil concentration in the aqueous emulsions.

After reviewing my results in Chapters 3, 4, 5 and 6, I concluded that low concentrations (0.30 - 0.50%) of MOs can effectively control TSM on beans through
combined effects on mite mortality, development of the immature stages and mite behaviour. nC24 AMO sprays applied as 0.30% aqueous emulsions killed 85% of eggs and no mites that hatched from eggs laid up to 7 d after the application of sprays reached adulthood. Deposits of this dose also suppressed feeding and oviposition by adult females by 70% and 55%, respectively, for up to 7 d after AMO application. This conclusion is supported by the successful field control of TSM using 0.3% to 0.5% v/v nC24 HMO (Cooper et al. 2002). This dose is also safe to use in TSM IPM programs with P. persimilis if sprays are strategically applied to upper canopies (Nicetic et al. 2001).

Impact of nC24 agricultural mineral oil deposits on functional and numerical responses of P. persimilis to TSM

My research indicated that although adult P. persimilis females consumed fewer prey and produced fewer eggs at low prey densities (4 and 8 adult TSM females/disc) on AMO deposits, they consumed more prey at higher densities (16, 32, 64 adult TSM females/disc) on the AMO deposits than they did at these prey densities on the water-sprayed control. I attributed this to the impact of the AMO on feeding behaviour of TSM (see Chapter 3), which led to smaller-sized prey for the predator in the AMO treatments. Under these circumstances, it appears that the predator must consume more prey to be satiated. Results of these experiments suggested that MO deposits can thus play an important role in regulating prey-predator interactions. On one hand, MO deposits reduce feeding and oviposition by TSM; and on the other hand, they enhance predation of P. persimilis at certain prey densities. This suggests MOs and P. persimilis are compatible, especially when TSM populations are high. The compatibility of MOs and P. persimilis in IPM programs for TSM on greenhouse tomato and roses has been reported (French et al. 1976, Nicetic et al. 2001, Nicetic et al. 2002). The impact of MO deposits on the functional and numerical response of the predator to its prey is probably a major factor contributing to this compatibility.
Impact of \( nC24 \) agricultural mineral oil on production and function of herbivore-induced plant volatiles

Volatiles from \( nC24 \) AMO had no positive or negative effect on the preference of TSM and \( P. \) persimilis to the herbivore-induced volatiles. However, French bean plants treated with 0.50% and 1.00% aqueous AMO emulsions were more attractive to \( P. \) persimilis than distilled-water treated control plants and plants treated with 0.25% AMO emulsions. These results suggest that the AMO induced emission of some of the same volatile chemicals that are released by plants in response to feeding by TSM. Many abiotic stress factors, such as heavy metals (Maksymiec et al. 2005) and ozone (Vuorinen et al. 2004), are known to induce the accumulation of secondary metabolites or the production of volatile compounds, which are typically induced during herbivore attack. However, my results indicate that if volatiles were induced by the AMO, they must have been qualitatively or quantitatively different to TSM-induced volatiles, as volatiles from bean plants treated with the AMO had no significant effect on the olfactory responses of adult TSM females.

The chemical analysis of the volatiles from French bean plants infested with TSM and the plants sprayed with AMO indicated that 0.50% and 1.00% AMO deposits elicited the production of volatile compounds, including all chemical components of TSM-induced volatiles:

- salicylate (MeSA),
- (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) 3-octen-1-ol,
- 2,3-hexanediol,
- (4E)-4-hexen-1-yl acetate,
- limonene,
- octamethyl cyclotetrasiloxane, and
- (3E)-4,8-dimethyl-1,3,7-nonatriene (DMNT).

The quantities of 2,3-hexanediol and DMNT were greater in the 1.00% AMO-induced volatiles than in the TSM-induced volatiles but quantities of MeSA and TMTT were higher in the TSM-induced volatiles than in 1.00% AMO-induced volatiles. The
qualitative difference between volatiles from TSM-infested plants and AMO-sprayed plants was that four chemicals were only present among those released from the oil sprayed plants. These chemicals were cyclooctatetraene, decamethyl cyclopentasiloxane, dodecamethyl cyclohexasiloxane and caryophyllen-13-al. Their function, biosynthesis and role in tri-trophic interactions between plants, phytophagous pests and their natural enemies could warrant for further research.

The impact of the AMO on the quantities of volatile chemicals released by sprayed bean plants was clearly related to increasing dose. Application of 0.25% AMO did not induce the production of detectable quantities of MeSA and TMTT, but higher concentrations did. Further research is required to understand the effect of multiple applications of lower AMO doses, for example, four 0.25% sprays at fortnightly intervals, on the production of plant volatiles, as my research has suggested that the most appropriate doses for use of MOs in IPM with *P. persimilis* would be no more than 0.50%.

MOs also have curative and preventative effects on susceptible plant pathogens (Beattie *et al.* 2002b, Childers 2002, Cooper *et al.* 2002, Nicetic *et al.* 2002) My results suggest that elicitation of plant defence mechanisms by MOs might be a possible reason for some of these effects, which should be an important further research topic.

TSM-induced synthetic activity has been reported (Bouwmeester *et al.* 1999). Genes involved in TSM-induced volatile formation have been discovered in some plant species (Arimura *et al.* 2000, Ament *et al.* 2004, Arimura *et al.* 2004, Kant *et al.* 2004, Mercke *et al.* 2004). Transgenic plants that emit some components of the volatiles have been developed, and these genetically modified plants attracted predatory mites (Kappers *et al.* 2005). Future investigation should be directed towards the possible defence mechanisms induced by AMO at the molecular level. The understanding of the metabolic pathways and their regulation on protein and gene level would play an important role in development of genetically modified plants.

The ecological effects of AMO-induced defence on the pests and natural enemy population dynamics in the field would also be an important research topic. The indirect
defences of plants can be operated in agricultural systems (Kessler & Baldwin 2001). MeSA can be a useful tool to manipulate the behaviour of natural enemies as a method of biological control against herbivorous pests in agroecosystems (Shimoda et al. 2002). The use of controlled-release MeSA in field crops can increase the recruitment and residency of populations of certain beneficial arthropods. This phenomenon has the potential to enhance the efficacy and reliability of conservation biological control in crop pest management (James & Price 2004, James & Castle 2005). The function of AMO seems more powerful than MeSA. AMO can kill pests directly, significantly impede the development of immature stages of TSM and suppress the feeding and oviposition of TSM, regulate prey-predator interactions, and enhance the predation of *P. persimilis*, besides inducing the production of volatiles, which include MeSA. The ecological effects of MO-induced defences would help us to more comprehensively understand the impacts of these old but effective crop protectants.
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## Appendix 1. Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>AMOs</td>
<td>agricultural mineral oils</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>cv</td>
<td>cultivar</td>
</tr>
<tr>
<td>DMNT</td>
<td>((3E)-4,8\text{-dimethyl-1,3,7-nonatriene})</td>
</tr>
<tr>
<td>DSS</td>
<td>down sampling step</td>
</tr>
<tr>
<td>ESEM</td>
<td>environment scanning electron microscopy</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatograph</td>
</tr>
<tr>
<td>GC-MS</td>
<td>gas chromatograph-mass spectrometer</td>
</tr>
<tr>
<td>HMOs</td>
<td>horticultural mineral oils</td>
</tr>
<tr>
<td>IPDM</td>
<td>integrated pest and disease management</td>
</tr>
<tr>
<td>kPa</td>
<td>kilo pascal</td>
</tr>
<tr>
<td>JA</td>
<td>jasmonic acid</td>
</tr>
<tr>
<td>LC\textsubscript{50}</td>
<td>medium lethal concentration</td>
</tr>
<tr>
<td>L:D</td>
<td>light:dark</td>
</tr>
<tr>
<td>MeSA</td>
<td>methyl salicylate</td>
</tr>
<tr>
<td>MOs</td>
<td>mineral oils</td>
</tr>
<tr>
<td>NLR</td>
<td>nonlinear regression</td>
</tr>
<tr>
<td>PDSOs</td>
<td>petroleum-derived spray oil</td>
</tr>
<tr>
<td>RH</td>
<td>relative humidity</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
</tr>
<tr>
<td>SPME</td>
<td>solid phase micro-extraction</td>
</tr>
<tr>
<td>TMTT</td>
<td>((3E,7E)-4,8,12\text{-trimethyl-1,3,7,11-tridecatetraene})</td>
</tr>
<tr>
<td>TSM</td>
<td>two-spotted mite</td>
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Appendix 2. Typical data for SK Enspray 99

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<tr>
<th>Specification</th>
<th>Test method</th>
<th>YK 70N</th>
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<tr>
<td>Distillation temperature: °C at 101.33 kPa</td>
<td>ASTM D 2887</td>
<td>344.0</td>
</tr>
<tr>
<td>10% point</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% point</td>
<td></td>
<td>394.5</td>
</tr>
<tr>
<td>90% point</td>
<td></td>
<td>426.0</td>
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<tr>
<td>Distillation temperature: °C at 1.33 kPa</td>
<td>ASTM D 1160</td>
<td>196.0</td>
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<tr>
<td>10% point</td>
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<tr>
<td>50% point</td>
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<td>243.5</td>
</tr>
<tr>
<td>90% point</td>
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<td>265.5</td>
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<tr>
<td>Median equivalent n-paraffin carbon number (nC)</td>
<td>ASTM D 2887</td>
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<td>10% point</td>
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<td>20</td>
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<tr>
<td>50% point</td>
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<td>24.3</td>
</tr>
<tr>
<td>90% point</td>
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<td>27.4</td>
</tr>
<tr>
<td>Mean molecular weight density 15°C</td>
<td>ASTM D 4052</td>
<td>-</td>
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<tr>
<td>Viscosity: Saybolt Universal Seconds at 37.8°C</td>
<td>ASTM D 2161</td>
<td>71.1</td>
</tr>
<tr>
<td>Viscosity: Kinematic</td>
<td>ASTM D 445</td>
<td></td>
</tr>
<tr>
<td>at 40°C</td>
<td></td>
<td>12.43</td>
</tr>
<tr>
<td>at 100°C</td>
<td></td>
<td>3.12</td>
</tr>
<tr>
<td>Pour point maximum (°C)</td>
<td>ASTM D 97</td>
<td>- 24</td>
</tr>
<tr>
<td>Unsulphonated residue: % min volume</td>
<td>ASTM D 483</td>
<td>99</td>
</tr>
<tr>
<td>Density at 15°C</td>
<td>ASTM D 1298</td>
<td>0.8299</td>
</tr>
<tr>
<td>Molecular types (%)</td>
<td>ASTM D 2140</td>
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</tr>
<tr>
<td>C&lt;sub&gt;p&lt;/sub&gt; (paraffins)</td>
<td></td>
<td>74</td>
</tr>
<tr>
<td>C&lt;sub&gt;n&lt;/sub&gt; (naphthenes)</td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>C&lt;sub&gt;a&lt;/sub&gt; (aromatics)</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Composition analysis (wt %)</td>
<td>ASTM D 2549 &amp; D 2786</td>
<td></td>
</tr>
<tr>
<td>iso-paraffins</td>
<td></td>
<td>41.8</td>
</tr>
<tr>
<td>total naphthenes (cycloparaffins)</td>
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<td>58</td>
</tr>
<tr>
<td>mono-cyclic naphthenes</td>
<td></td>
<td>25.8</td>
</tr>
<tr>
<td>di-cyclic naphthenes</td>
<td></td>
<td>16.1</td>
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<tr>
<td>tri-cyclic naphthenes</td>
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<td>14.1</td>
</tr>
<tr>
<td>aromatics (not detectable)</td>
<td></td>
<td>&lt; 0.2</td>
</tr>
</tbody>
</table>

food grade = medicinal paraffin; UV stability P>N>A; Volatility A>N>P;
Skin absorption A>N>P; pour point – 24°C
Appendix 3. Mass spectra of detected volatiles

2,3-Hexanediol

Cyclooctatetraene
3-Octen-1-ol

(4E)-4-Hexen-1-yl acetate
Limonene

Octamethyl cyclotetrasiloxane
(3E)-4,8-Dimethyl-1,3,7-nonatriene

Methyl salicylate
Decamethyl cyclopentasiloxane

![Graph and Molecular Structure]

Dodecamethyl cyclohexasiloxane

![Graph and Molecular Structure]
Caryophyllen-13-al

(3E,7E)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene
$n$-Eicosane (internal standard)

Hexadecaahydropyrene
Unknown 1

BP 232 (G3597=100%)

Unknown 2

BP 232 (102174=100%)