# Chapter 1

# General introduction

## 1.1 The western flower thrips

Thrips are small, slender, usually winged, insects belonging to the order Thysanoptera, which is composed of two sub-orders, the Terebrantia and the Tubulifera. The seven families of the Terebrantia, the Uzelothripidae, Merothripidae, Aeolothripidae, Adiheterothripidae, Fauriellidae, Heterothripidae and Thripidae, account for approximately 2065 of the 5000 or so species in this order (Mound, 1997). The remaining 3100 species belong to the single Tubuliferan family Phlaeothripidae. The majority of species are phytophagous and some of these are serious pests of protected crops, whilst a few species are mycophagous or predatory in their feeding habits. Pest species occur in the four subfamilies of the Thripidae (the Thripinae, Panchaetothripinae, Dendrothripinae and Sericothripinae) and in phlaeothripid subfamily Phlaeothripinae. Since the mid 1980's the western flower thrips, *Frankliniella occidentalis* (Pergande), a highly polyphagous species, has spread from its native western USA to a worldwide distribution. This species is currently a major pest of both horticultural and floricultural protected crops in the UK. Cucumbers and chrysanthemums are particularly affected.

## 1.2 Biology

In common with the majority of Terebrantia, the *F. occidentalis* life cycle has six developmental stages: the egg, two feeding larval stages, two non-feeding pupal stages and the adult stage (Bryan & Smith, 1956; Lewis, 1973). The development of thrips is neither truly holometabolic or hemimetabolic. Although the appearance of larval stages closely resembles the adults, suggesting hemimetabolic development, the pupal stages undergo major internal reorganisation, which is typical of holometabolic development. Whilst thrips development is intermediate between holometabolism and hemimetabolism, the majority of researchers refer to thrips as holometabolic insects (Lewis, 1973).

### 1.2.1 Egg

The *F. occidentalis* are haplodiploid and reproduce by arrhenotokous parthenogenesis (Bryan & Smith, 1956; van Rijn *et al.*, 1995). Unfertilised haploid eggs will result in male offspring, and fertilised diploid eggs in female offspring, although mated females may produce both male and female offspring. The kidney-shaped egg has a smooth exterior, is pale white in colour, approximately 200 μm in length, and is laid directly into plant tissue (Moritz, 1997). During embryogenesis, the cells that would form the right mandible die and, when the egg is about to hatch, red eyespots can be clearly seen through the egg chorion. At the anterior end of terebrantian eggs there is an operculum, which is removed by a saw-shaped oviruptor at hatching. The larva emerges in an embryonic cuticle. In *Kakothrips pisivorus* (Westwood) this embryonic cuticle splits by movements of the pronotum and is then forced down to the tip of the abdomen by peristaltic movements, exposing the antennae and legs (Kirk, 1985). The larva, which is still attached to the egg by the tip of the abdomen, then rotates its body until the legs come into contact with the substrate, enabling the larva to detach itself from the egg (Lewis, 1973). This developmental stage requires approximately 2.6 d at 25°C (van Rijn *et al*, 1995).

### 1.2.2 Larvae

There are two larval stages in the *F. occidentalis*: larvae I and larvae II, which require approximately 2.3-2.8 d and 3.6-3.8 d to complete at 25°C, respectively (van Rijn *et al*, 1995). After hatching, larvae I are translucent, but soon develop a yellow colouration. Late larvae I and early larvae II are very similar in appearance, and can only be distinguished microscopically. Larvae I possess one pair of setae on the sternites of segments IV-VIII, whilst larvae II have three pairs of setae on these segments. Similarly,

the sex of larvae can only be distinguished microscopically. Female larvae I have three pairs of setae on abdominal segment IX, whilst males have four pairs. In larvae II, females have five pairs of setae and males have six pairs (Heming, 1991).

Larvae are able to walk and feed immediately after hatching. Walking is aided by arolia, or foot bladders, on each leg at the end of the tarsi. The arolia can be extruded and retracted, to grip the substrate, enabling larvae to walk on very smooth surfaces. The mouthparts are grouped into a structure called the mouthcone, which protrudes from beneath the head. Thrips use the 'punch and suck' mode of feeding. The left mandible is used to pierce a plant cell, creating a hole through which the maxillae, which form a tube, are inserted to suck up the cell contents (Chisholm & Lewis, 1984).

Although larvae, being soft bodied, appear vulnerable to attack by predators, they are capable of mounting a defence. *F. occidentalis* larvae repel attacks by vigorously jerking and wagging their abdomen at the predator. This defence is probably most effective in larvae II (van der Hoeven & van Rijn, 1990), as is the case in *Thrips tabaci* Lindeman (Bakker & Sabelis, 1989). In addition to defensive abdominal movements, the larvae of many thrips species will usually produce a drop of clear fluid from the tip of the abdomen when attacked. In the *F. occidentalis*, this anal droplet (AD) contains decyl acetate and dodecyl acetate, which are reported to function as an alarm pheromone, causing nearby larvae to move away from the emitter of the AD (Teerling, 1992; Teerling *et al.*, 1993).

### 1.2.3 Pupae

Late larvae II of the *F. occidentalis* leave the host plant and pupate in the soil underneath (Bennison *et al.*, 2001) at a depth of 1-5 mm (Helyer *et al.*, 1995). There are two quiescent pupal stages in the *F. occidentalis*: propupae and pupae, which require 1.1-1.2 d and 2.6-2.8 d to complete at 25°C, respectively (van Rijn *et al*, 1995). Propupae are distinctly different from larvae II. They are paler, possess wing buds, have erect antennae and are almost unable to walk, although they will move a little when disturbed. Pupae are distinct from propupae. The antennae are laid flat against the head and the wing buds, which are longer than in propupae, are initially clear but darken shortly before emergence of the adult. Unlike larvae, the pupal stages do not produce an anal droplet in response to attack, although they jerk the abdomen in a manner similar to that in larvae (personal observation).

### 1.2.4 Adults

Upon hatching, both adult males and females have a pale colouration (Bryan & Smith, 1956). Females are inactive for the first 24 h, and within 48 h have undergone teneral development which is probably temperature dependent (Lewis, 1973), and gained their final colouration. Whilst males remain pale in colour, females will develop one of three different colour forms (Bryan & Smith, 1956). According to Bryan & Smith (1956), these colour forms are under genetic control, the pale form being dominant, the dark form recessive and the intermediate form heterozygous. Sexual dimorphism is apparent, with females being larger than males.

After completion of teneral development, the adults of *F. occidentalis* are extremely active, both in walking and in flight. The flight periodicity, in Portuguese glasshouses, for males and females is the same with one peak of flight activity mid-morning and another mid-afternoon (Mateus *et al.*, 1996). There are no reports on the genetics of this periodicity in the literature. Higgins (1992) found that, at low population densities, 70-90% of thrips caught in traps are male, but at high densities this is reversed, with 65-90% of trapped thrips being female. Very few male *F. occidentalis* are found on the flowers and leaves of crops, 2-12% and 1-3%, respectively, even when high numbers are being trapped. The reason for this is unclear. It may be that males only remain on flowers long enough to mate with females, which concentrate in cucumber flowers (Higgins & Myers, 1992) and are more likely to be trapped as they spend more time flying (Higgins, 1992).

Females do not begin to lay eggs until approximately 1.8 d after eclosion at 25°C (van Rijn *et al.*, 1995). Oviposition rate peaks within the first 10 d after eclosion and then declines gradually, although the number of eggs laid is influenced greatly by diet (Trichilo & Leigh, 1988). Oviposition is known to occur mainly during daylight (Kirk *et al.*, 1999; Kiers *et al.*, 2000) and, in cucumber crops, most eggs are laid in the leaves, with younger leaves being selected over older leaves (de Kogel *et al.*, 1997). Few eggs are laid in the plant stems or flowers, which are ephemeral (Kiers *et al.*, 2000). The longevity of females thrips is longer than that for males (Lewis, 1973). In *F. occidentalis* this can be as long as three months at 15°C, although this reduces with increasing temperature (Katayama, 1997).

## 1.3 Economic impact

### 1.3.1 Introduction to the UK

The *F. occidentalis* began to spread within the USA in about 1980 and its presence was detected in Europe, in The Netherlands, in 1983 (Mantel & van de Vrie, 1988; Baker *et al.*, 1993). In June 1986, an outbreak of *F. occidentalis* was confirmed on chrysanthemums in Cambridge at a single location. Phytosanitary measures were initiated by the Ministry of Agriculture, Fisheries and Food (MAFF) in an attempt to eradicate the infestation, but this strategy soon changed to one of limiting the spread. In March 1987 the first infestation of a cucumber crop was confirmed and by the end of that year, 154 horticultural sites were confirmed to have *F. occidentalis*. In June 1989, MAFF decided that the *F. occidentalis* should be classed as established in the UK, and ceased attempts to limit its spread (Baker *et al.*, 1993).

### 1.3.2 Crops affected in the UK

In California, *F. occidentalis* have been collected from plants belonging to almost every order of Spermatophyta (Bryan & Smith, 1956). In the first two years or so of the presence of *F. occidentalis* in the UK, this species had been found on 69 genera of plants (Baker *et al.*, 1993). Although *F. occidentalis* may be able to survive mild winters outside glasshouses in the UK (McDonald *et al.*, 1997), they are primarily a pest of crops grown in glasshouses, and are considered to be of major importance in cucumbers (figure 1.1), sweet peppers, chrysanthemums and bedding plants (Jacobson, 1997). Control of *F. occidentalis* is largely through Integrated Pest Management (IPM, §1.3.4), and this is estimated to cost cucumber and sweet pepper growers £15,000 $ha^{-1}$ $season^{-1}$ and £7,200 $ha^{-1}$ $season^{-1}$, respectively (Jacobson, 1997). Of these costs, between approximately 20-30% are accounted for by biological control agents against thrips (§1.3.4.1).

### 1.3.3 Damage caused by *F. occidentalis*

*F. occidentalis* can affect crops, such as cucumber and sweet pepper, either directly, through feeding and oviposition activity, or indirectly, through the transmission of plant viruses.

Feeding and oviposition activity can affect the crop through activity on the fruit, and through activity on the rest of the plant that ultimately has a detrimental impact on the crop. Adult male and female *F. occidentalis* exhibit differential feeding behaviour (van de

Wetering *et al.*, 1998). Females feed more frequently and intensively than males, and so cause more scar formation on fruit. In cucumbers, this can result in curvature of rapidly growing fruit (figure 1.2), and bronzing and silvering in both cucumbers (figure 1.3) and sweet peppers, resulting in significant downgrading of fruit and so representing a financial loss to the grower (Shipp *et al.*, 1998; Hao *et al.*, 2002). *F. occidentalis* activity on the rest of the plant can reduce plant growth, photosynthesis (figure 1.4) and marketable yield, although such effects are not as serious as the effects on fruit (Shipp *et al.*, 1998; Hao *et al.*, 2002). In both cucumber and sweet pepper crops, plants are slow to recover from thrips damage once yield loss has occurred, highlighting the importance of monitoring pest populations (§1.3.4.3).

Indirectly, *F. occidentalis* are vectors of two commercially important plant viruses: tomato spotted wilt virus (TSWV) and impatiens necrotic spot virus (INSV). The transmission of TSWV and INSV by *F. occidentalis* can have a greater impact than the direct effects that this species may have on a crop. Whilst *F. occidentalis* can be controlled through IPM and serious crop damage avoided, plants infected with these viruses cannot be treated. Therefore, such virus diseases can only be controlled through preventative means. As in the direct effects *F. occidentalis* have on crops, the different feeding behaviour of adult males and females is important in the transmission of viruses. Adult male *F. occidentalis* have a higher vector efficiency in transmitting TSWV than females (van de Wetering *et al.*, 1999b). Viruses are obligate parasites, and TSWV needs to be injected into a plant cell, in saliva through feeding activity, which is still viable after feeding. Unlike adult females, whose intensive feeding activity results in plant cells being destroyed, males have a greater probing activity that is less likely to destroy the cells (van de Wetering *et al.*, 1998). This results in TSWV being injected into viable plant cells, causing differential vector competency (van de Wetering *et al.*, 1999a).

### 1.3.4 Control: Integrated Pest Management

IPM may be defined as “a pest management system that, in the socio-economic context of farming systems, the associated environment and the population dynamics of the pest species, utilises all appropriate techniques in a compatible manner to maintain pest population levels below those causing the economic damage” (Dent, 1995). IPM is composed of four different control components: biological, chemical, physical and cultural control.

### 1.3.4.1 Biological control

Biological control is a crucial IPM component used to control *F. occidentalis* in UK glasshouse crops, especially cucumbers, and may be defined as "the use of a living organism (the beneficial) for the regulation of populations of another (the pest)" (Jacobson, 1997). The principal agents used in the biological control of *F. occidentalis* are predatory phytoseiid mites and heteropteran bugs (reviewed by Sabelis & Van Rijn, 1997). The principle predatory mite used is *Neoseiulus* (=*Amblyseius*) *cucumeris* (Oudemans) (figure 1.5), although 15 other *Amblyseius* spp. are reported as attacking *F. occidentalis* (Sabelis & Van Rijn, 1997). This mite only feeds succesfully on the egg and larva I stages of *F. occidentalis* (Gillespie & Ramey, 1988), as all other stages are capable of either escaping or defending themselves by jerking the abdomen (van der Hoeven & van Rijn, 1990), a response similar to that shown by *T. tabaci* to *N. barkeri (=Amblyseius mckenziei)* (Hughes) (Bakker & Sabelis, 1989). However, in the presence of another pest, the spider mite *Tetranychus urticae* Koch, thrips larvae move into the web produced by the spider mite to escape predation following detection of volatile cues associated with *N. cucumeris* (Pallini *et al.*, 1998). This however results in reduced thrips developmental rate, due to interspecific competition for food between the thrips and the spider mites. *N. cucumeris* is most effective when used prophylactically and released onto the crop at the time of planting (Jacobson *et al.*, 2001b). Using this strategy *F. occidentalis* populations can be almost entirely suppressed. If the release of the mite is delayed there is an increased risk of control failure, which may require the use of remedial chemical control that could disrupt biological controls in place for the control of other pest species, e.g. the hymenopteran parasitoid *Encarsia formosa* Gahan used to control the whitefly *Trialeurodes vaporariorum* (Westwood). Predatory heteropterans, such as the minute pirate bug *Orius majusculus* (Reuter), are occasionally used as a remedial treatment, but tend to be ineffective as they are slow to reproduce and oviposit into crop parts that are removed during normal crop maintenance, in cucumbers (Jacobson, 1993). Preventative release of *Orius laevigatus* (Fieber) on cucumber is ineffective as this species does not fully establish, but does on pepper (Chambers *et al.*, 1993), possibly due to the presence of pollen as an alternative food source. Supplying sweet pepper pollen on cucumber for *O. laevigautus* serves as an effective alternative food source in the absence of thrips, but does not prevent searching for thrips when they are available (Hulshof & Jurchenko, 2000). This approach may allow the preventative use of *Orius* spp. against *F. occidentalis* in the

future. However, the presence of additional pollen, which *F. occidentalis* feed on and improves their reproductive fitness (Trichilo & Leigh, 1988), may result in a pest population build-up, as well as reducing the predation rate of predatory mites, such as *N. cucumeris* (van Rijn & Sabelis, 1993).

To a lesser extent, entomopathogenic fungi are also being used in IPM systems (reviewed by Butt & Brownbridge, 1997). *Verticillium lecanii* (Zimmermann) and *Metarhizum anisopliae* Zimmermann can cause 53% and 75% mortality of pupal stages, respectively, when larvae II are exposed to the fungal spores (Helyer *et al.*, 1995). Newly emerged adults exposed to *Paecilomyces fumosoroseus* (Wize) suffer 58-90% mortality, within 4 d of exposure (Gindin *et al.*, 1996) and *Beauveria bassiana* (Balsama) Vuillemin can reduce numbers of immature stages by 75% over a three week period on glasshouse cucumbers, without impairing control by *N. cucumeris* (Jacobson *et al.*, 2001a). The compatibility of *B. bassiana* and *N. cucumeris* may therefore provide effective control of *F. occidentalis* without the need for remedial insecticidal treatment: prophylactic release of *N. cucumeris* would control *F. occidentalis* at acceptable levels and treatment with *B. bassiana* could be used if the *F. occidentalis* population exceeded the ability of the mites to control them, without affecting the mite population (Jacobson *et al.*, 2001a).

In the future it is likely that parasitic nematodes (reviewed by Loomans *et al.*, 1997) will also be used in the regulation of *F. occidentalis* populations. The parasitic nematode species *Heterorhabditis megidis* Poinar, *Steinernema feltiae* (Filipjev) and *S. carpocapsae* Weiser caused 63%, 60% and 77% mortality of pupae in compost, compared to an untreated control mortality of 31% (Helyer *et al.*, 1995), whilst *H. bacteriophora* (Poinar) reduced adult emergence to 40% of the control (Chyzik *et al.*, 1996). It is likely that nematodes would only be of use in IPM when combined with predatory mites and bugs controlling the larval stages.

#### 1.3.4.2 Chemical control

There are several problems associated with the use of chemical insecticides to control *F. occidentalis*. The thigmotropic behaviour of thrips results in inefficient targeting of the insecticides and is likely to have a detrimental impact on biological control in use. There is also growing evidence of *F. occidentalis* resistance to existing insecticides, e.g.the pyrethroids permethrin and cyfluthrin, the carbamates methomyl and methiocarb, the organophosphates dimethoate andacephate, and the organochlorine DDT (Brødsgaard,

1994; Immaraju *et al.*, 1992; Robb *et al.*, 1995; Jensen, 2000). This situation is compounded by insecticides being removed from commercial use on food crops and the fact that producers of insecticides are not registering new products for use against *F. occidentalis*. Supermarkets have considerable power in determining the usage of insecticides on food crops, and in recent years, have been demanding that the use of insecticides is reduced.

1.3.4.3 Physical control

The main physical control measures against *F. occidentalis* in glasshouses are the use of traps and screens.

Traps have five roles: (1) to detect thrips; (2) to enable comparison of *F. occidentalis* activity before and after treatments; (3) to trap thrips as they enter glasshouses through vents; (4) to 'mop up' thrips in empty glasshouses and (5) to reduce the numbers of thrips in localised 'hot spots' of activity within the crop (Jacobson, 1997). The colour and position of traps within the crop affect trap effectiveness. Blue, yellow, violet and white -UV coloured traps have been reported as being more attractive than white +UV or black traps (Gillespie & Vernon, 1990; Vernon & Gillespie, 1990), although Brødsgaard (1989) found that *F. occidentalis* were attracted to a specific shade of blue more than other shades of blue, yellow or white traps. Females are relatively more attracted to blue traps than males compared to yellow traps (Gillespie & Vernon, 1990). Traps placed against a contrasting colour are more attractive than traps placed against the same colour (Vernon & Gillespie, 1995) and colours that reflect UV are not attractive to flower thrips (Kirk, 1984). In cucumber crops, significantly more *F. occidentalis* are caught at a height of 2.4 m, compared to heights of 0.6 m and 1.2 m in a crop 2.1 m high (Gillespie & Vernon, 1990). The attractiveness of traps to thrips can be further enhanced by baiting the traps with attractive chemicals (§1.4.1), e.g. *p*-anisaldehyde (Brødsgaard, 1990; Teulon *et al.*, 1993).

Screens may be used to prevent thrips gaining access to the glasshouse via the vents, although the extremely small mesh size required for thrips exclusion may impede airflow. Mesh, with an aperture size that does not exclude thrips, coated with aluminium, which reflects UV, does discourage thrips from getting into glasshouses, as does aluminium tape placed around vents (McIntyre *et al.*, 1996), and can reduce the ingress of thrips by as much as 55%. The use of materials that reflect or transmit UV to reduce the number of thrips on crops (reviewed by Terry, 1997; Antignus, 2000) also extends to UV reflective

mulches between crop rows although the effectiveness is reduced as the crop canopy grows and shades the mulch.

1.3.4.4 Cultural control

Cultural control is based on techniques that modify the agroecosystem to the disadvantage of the pest species. This may be achieved through modification of the host, e.g. using crop cultivars that are resistant to the pest (§3.1.1.2) and reduce developmental rate, or the environment, e.g. nursery hygiene, intercropping, the use of trap crops, supplementary lighting and manipulation of temperature and humidity.

Nursery hygiene is extremely important in controlling *F. occidentalis* in glasshouses. Weeds outside the glasshouse and crop residue within the glasshouse may serve as a reservoir of *F. occidentalis*. This is especially important if replanting occurs during the growing season, as is the practice in UK cucumber crops. The replanted crops are vulnerable to *F. occidentalis* attack and biological controls applied to the plants may not be able to control a large, sudden invasion of *F. occidentalis*. This would result in remedial chemical treatments being used to control the invasion, causing collapse of biological control.

Intercropping leek with clover results in a large reduction in the number of adult *T. tabaci* present on the intercrop compared with the monocrop (Theunissen & Schelling, 1993). This may be due to clover altering the host-plant quality resulting in an increased emigration rate of *T. tabaci* from the host-plant (den Belder *et al.*, 2000). However, intercropping chrysanthemums with clover to combat *F. occidentalis* has resulted in increased thrips damage, possibly as a result of changes in the reaction of the chrysanthemum to thrips mediated by the clover (den Belder *et al.*, 1999). At present, the use of intercropping in glasshouse cucumber is unlikely.

Trap crops are used to selectively attract pests away from the main crop. The trap crop is then selectively treated, with either insecticides or biological control agents. The selective application of treatment to trap crops, such as the *Verbena* cultivar ‘Sissinghurst Pink’ (Bennison *et al.*, 1999), not only reduces the expense of treatment to the grower, but also avoids inducing the breakdown of biological control throughout the crop by the application of insecticides.

Biological control agents, such as *Orius insidiosus* (Say), enter reproductive diapause under the short photoperiod conditions that occur during the temperate winter months. The

reduced reproductive rate could lead to the failure of biological control under such conditions. Blue-light supplementation under these conditions enhances the reproduction of *O. insidiosus*, reducing the chance of control failure (Stack & Drummond, 1997).

Increasing glasshouse temperature and humidity can enhance the efficacy of insecticides and bioinsecticides, as a result of higher temperatures causing increased activity of *F. occidentalis* (Shipp & Zhang, 1999), and therefore increased pickup of insecticides and spores, and increased germination of entomopathogenic spores due to higher humidity.

## 1.4 Semiochemicals

"Semiochemicals" is the term used to describe chemicals involved in the chemical interactions between organisms (Nordlund & Lewis, 1976). Such chemicals may be separated on the basis of whether their effect is intraspecific or interspecific. Chemicals having an intraspecific effect are termed pheromones whilst those having an interspecific effect are termed allelochemicals (Dicke & Sabelis, 1988). Allelochemicals are divided into allomones, kairomones and synomones based on whether the emitter or receiver benefits. Allomones benefit the emitter, e.g. defensive secretions, kairomones benefit the receiver, e.g. pheromones, allomones and floral scents attracting phytophagous insects, and synomones benefit both emitter and receiver, e.g. floral scents attracting pollinators.

Under such terminology, chemicals can have more than one designation. For example, alarm pheromones may not only induce alarm behaviour in conspecifics, but also function as a kairomone to a predator or parasitoid of the emitter.

### 1.4.1 Thrips semiochemicals

Numerous chemicals have been identified from whole body extracts of, and the anal droplets produced by, thrips (Appendix 1). Most of these appear to be defensive secretions and repellents, although, in the majority of cases, insufficient behavioural observations have been made to determine their function. In only one species, the *F. occidentalis*, has a pheromone, that releases alarm behaviour, been identified (Teerling, 1992; Teerling *et al.*, 1993). Floral scents, such as *p*-anisaldehyde (Brødsgaard, 1990; Teulon *et al.*, 1993) and geraniol (de Kogel *et al.*, 1999; Koschier *et al.*, 2000) and host plant odours, such as (*E*)-β-farnescene (Pow *et al.*, 1999: Koschier *et al.*, 2000; Bennison *et al.*, 2001) have been identified as attractive to *F. occidentalis*. It is possible to use these chemicals to increase the attractiveness of traps to *F. occidentalis* for cultural control and as part of a push-pull strategy to attract, or pull, thrips onto trap crops (Pickett *et al.*, 1997). Such a strategy also requires that the pest be pushed from the crop, e.g. by a repellent or oviposition deterrent. The alarm pheromone of the *F. occidentalis* may potentially be a suitable solution for the push part of this strategy.

### 1.4.2 The *F. occidentalis* alarm pheromone

The *F. occidentalis* alarm pheromone is produced by larvae in the form of a droplet produced from the tip of the abdomen in response to attack from a predator and consists of decyl acetate and dodecyl acetate (Teerling, 1992; Teerling *et al.*, 1993) (Chapter 4).

These compounds cause larvae to move away or drop from the host plant (Chapter 5), and adults to move away (Chapter 6). The alarm pheromone also reduces the oviposition rate of adult females (Teerling, 1992; Teerling *et al.*, 1993; Kirk *et al.*, 1999).

The moving away and dropping from host plants responses are similar to those shown by many species of aphids to their own alarm pheromone. In the control of aphids, the alarm pheromone has been used to manipulate their behaviour in order to improve the effectiveness of some control methods.

### 1.4.3 The aphid alarm pheromone

The chemical ecology of aphids has been reviewed by Pickett *et al.* (1992). Many species of aphids produce a droplet from their cornicles in response to predator attack. The main components of this secretion are triglycerides (Strong, 1967), which may have a defensive function. Kislow & Edwards (1972) noted that these cornicle droplets elicited alarm responses in conspecifics, causing other aphids to walk away from the source or drop from the host plant. The compound responsible for these behavioural responses was later identified as (*E*)-β-farnesene (EBF) in four aphid species (Bowers *et al.*, 1972). EBF has been successfully used to increase the efficacy of both chemical and biological insecticides (Griffiths & Pickett, 1980; El-Agamy & Haynes, 1992; Ester *et al.*, 1993) due to increased aphid activity, although the aphids may rapidly habituate or undergo sensory adaptation (El-Agamy & Haynes, 1992). Wohlers (1982) reported that EBF also caused flying aphids to avoid contaminated plants, and induced alate aphids to take off from such plants.

A similar approach may also be possible in the *F. occidentalis*. However, the limited information available on the chemistry and behavioural effects of this alarm pheromone warrants further research to assess its potential in *F. occidentalis* control.

## 1.5 Aims

The overall aim was to investigate whether the alarm pheromone of the *F. occidentalis* can be used to enhance control measures.

The specific aims of the work were to:

(1) Analyse the chemistry of alarm pheromone production in greater detail;

(2) Analyse the effects of alarm pheromone on larval movement;

(3) Assess if larvae habituate readily to alarm pheromone;

(4) Analyse the effects of alarm pheromone on adult movement.

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**Figure 1.1** View of a cucumber crop. Photo: K.M. Macdonald.

**Figure 1.2** Extreme curvature of so-called 'pig-tail' cucumber fruit, the result of *F. occidentalis* feeding activity on rapidly developing fruits. Photo: K.M. Macdonald.

**Figure 1.3** Silvering of cucumber fruit, the result of *F. occidentalis* feeding activity on mature cucumber fruit. Note the feeding yellow larvae. Photo: K.M. Macdonald.

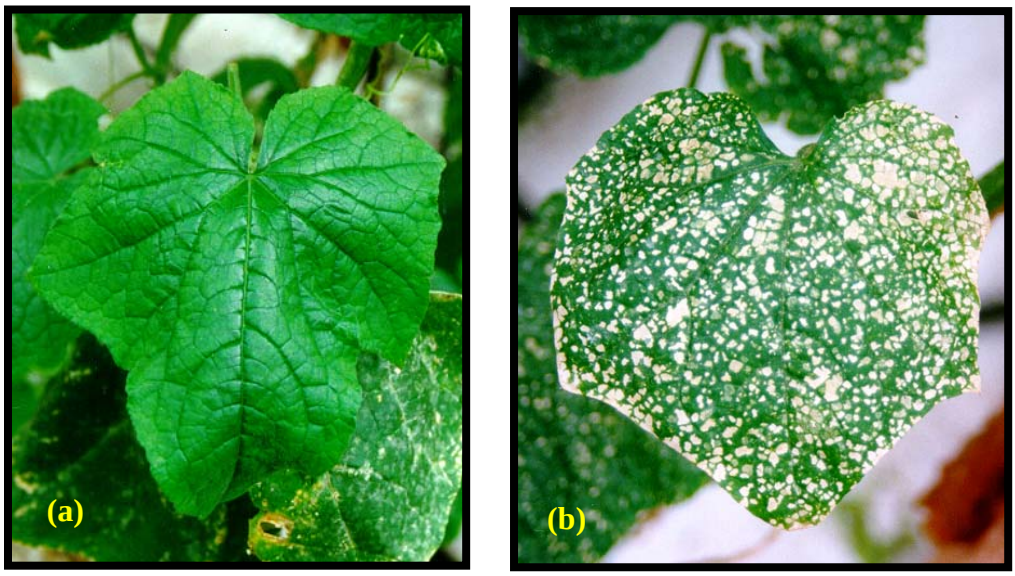


**Figure 1.4** The impact of *F. occidentalis* feeding activity on cucumber leaves that results in reduced photosynthetic capability. (a) an undamaged leaf, (b) the result of thrips damage. Photos: K.M. Macdonald.


**Figure 1.5** Culture pack used to introduce *Neoseiulus (=Amblyseius) cucumeris* into cucumber crops as a biological control for *F. occidentalis*.
Photo: K.M. Macdonald.

# Chapter 2

# General methods

## 2.1 Introduction

This chapter describes methods used in more than one chapter. It covers the rearing of thrips, lighting and temperature control, preparation of solutions, cleaning of equipment, handling of thrips and analysis of data. Methods specific to a particular chapter are detailed in that chapter.

## 2.2 Rearing of thrips

Thrips may be reared using a variety of methods (reviewed by Loomans & Murai, 1997). Adult *F. occidentalis* are very active, so rearing must be contained within a 'thrips-proof' container to prevent their escape. The container requires ventilation to prevent condensation, which can easily trap and drown thrips, forming inside the cage. Such containers may take the form of a frame covered by very fine mesh, or a plastic cage with vents covered by either very fine mesh or precision paper, to provide ventilation. Within the container, suitable plant material must be provided. This is usually determined by the purpose of the culture. Where the purpose is to rear mixed-age adults, e.g. a stock culture, whole plants, such as flowering chrysanthemum (Helyer & Brobyn, 1992) are normally used, as they last longer and so reduce the maintenance time of the culture. Where the culture purpose is the production of even-age, synchronised cohorts of larvae or adults, plant parts, such as bean-pods (Bryan & Smith, 1956), flowers (McDonald *et al.*, 1997) or leaf discs (Brødsgaard, 1994) may be used. Methods that are more complicated use artificial oviposition substrates and diets (Teulon, 1992). As *F. occidentalis* do not normally pupate on the host plant, suitable pupation sites, such as compost (Helyer & Brobyn, 1992), vermiculite or layers of paper and tissue (Bryan & Smith, 1956), must be provided in the bottom of the container. In some methods relative humidity in the culture

container is kept high by wet capillary mats underneath compost (McDonald *et al.*, 1997), wet cotton underneath leaf discs (Brødsgaard, 1994) or with water-filled containers covered with stretched Parafilm (de Kogel *et al.*, 1997).

### 2.2.1 Stock culture

This culture provided mixed-age adults for bioassay and for use in the production of known-age larvae (§2.2.2.1) and adults (§2.2.2.2).

#### 2.2.1.1 Source of stock culture

The stock culture was started in 1997 from adult *F. occidentalis* cultured on chrysanthemums at Horticulture Research Internationals (HRI) Wellsbourne site. This culture was known to have originated from a commercial chrysanthemum nursery, but it was not known how long the thrips had been cultured at HRI before this culture was started.

#### 2.2.1.2 Maintenance of stock culture

*F. occidentalis* were reared on a susceptible variety of potted chrysanthemum, in flower (*Dendranthema grandiflora* Tzvelev, variety Yellow Princess Anne, Rydale Nurseries, Carlisle), in ventilated perspex containers (modified from Helyer & Brobyn, 1992). Active ventilation was provided in each container by an electric fan on the back of the cage, which drew air out of the cage (Papst axial fan, 12 v DC, 8x8x2.5 cm, air flow = 33 $m^3h^{-1}$, RS Components, Northants, UK). The chrysanthemums were propagated on site (as described in Appendix 2). This culture was maintained at 25 ± 2°C (§2.3.2) and an 18:6 light:dark photoperiod (§2.3.1), as these are close to the ideal temperature and photoperiod (§3.1.1.1 and §3.1.1.4, respectively) to maximise population growth rate. Known-age *F. occidentalis* were reared at the same photoperiod, but at a more accurate temperature (§2.2.2). Each perspex container (figure 2.1, for dimensions) had a layer of vermiculite (depth 3-4cm) onto which four plants, in white plastic dishes (height 6cm, diam. 17cm), were placed. Plants were watered every 1-2 d by placing water into the white plastic dishes. This avoided inundating the top layer of compost in the pots, which may serve as a pupation site.

Each week the oldest plant was replaced. The flower heads of the oldest plant were removed and placed back into the container on a plastic dish in the centre of the floor, so that any thrips in them could leave as the flowers dried out. This reduced the numbers of

*F. occidentalis* removed unnecessarily from the container. Adults were collected from the container by tapping flower heads over a suitable container and collecting adults with an aspirator (§2.6.1). Any larvae present in the container were returned to the culture. As it was possible that other thrips species could be introduced with fresh plants adult thrips were collected from the culture and identified every 1-2 months, using Kirk (1996). Thrips were collected and stored in 70% ethanol for at least 24 h, before being mounted on glass slides in polyvinyl lactophenol (BDH, UK). The specimens were allowed to 'clear' for at least 24 h after mounting, to aid identification.

2.2.1.3 Control of insect pests

Aphids and whitefly in the culture were controlled by the release of parasitic wasps in the containers. Aphids were controlled with *Aphidius colemani* Viereck (Aphi-line c, Novartis BCM, UK) and whitefly with *Encarsia formosa* Gahan (Encar-f, Syngenta Bioline Production Ltd., UK). No insecticides were ever applied to the culture, or to the potted chrysanthemums, which were propagated at Keele (Appendix 2).

### 2.2.2 Rearing of known-age *F. occidentalis*

Known-age *F. occidentalis* were reared, for chemical analysis (Chapter 4) and bioassay (Chapters 5 and 6), using a modification of the bean pod method (Bryan & Smith, 1956; Loomans & Murai, 1997). Six bean pods were placed into a culture pot containing approximately 100-150 adults collected from the stock culture (the oviposition pot). The adults were allowed to lay eggs for 1-3 d, depending on the purpose of the culture. Bean pods were bought in shops, usually originated from Kenya or Egypt, and were refrigerated until use. Before use, the bean pods were washed in a weak detergent solution (Teepol L, BDH, UK), rinsed in tap water and dried, to remove any insecticide residues. They were not used once the 'display by' date was reached, therefore reducing the risk of using low quality bean pods in the culture, which could affect the developmental rate. As the inclusion of pine pollen in the diet of *F. occidentalis* is known to maximise their reproductive fitness (Trichilo & Leigh, 1988), about 3-4 mg pine pollen was scattered over the bean pods. Pine pollen was used as thrips reproduce particularily well when fed on it (Hulshof, pers. com.), and it can be collected in the large quantities required, with relative ease (§2.2.3). The bean pods were then cleaned of adults and transferred to another pot (the rearing pot). The rearing pot was the same as the oviposition pot, but contained no

adults. The eggs hatched from the bean pods in the rearing pot and the larvae remained in the pot until use (§2.2.2.1), or until they had become adults (§2.2.2.2).

The oviposition and rearing pots were made from clear plastic with a screw-on lid (500 ml, height 8.6 cm, diam. 11.7 cm, Nalge Company, USA). The lid had a large hole cut in it (diam. 8 cm). Each pot contained 20 filter paper discs (no. 1, Whatman International, UK), scattered on the base. These provided a pupation site in the rearing of known-age adults, but were present in all pots for simplicity. Each pot contained a humidifier, constructed from a small glass jar (height 2.3 cm, diam. 2.3 cm, Agar Scientific Ltd., UK) filled with distilled water, with a wick (Parasene, M.H. Berlyn Co. Ltd., UK) projecting approximately 2 cm through the snap-on plastic lid. This kept the relative humidity high, at about 70%. The pots were made 'thrips-proof' by securing a piece of precision paper (Kimwipes Lite, Kimberly-Clark, UK) over the pot mouth with the screw-on lid (figure 2.2). The pots were placed under a light rig with a 18:6 L:D photoperiod at 25 ± 0.3°C, for consistency with the stock culture. Accurate temperature control was required principally for larval rearing, as even small temperature fluctuations of 1-2°C would have a significant effect on their developmental rate.

#### 2.2.2.1 Rearing of known-age larvae

The bean pods were placed into the oviposition pot at 1400 GMT on day one, removed at the same time on day two and placed into a rearing pot, i.e. the bean pods were exposed to ovipositing females for only 24 h. The bean pods were checked daily for the emergence of larvae. This normally started between days three and four. On day four, any larvae on the bean pods were removed. On day five a 0-24 h old cohort (from hatching) of larvae were collected by blowing $CO_2$ gas (medical grade, BOC Gases, UK) into the rearing pot for a few seconds. The larvae were then dislodged from the bean pods by tapping the pot on a hard surface. The bean pods were then replaced with new pods and fresh pollen scattered over them. Larvae were kept in the rearing pot until use. The age of larvae was always the time from hatching, not from oviposition.

#### 2.2.2.2 Rearing of known-age adults

Bean pods were left in the oviposition pot to be oviposited in for 3 d, before being transferred to a rearing pot. Due to the large number of larvae present the bean pods were replaced every 3 d. Humidifiers were refilled as required. Rearing pots were checked daily for the emergence of adults, which took about two weeks from oviposition (Chapter

3). Four days after adult emergence started the adults were transferred to a new rearing pot with fresh bean pods and pollen, and kept for 4 d until 4-8 d old.

### 2.2.3 Collection of pine pollen

Pine pollen was collected from pine trees (*Pinus brutia* Tenore) in the spring of 1998. Pollen cones were spread out onto paper and air-dried for 3 d. The pollen was then separated from the pollen cones with a plastic mesh (1 mm) to remove large debris followed by a fine mesh to remove all remaining debris. The pollen was then placed into Eppendorf tubes (1.5 ml, Life Sciences International, UK) and stored at approximately -20°C until use. Pollen, when prepared and stored in this way, can last for many years (Loomans & Murai, 1997).

## 2.3 Lighting and temperature control

### 2.3.1 Lighting

Illumination for rearing and laboratory bioassays was provided by four fluorescent tubes (length 1.5 m, type F65W/35) mounted horizontally on a frame constructed from square-section mild steel. Three separate light rigs were used (table 2.1 and figure 2.3). The light rigs used for cultures were automatically switched on at 04:00 and off at 22:00 GMT using time switches. All light measurements were taken using a hand held light meter (light sensometer, Philip Harris, UK). Light measurements were taken directly, i.e. the exposed silicon photodiode was held directly underneath the light source. The light intensity on the bench beneath the light rigs was 1000 lux.

### 2.3.2 Temperature control

Two constant temperature rooms were used, one to house the stock culture (room A) and one for known-age cultures and laboratory bioassays (room B). In room A the temperature was controlled by an in built heated air system. In room B heating was provided by a fan heater (2 kW Dimplex, UK) mounted vertically on a wall, so that heated air was directed towards the floor. This helped disperse the heated air and prevent a hot spot in the room. The fan heater was controlled by a thermostat (Allen-Martin, UK), set to 26°C, mounted on the rearing light rig, 30 cm below the fluorescent tubes. This arrangement provided a very accurate temperature of 25 ± 0.3°C at a height of 26 cm above the bench, irrespective of whether the lights were on or off. A metal grid was placed at this height, held by retort stands (the rearing platform). Culture pots were placed onto the rearing platform (figure

2.3). Temperature control was confirmed by placing a thermocouple, linked to a data recorder (Squirrel meter/logger, Grant Instruments, UK), inside a culture pot on the rearing platform, and monitoring the temperature over a 24 h period.

## 2.4 Preparation and storage of bioassay solutions

Solutions of decyl acetate (98%, Sigma, UK) and dodecyl acetate (97%, Aldrich, UK) in n-hexane (95%, residue analysis grade, Fisher Scientific Ltd., UK) were made in disposable glass vials. The absence of dodecyl acetate in decyl acetate, and vice versa, was confirmed by GC analysis. The n-hexane was not contaminated by either decyl acetate or dodecyl acetate. The required amount(s) of decyl acetate and/or dodecyl acetate were placed into a vial and the volume made up to 1 ml with n-hexane. The vials were made from disposable Pasteur pipettes (length 145 mm, Volvac disposable glass Pasteur pipettes, John Poulten Ltd., UK). These were heated over a Bunsen burner to drive off any contaminants, before sealing the narrow end of the pipette. Dilute solutions were made through serial dilution of more concentrated solutions. Final solutions were placed into sealed glass vials, made from disposable Pasteur pipettes, and stored at approximately -20°C until use. Before solutions were used in bioassays, except in §5.2.1.1, the amount of alarm pheromone component(s) and ratio were checked by GC.

## 2.5 Cleaning of bioassay equipment

As insect pheromones are active in very small amounts it was of the utmost importance that all items used in bioassays were as free from contamination as possible. Bioassay items were cleaned in the following ways:

### 2.5.1 Glass items

All glass items were cleaned by immersion in a sonication bath (Decon Ultrasonics Ltd., UK) containing a detergent solution (2% Decon 90, Decon Laboratories Ltd., UK) for at least 15 min. Items were then rinsed with copious amounts of distilled water, followed by acetone (reagent grade, Bamford Laboratories, UK). Items were then heated to approximately 200°C in an oven (Carbolite, Camlab, UK) for approximately 12 h.

### 2.5.2 Teflon items

All items made from Teflon were washed as described in §2.5.1, except that the items were heated in the oven for approximately 2 h before use, to dry them.

### 2.5.3 Plastic items

All plastic items were soaked in a weak detergent solution overnight (Teepol L, BDH, UK), rinsed in tap water and air-dried before use.

## 2.6 Handling of thrips

*F. occidentalis* are normally very active and difficult to handle without causing them injury. Two different methods were employed in handling them: aspirators and brushes.

### 2.6.1 Aspirators

Two types of aspirator were used. To collect large numbers of *F. occidentalis* an aspirator (Watkins and Doncaster, UK) was used. This was modified by fitting a plastic pipette tip (1.5 ml, Eppendorf, USA), trimmed so that the small opening was approximately 2mm in diameter, over the aspirator intake tube. This modification made it easier to suck up the thrips. The aspirator could also be used to remove adults from bean pods, when removing bean pods from the oviposition pot, by blowing through the aspirator and using the resulting fine jet of air to dislodge them, without causing damage. Collected thrips could be anaesthetised in the aspirator by applying $CO_2$ gas to the pipette tip for a few seconds.

To collect individual thrips an aspirator made from a thin tube (external diam. 4 mm, length 40 cm, clear Teflon tubing, Aldrich, UK) with fine cotton mesh held over one end with a trimmed pipette tip (10 µl, Eppendorf, USA) was used. Thrips could be held in the aspirator by sucking gently and expelled from the tip by blowing gently.

### 2.6.2 Brushes

Where manipulation by aspirator was unsuitable, a moistened brush, trimmed to just a few bristles, was used.

## 2.7 Statistical analysis

All data were analysed using Minitab (version 13.30, Minitab Inc., USA). The data were tested initially with a parametric test. The residuals were then tested for normality with the Anderson-Darling test. If the residuals were normally distributed ($P > 0.05$) the parametric test was used. If the residuals were not normally distributed ($P < 0.05$), the data were transformed to normalise the residuals or, if this was not possible, a suitable non-parametric test was used. Where the 95% confidence interval is given for a median, this was calculated by Minitab (version 13.30, Minitab Inc., USA) by a non-linear interpolation

procedure. Where multiple comparisons were made, the *P* values were adjusted using Holm's method (Holm, 1979), as recommended by Wright (1992). These were calculated with PEPI (Computer Programs for Epidemiologists) version 3.01 (Abramson & Gahlinger, 1999). Where used, level of significance is indicated thus: ns = not significant, * = $P < 0.05$, ** = $P < 0.01$ and $P < 0.001$.

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**Table 2.1** Dimensions of light rigs used in the rearing and bioassay of *F. occidentalis*.

| Use | Height (m) | Width (m) | Depth (m) |
|---|---|---|---|
| Stock culture | 0.89 | 1.70 | 0.60 |
| Known-age cultures; Oviposition bioassay | 1.26 (with extension) | 1.70 | 0.60 |
| Bioassay | 0.89 | 1.50 | 0.60 |

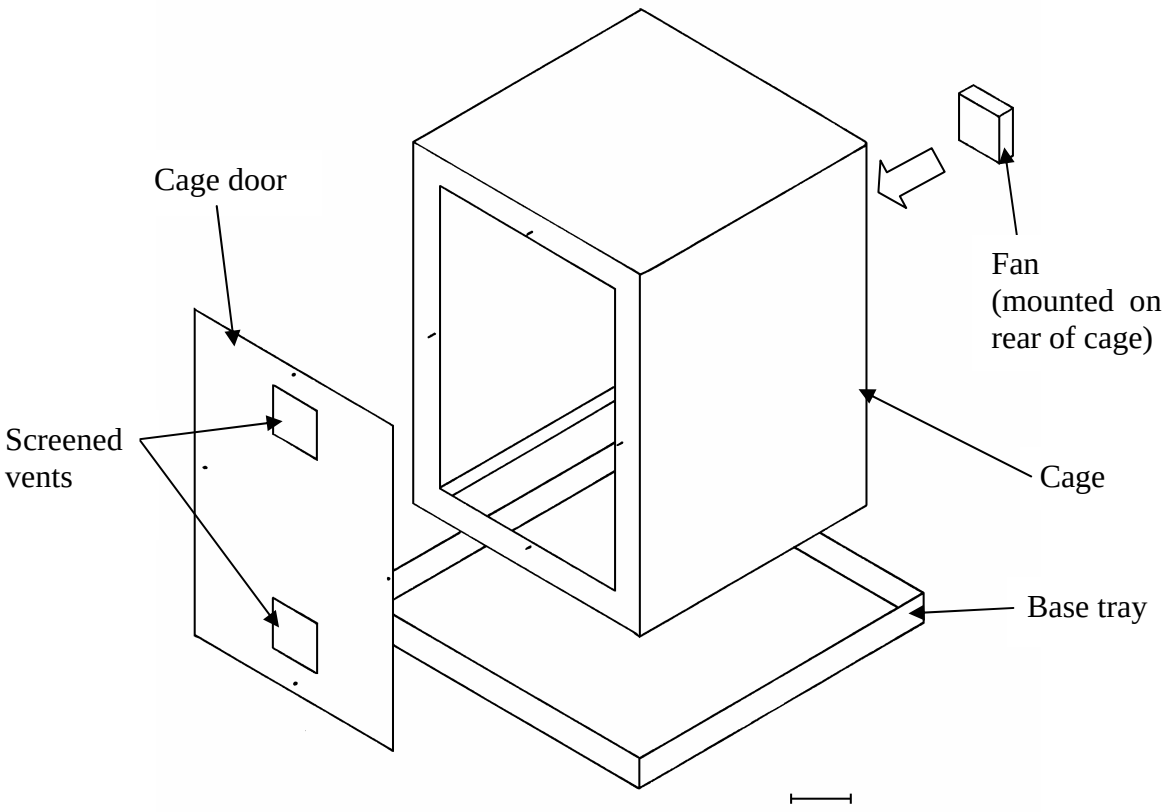


**Figure 2.1** Partially exploded isometric view of perspex cage used to contain the *F. occidentalis* stock culture on potted chrysanthemums (bar = 10cm).

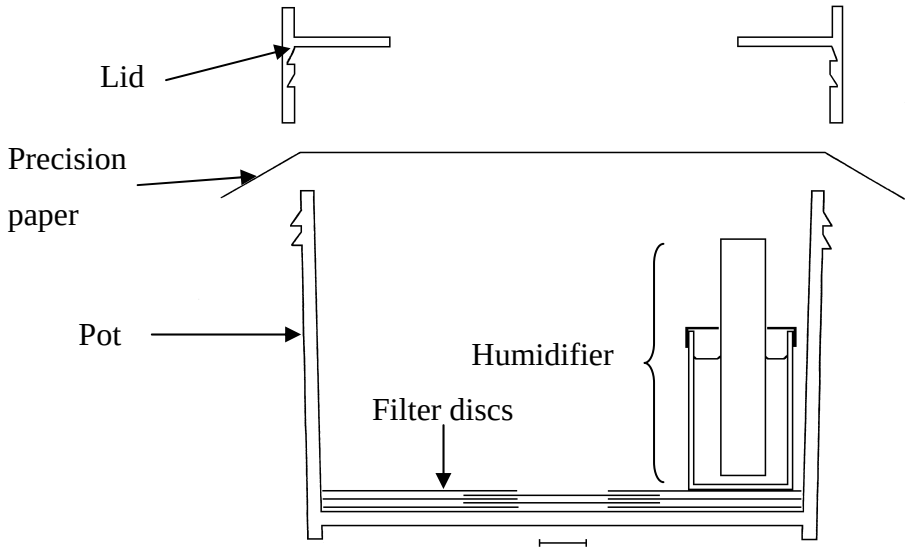


**Figure 2.2** Cross-section view of rearing pot used in the production of known-age *F. occidentalis* larvae and adults (bar = 1cm).

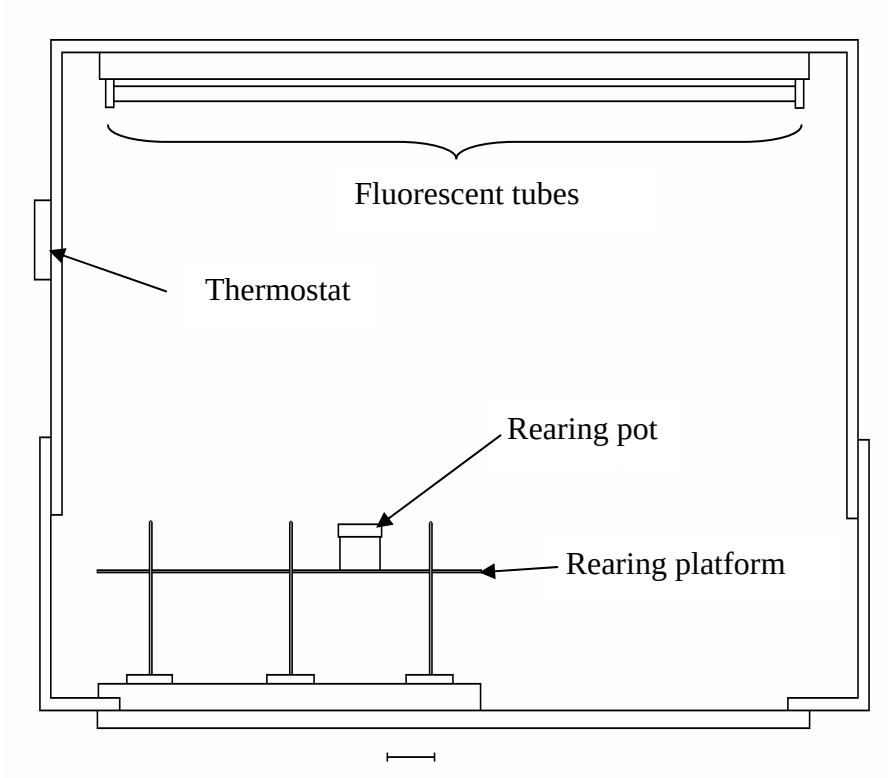


**Figure 2.3** Side view of light rig used for rearing known-age *F. occidentalis* larvae and adults, and for conducting oviposition bioassays (bar = 10cm).

# Chapter 3

# Developmental rate

## 3.1 Introduction

*F. occidentalis* larvae develop rapidly, and alarm pheromone production may change as they develop. To study changes in the production of alarm pheromone, larvae have to be reared in a consistent and repeatable manner. This would allow the results of chemical analysis to be applied to behavioural bioassays using larvae of known developmental stage. To achieve consistent and repeatable rearing one must first understand what factors influence developmental rate in insects.

### 3.1.1 Factors affecting developmental rate

#### 3.1.1.1 Temperature

Due to their high surface area to volume ratio, thrips rapidly equilibrate to the temperature of their surroundings, i.e. they are poikilothermic (Lowry *et al.*, 1992). Consequently, temperature is one of the most important factors affecting developmental rate. The relationship between developmental rate and temperature is largely linear, with developmental rate increasing with increasing temperature (Davidson, 1944; Danks, 2000). Exceptions occur at extreme temperatures, with no development occurring below or above certain temperatures (the developmental zero and the upper lethal temperature limit, respectively). Reported values for the developmental zero of the *F. occidentalis* vary between 6.5°C and 10.9°C, with an upper limit of between 30°C and 34°C (Robb, 1989; Lowry *et al.*, 1992; van Rijn *et al.*, 1995). The optimal temperature for *F. occidentalis* development is close to 30°C (Lublinkhof & Foster, 1977; Robb, 1989; Gaum *et al.*, 1994a; van Rijn *et al.*, 1995).

3.1.1.2 Diet

The development rate of thrips can be affected by the host plant tissue and by the availability and species of pollen. The effect of host plant on *F. occidentalis* development rate has been demonstrated on cucumber (Soria & Mollema, 1995; de Kogel *et al.*, 1997), rose (Bergh & Le Blanc J-P, 1997) and cotton (Trichilo & Leigh, 1988), where resistant host varieties caused a slowing of development. This may be the result of chemicals altering *F. occidentalis* behaviour, e.g. a toxin or feeding deterrent (antixenosis), or that the resistant variety is nutritionally deficient, e.g. contains reduced amounts of aromatic amino acids important in insect development (antibiosis) (Dadd, 1973). Studies on cucumber (Soria & Mollema, 1995) have shown that larva II development on resistant varieties (9127 and 9140), compared to that on a susceptible variety (G6), may be prolonged by 62-102%. Resistant variety 9140 was found to have a aromatic amino acid content not significantly different to the susceptible variety G6, suggesting that reduced development rate was the result of antixenosis. Such behavioural alteration has also been observed in larvae reared on resistant rose cultivars known to prolong development. The commencement of feeding in newly emerged larva I may be delayed (Gaum *et al.*, 1994b) and larvae may spend significantly more time wandering on resistant, compared to susceptible, cultivars (Bergh & Le Blanc J-P, 1997). The effects of host resistance on development rate can be removed if an alternative food source, such as pollen is provided (Trichilo & Leigh, 1988). Whilst there are no published comparative studies on the effect of pollen species on *F. occidentalis* development, significant differences have been found in *Thrips obscuratus* Crawford, indicating that pollen species is important (Teulon & Penman, 1991).

3.1.1.3 Strain of thrips

Strain effects have been found in the resistance of *F. occidentalis* to insecticides (Brødsgaard, 1994a), and in their reproductive performance on susceptible and resistant cucumber varieties (de Kogel *et al.*, 1997). Whilst total developmental duration was prolonged by 20% to 35% on resistant varieties, no difference in total developmental duration was observed between strains (de Kogel *et al.*, 1997).

3.1.1.4 Photoperiod

Photoperiod affects developmental rate in the *F. occidentalis*. Brødsgaard (1994b) measured developmental duration of *F. occidentalis* at 4:20, 8:16 and 16:8 L:D. Larvae II developed significantly faster as photophase increased, requiring 8% and 18% less time at

8:16 and 16:8 L:D, respectively, compared to 4:20. Propupae and pupae reared under 8:16 and 16:8 L:D developed significantly faster, by 3 % to 11%, than those reared at 4:20 L:D. This may be the result of increased activity during the photophase. No photoperiod effect was observed in the egg and larva I stages. The effect on larvae II is not surprising, as this is the most active immature stage. The weaker effect on the pupal stages is likely to be the result of the increased larvae II vigour.

### 3.1.2 Aim of work

Larvae develop rapidly and may change physiologically, e.g. in the production of alarm pheromone, and behaviourally, e.g. in the way they respond to alarm pheromone, over a short period of time. The duration of larvae I and larvae II combined is typically about 5 d at 30°C (Lublinkhof & Foster, 1977; Gaum *et al.*, 1994a). To (1) measure the production of alarm pheromone and (2) test the response of known developmental stage larvae to a synthetic 'copy' of alarm pheromone produced by larvae of the same developmental stage, there must be as little variation in the developmental rate as possible. The aim of the present study was to measure the duration of each developmental stage and thus be able to produce cohorts of larvae of a particular developmental stage as required for pheromone measurements or bioassays.

## 3.2 Materials and methods

### 3.2.1 Experimental conditions

The temperature used in the present study was the same as that used in the normal rearing of known-age thrips (§2.2.2), i.e. 25 ± 0.3°C. Accurate temperature control was required, as a fluctuating temperature could result in greater variation in the duration of the developmental stages. Although the optimal temperature for development in *F. occidentalis* is about 30°C (§3.1.1.1), maintaining the rearing room at this temperature would have been difficult and would have been too warm for the pot chrysanthemums used in the stock culture. A temperature of 25°C was selected as a reasonable compromise, as it was easier to maintain the room at this temperature. The same light source and photoperiod used for the rearing of known-age thrips was used for this study, i.e. fluorescent light with a 18:6 L:D photoperiod. This produced a light intensity of 330 lux inside the culture pots used in these experiments. In keeping with the normal rearing of known-age thrips, the host was shop-bought bean pods. Pine pollen was also provided, as this is used in the rearing of known-age thrips. The origin of the *F. occidentalis* is as described in §2.2.1.1.

### 3.2.2 Rearing of immature instars for calculation of stage durations

The procedure described here is summarised in table 3.1. Bean pods were exposed to adult females for 24 h, as described in §2.2.2. The bean pods were placed in the oviposition pot at 14:00 GMT (day one), removed at 14:00 GMT the next day (day two) and placed into a rearing pot without any adult females. On day five, at 14:00 GMT, a cohort of larvae I, with an age range of 0-1 d, from hatching, was collected. These larvae were then isolated on bean pod segments (length 1.5 cm), the cut ends of which had been sealed with paraffin wax, so that they could be followed individually. This would not only prevent the pod segment from drying out but also prevent larvae from getting into the pod segment. A small quantity (0.3 mg) of pine pollen was placed onto the pod segment before it was placed into a modified Eppendorf tube (1.5 ml, Life Sciences International, UK, figure 3.1). The tube had been modified by melting a hole (diam. 6 mm) in the lid. When the tube was closed a piece of precision paper (2 x 2 cm, Kimwipes Lite, Kimberly-Clark, UK) was placed over the tube mouth. This arrangement allowed ventilation of the tube, reducing the chance of condensation forming inside the tube, but prevented the larva from getting out. The tube was sufficiently transparent to allow observation of the larva with a

dissection microscope (magnification = x40). Forty of these tubes were placed into culture pots (20 tubes $pot^{-1}$).

The development stage present in each tube was recorded once a day at 14:00 GMT, using a dissection microscope and a cold light source for illumination. As larva I and larva II can only be distinguished microscopically, the presence of a cast skin was used to define the moult from larva I to larva II. The moults from larva II to propupa, and from propupa to pupa, were determined by the easily visible, characteristic morphological changes that occur with each moult, namely the shorter antennae and appearance of wing buds in the propupae, and the longer wing buds with antennae laid flat on top of the head in the pupae. The start of a developmental stage was recorded as taking place at the midpoint between the time the stage was first observed and the time of the previous observation (Robb, 1989). Data from individuals that died were excluded, even if they had completed some stages successfully.

### 3.2.3 Rearing of eggs for calculation of stage duration

For completeness, the duration of the egg stage was determined. *F. occidentalis* lay their eggs into the host tissue, and so are not easily visible. As a result, the appearance of larvae is the only indication of eggs.

Bean pods were exposed to adult females, as described in §3.2.2. A single bean pod was removed from the oviposition pot on day two and placed into a rearing pot (§2.2.2). The bean pod was examined daily, at 14:00 GMT, for larvae. Any larvae present were counted and removed. Daily examination stopped after two days in which no further emergence was observed. Stage duration was calculated as described in §3.2.2. Oviposition was assumed to be at the midpoint of the time available for oviposition

### 3.2.4 Does age affect behaviour in late larvae II?

During the normal rearing of known-age larvae, a change in behaviour was noted in late larvae, whose age range was 4-5 d (post-hatching). Whilst some larvae remained on the bean pods feeding, others started to wander around the pot. It was suspected that these different behaviours were due to a slight difference in physiological and/or actual age, with the wandering larvae being slightly older and actively searching for a suitable pupation site (Okada, 1981). To test the hypothesis that the observed behavioural difference was due to a difference in age, 30 4-5 d old feeding and 30 4-5 d old wandering larvae were isolated as described in §3.2.2. If a developmental difference was present, the wandering larvae

would moult to the propupal stage sooner than the feeding larvae. As the age difference may be small, the larvae were observed every 6 h and the stage recorded, until all larvae had moulted. The moult was assumed to be at the midpoint of time between the first observation of the propupa and the previous observation.

## 3.3 Results

### 3.3.1 Rearing of immature instars for calculation of stage durations

The mean durations of each developmental stage are summarised in table 3.1. Of the 40 larvae isolated, only 17 reached adulthood. Of the 23 that did not complete development, four died (three as larvae I and one as a pupa), and 19 escaped (eight as larvae I and 11 as larvae II). Death was caused either by the individual becoming trapped in a condensation droplet, or by being squashed between the bean pod segment and the tube. Larvae I were usually found on the bean pod segment. Larvae II were more active than larvae I, and were sometimes found walking on the inner surface of the tube. In the majority of cases, escapes could be attributed to a small tear in the precision paper covering the hole in the Eppendorf tube lid.

### 3.3.2 Rearing of eggs for calculation of stage duration

Larvae started to hatch 3 d after the bean pod was removed from the oviposition pot, i.e. first observed on day five (table 3.1). Hatching occurred over 2 d, with 66% of hatching occurring on the first day. The mean duration of the egg stage was $3.3 \pm 0.1$ d (table 3.1).

### 3.3.3 Does age affect behaviour in late larvae II?

Larvae II that were wandering at 4-5 d post hatching moulted to the propupal stage after a further $1.460 \pm 0.114$ d. Larvae II that were feeding at the same age moulted after $2.300 \pm 0.183$ d. This difference was significant (Mann-Whitney U-test, W=611.0, $P < 0.001$, table 3.2). Wandering larvae were therefore more developed than feeding larvae. The age range of these larvae was only 1 d, but there was a mean difference in physiological age of almost as much, indicating that some thrips had developed faster than others.

## 3.4 Discussion

The developmental stage durations found in the present study are broadly in agreement with those reported by others at 25°C (figure 3.2). Variation between reported durations is restricted to the egg and larval stages, with the propupal and pupal durations being very similar in all cases, with the exception of Lowry *et al*. (1992), who concluded that the host used (peanut) was not very suitable for *F. occidentalis*. The greatest variation, excluding the egg duration reported by Lowry *et al*. (1992), was in the duration of larva II. This is the stage that is most likely to be affected by diet (Soria & Mollema, 1995), which indicates that the diet provided in this study (bean pod and pine pollen) is particularly beneficial to *F. occidentalis* development. Interestingly, the duration of larva II reported by Robb (1989) is the second longest, even though apple pollen was provided, suggesting that this pollen species is not nutritionally beneficial to *F. occidentalis*. Variation within the developmental stage durations found in this study was reasonably low, indicating that it would be possible to bioassay larvae of known developmental stage with a synthetic version of the alarm pheromone produced by that, or other, developmental stages. Whilst the inclusion of pine pollen in the *F. occidentalis* diet may increase the number of eggs laid by females, and so maximise the number of larvae available for bioassay, it seems likely that it is also responsible for shortening the developmental duration of larva II. This is unfortunate, as a longer duration for larvae II would allow pheromone production and behaviour to be studied at more points in their development.

The developmental stage durations (table 3.2) are for *F. occidentalis* reared singly. However, to rear the large numbers of larvae required for bioassay, large groups needed to be reared in pots (§2.2.2.1). Table 3.1 shows the predicted occurrence of developmental stages in a rearing pot at 14:00 GMT each day of rearing, assuming that the developmental rate is the same for larvae reared in pots as for larvae reared singly. The predominant stage in the pots each day was correctly predicted. Variation in age was reduced further by ensuring that the cohort of newly emerged larvae I collected on day five of rearing (table 3.1) were no older than 1 d. This was achieved by examining the bean pods for newly emerged larvae at 14:00 GMT on day 4 of rearing and removing any that were present. Direct observation then showed that the predicted developmental stages were present in the rearing pots at 14:00 GMT each day, and that only one stage was present at 14:00 GMT each day.

The different behaviours observed in late larvae II were due to an age difference of 0.8 d, with the older larvae exhibiting the wandering behaviour. *F. occidentalis* are often reported as pupating in the soil beneath the host plant, i.e. somewhere dark and with a high relative humidity (Helyer *et al.*, 1995; Bennison *et al.*, 2001). It is probable that the older larvae were about to moult and were searching for a suitable pupation site, as occurs in *Scirtothrips dorsalis* Hood (Okada, 1981). Larvae 4-5 d post hatching could therefore be selected according to their behaviour to restrict them to a narrower range of physiological development.

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**Table 3.1** Developmental stages of *F. occidentalis* reared on bean pods with pine pollen at 25 ± 0.3°C and 18:6 L:D.

| Day of rearing | Action | Time from oviposition (d) | Time from hatching (d) | Developmental stage |
|---|---|---|---|---|
| 1 | Beans placed with adults | - | - | - |
| 2 | Beans moved to rearing pot (no adults) | 0-1 | - | Egg |
| 3 | - | 1-2 | - | Egg |
| 4 | Any hatched larvae discarded | 2-3 | - | Egg |
| 5 | Hatched larvae moved to new beans | 3-4 | 0-1 | Larva I (early) |
| 6 | - | 4-5 | 1-2 | Larva I (late) |
| 7 | - | 5-6 | 2-3 | Larva II (early) |
| 8 | - | 6-7 | 3-4 | Larva II (mid) |
| 9 | - | 7-8 | 4-5 | Larva II (late) |
| 10 | - | 8-9 | 5-6 | Propupa |
| 11 | - | 9-10 | 6-7 | Pupa |
| 12 | - | 10-11 | 7-8 | Pupa |
| 13 | - | 11-12 | 8-9 | Adult |

**Table 3.2** The mean duration ± SE of developmental stages of *F. occidentalis* on bean pods with pine pollen at 25 ± 0.3°C and 18:6 L:D.

| | Time to complete developmental stage (d) | | | | |
|---|---|---|---|---|---|
| | Egg | Larva I | Larva II | Propupa | Pupa |
| Mean ± SE | 3.3 ± 0.1 | 1.9 ± 0.1 | 2.7 ± 0.3 | 1.1 ± 0.1 | 2.6 ± 0.1 |
| n | 67 | 28 | 17 | 17 | 17 |

**Table 3.3** The mean times ± SE taken for feeding and wandering *F. occidentalis* late larvae II to moult to the propupal stage. Times to moulting were compared using a Mann-Whitney U-test.

| | Behaviour 4-5 d after hatching | | |
|---|---|---|---|
| | Feeding | Wandering | Significance |
| Days to moult (mean ± SE) | 2.30 ± 0.18 | 1.46 ± 0.11 | $P<0.001$*** |
| n | 20 | 25 | |

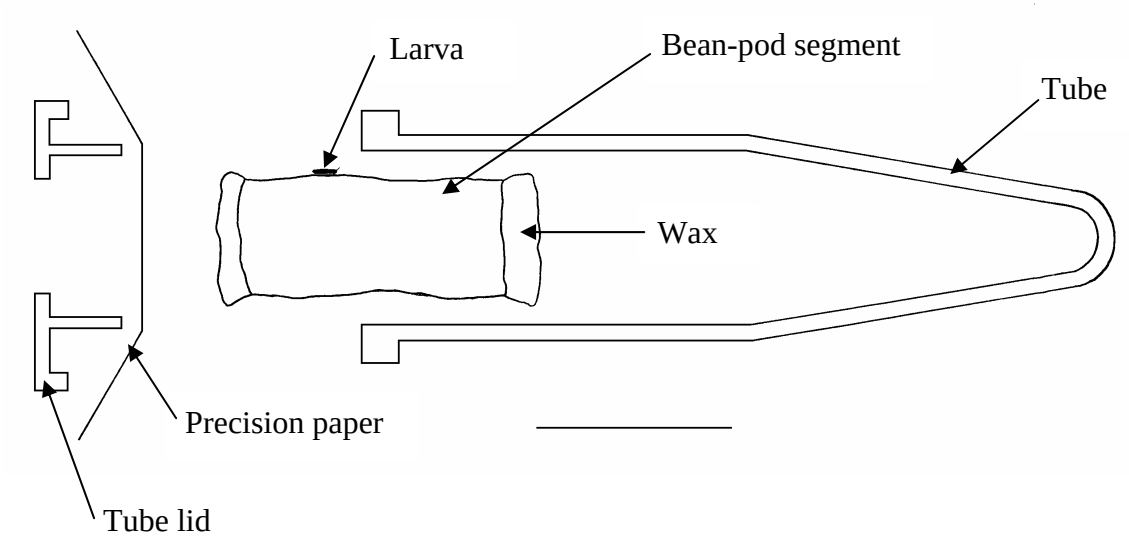


**Figure 3.1** Partially exploded cross-section view of the modified Eppendorf tube used to isolate *F. occidentalis* for observation during measurement of developmental stage duration (bar = 1cm).

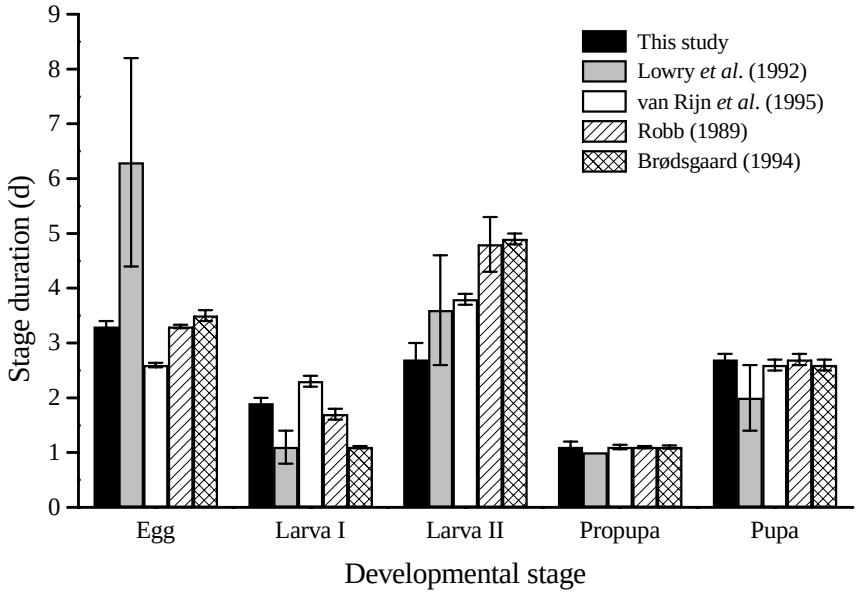


**Figure 3.2** Comparison of *F. occidentalis* developmental stage durations (mean ± SE) at 25°C (this study 18:6 L:D on bean pod segment with pine pollen; Lowry *et al*. (1992) 14:10 L:D on peanut leaf disc; van Rijn *et al*. (1995) 16:8 L:D on cucumber leaf disc; Robb (1989) 14:10 L:D on chrysanthemum leaf with apple pollen; and Brødsgaard (1994) 16:8 L:D on bean leaf disc).

# Chapter 4

# The alarm pheromone

## 4.1 Introduction

### 4.1.1 Responses of thrips to predatory attack

When attacked by a predator, thrips will often raise and jerk the abdomen in response, as a means of deterring the attack. This defensive behaviour is often accompanied by the release of a droplet of fluid from the tip of the abdomen (Lewis, 1973). This behaviour, exhibited by *F. occidentalis* (van der Hoeven & van Rijn, 1990) (figure 4.1), may be aggressive and, in many species, the thrips will attempt to place the anal droplet (AD) onto the attacker. Other thripid pest species, which exhibit similar behaviour, are *Thrips tabaci* Lindeman (Bakker & Sabelis, 1989) and *Thrips palmi* Karny (personal observation). Although the former is still a major crop pest and the latter is a notifiable species in the UK, the chemical composition of the AD's produced by these species is yet to be investigated. Many phlaeothripid species also show this defensive behaviour. One phlaeothripid species, *Leeuwenia passanii* (Mukaigawa), sprays the AD not only around themselves but also at the aggressor, to deter the attack (Suzuki *et al.*, 1988). An exception to such behaviour is *Heliothrips haemorrhoidalis* (Bouché), which walks around with the AD held aloft on the tip of the abdomen (Zabaras *et al.*, 1999). The AD chemical composition for many phlaeothripid species, and only two thripid species, has been elucidated to date.

### 4.1.2 Chemicals produced by thrips

To date, the chemicals produced by 37 species of thrips have been analysed (Appendix 1). Two species belong to the Thripidae, and 35 to the Phlaeothripidae (18 in the sub-family Phlaeothripinae and 17 in the Idolothripinae). Seven classes of chemical are represented: alkanes, alkenes, alcohols, aldehydes, acetates, ketones and fatty acids. Usually more than

one chemical is produced by each species, with only four species, all from the Phlaeothripinae, producing a single compound. All seven chemical classes are produced by the Phlaeothripinae, three in the Thripinae (alkanes, acetates and ketones) and two in the Idolothripinae (ketones and fatty acids).

The *F. occidentalis*, in common with four other species, all from the Phleaothripinae, produce acetates. *F. occidentalis* larvae produce decyl acetate and dodecyl acetate, which are present not only in whole body (WB) extracts, but also in the AD (Teerling, 1992; Teerling *et al.*, 1993b). From two individual larvae II, these acetates were reported as occurring in a molar ratio of 2:1 and 1.5:1 (decyl acetate:dodecyl acetate). However, the age and sex of the larvae analysed, and the mass of decyl acetate and dodecyl acetate produced, was not reported.

### 4.1.3 Function of compounds present in AD's

The function of compounds present in AD's released by thrips can be placed into three categories: alarm pheromones, allomones and kairomones. *F. occidentalis* is the only species that has had the chemicals present in the AD classified, through bioassays, as an alarm pheromone. Both decyl acetate and dodecyl acetate are found in the defensive secretions of some species of ant (Lofqvist, 1977) and beetles (Will *et al.* 2000), and so may also function as allomones, as well as an alarm pheromone, to *F. occidentalis*. Two species of *F. occidentalis* predator, *Orius tristicolor* (White) and *Neoseiulus cucumeris* (Oudemans), have been demonstrated to use the *F. occidentalis* alarm pheromone as a kairomone (Teerling *et al.*, 1993a). Chemicals present in the AD's produced by other thrips species have been assigned as alarm pheromones based solely on casual observations, as is the case in *L. passanii* (Suzuki *et al.*, 1986), *Hoplothrips japonicus* (Karny) (Haga *et al.*, 1989) and *Thlibothrips isunoki* Okajima (Haga *et al.*, 1990). Defensive allomones have been identified in several thrips species. *Gynaikothrips ficorum* (Marchal) (Howard *et al.*, 1987), produces compounds, including hexadecyl acetate, which act as a topical irritant against the predatory ant *Wasmannia auropunctata* (Roger), *Haplothrips leucanthemi* (Schrank) (Blum *et al.*, 1992), also produces a chemical, mellein, that repels ants as does *Bagnalliella yuccae* (Hinds) (Howard *et al.*, 1983), which produces γ-decalactone. Teerling *et al.* (1993b) concluded that the function of the *F. occidentalis* AD was purely pheromonal and of little use for defence.

### 4.1.4 Location of chemical production/storage in thrips

Little is known of the location of AD chemical biosynthesis in thrips. In the Thripidae, a pygidial, or abdominal, gland, of unknown function, has been found in four species, including the *F. occidentalis* (Moritz, 1988, 1997). In the other three species, the gland joins the rectum near the anus. This gland, in all species, is not present in the imago. In the Phleaothripinae no similar gland has been found. However, the chemical secreted by *B. yuccae* (Howard *et al.*, 1983) has been located specifically in the hind gut.

### 4.1.5 Aims of the work

The aims of this chapter are to (1) quantify the mass of alarm pheromone contained within and produced by known-age *F. occidentalis* in the form of an AD, (2) determine the naturally occurring ratio of decyl acetate and dodecyl acetate produced by known-age larvae, (3) determine if there are additional compounds present in the AD and (4) determine if alarm pheromone is stored in the region of the abdomen where the pygidial gland is located.

## 4.2 Materials and methods

### 4.2.1 Alarm pheromone quantification

#### 4.2.1.1 Sample collection

Samples for GC analysis were collected from a single cohort of known-age thrips reared at 25 ± 0.3°C and 18:6 L:D photoperiod on bean pods with pine pollen, as described in §2.2.2. Samples were collected from a single cohort, as this would allow direct comparisons of the mass and ratio of alarm pheromone produced at different ages.

After egg hatching, samples were collected daily (sample points). It was described in Chapter 3, how the developmental stages being sampled could be predicted (table 3.1). Larvae I occurred 0-1 d and 1-2 d post-hatching, larvae II 2-3 d to 4-5 d post-hatching, propupae 5-6 d post-hatching, pupae 6-7 d and 7-8 d post-hatching and adults from 8-9 d post-hatching onwards. Larvae II 4-5 d post-hatching exhibit two distinct age related behaviours (§3.3.3). Physiologically younger larvae remain on the bean pods, presumably feeding, and the physiologically older larvae wander around the rearing pot, presumably searching for a suitable pupation site. Consequently, there were two sample points for 4-5 d post-hatching larvae: one for 'feeding' larvae (4-5 d f), and one for wandering larvae (4-5 d w). Although pupae were present over two days, they were sampled only on the first day of their appearance (6-7 d post-hatching), as it was unlikely there would be any difference between the pupae on the two days. Adults were not sampled until 4-8 d post-eclosion, corresponding to 11-12 to 15-16 d post-hatching, as this would allow completion of teneral development.

Two types of sample were collected: whole body (WB) and anal droplet (AD). Whilst WB samples were collected at all sampling points, with the exception of pupae 7-8 d post-hatching, AD samples were collected at only four sampling points, corresponding to larvae II (2-3 to 4-5 d post-hatching). AD samples were not collected from larvae I, due to their small size. No AD samples were collected from propupae, pupae and adults as these developmental stages do not produce an AD in response to simulated predator attack, although they will often raise the abdomen in a manner similar to larvae producing an AD.

WB samples were collected by placing a thrips directly into a glass vial, adding 5 µl n-hexane to extract the alarm pheromone and sealing the vial. Thrips were first anaesthetised by blowing $CO_2$ (medical grade, BOC, UK) into the rearing pot for approximately 15 s. This inactivated the thrips for the few minutes required to collect the samples, and

prevented larvae from producing an AD in response to handling. Individual anaesthetised thrips were transferred, on the tip of an n-hexane rinsed trimmed brush, to a glass vial. The glass vials were made in the same way as described in §2.4, with the exception that a constriction was placed near the open end of the vial before sampling. This constriction made sealing the glass vial, using a Bunsen burner, easier than without the constriction, and so required less heat to be applied to the glass vial, thus reducing possible evaporation of n-hexane and sample. Once the thrips was in the glass vial, the vial was held in ice, preventing the thrips from becoming active, and n-hexane added using a microsyringe (10 µl, SGE, Australia) before sealing the glass vial. Five samples were collected for each age. Control samples, consisting of 5 µl n-hexane in a glass vial, were also collected. All glass vials were then labelled and stored at approximately -20°C until analysis.

AD samples were collected at the same time as WB samples. Once the WB samples had been collected, and whilst the larvae in the rearing pot were still inactive, a small number of larvae were transferred to a glass Petri dish, cleaned as described in §2.5.1. When the larvae in the Petri dish became active a small piece of glass micro-fibre disc (GMD, approximately 1.5x1.5 mm, Whatman International Ltd., UK), which had been rinsed in n-hexane and baked at approximately 200°C overnight, held in n-hexane rinsed forceps, was gently pressed onto the dorsal surface of the larva. This simulated an attacking predator, and the larva normally responded by producing an AD and placing this onto the GMD. If this happened, the GMD was immediately transferred to a glass vial and 10 µl n-hexane added immediately. A greater volume of n-hexane, compared to that used to extract WB samples, was required to ensure the GMD was fully immersed in the n-hexane. Five samples were collected at each age. Vials were then sealed and stored at approximately -20°C, until analysis. Control samples, consisting of a GMD in 10 µl n-hexane in a glass vial were also collected. The larvae from which AD samples had been collected were placed into screw top glass vials (12x32 mm, Alltech, USA) containing 70% ethanol, so that the developmental stage and sex of the larva from which an AD had been collected could be confirmed later.

#### 4.2.1.2 GC analysis

GC analysis was carried out on a Shimadzu GC-15A (Dyson Instruments, UK) fitted with a split/splitless injector, used in the splitless mode with a sampling time of 0.6 min, and a flame ionisation detector (FID). The column was a 30 m fused silica capillary column

(DB-1 phase, 0.32 mm i.d., 0.25 μm film thickness, J & W Scientific, USA). The temperature programme was as follows: 45°C for 2 min, then increased to 100°C at 20°C $min^{-1}$ and held for 1 min, then increased to 150°C at 15°C $min^{-1}$ and held for 10 min and then increased to 280°C at 40°C $min^{-1}$ and held for 4 min. The carrier gas was helium at 1 ml $min^{-1}$ and the injector and detector temperatures were 180°C and 280°C, respectively.

Peak areas and retention times were then calculated by Millennium 2010 chromatography manager software (v 2.10, Millipore Corp., M.A., USA). Quantification of the mass of decyl acetate and dodecyl acetate present in samples was by an eight-point calibration curve (0.5, 1, 5, 10, 15, 20, 25 and 30 ng). Each calibration point was replicated six times using independent standards.

### 4.2.2 AD composition

#### 4.2.2.1 AD volume analysis

In order to ascertain if the AD produced by larvae is composed solely of decyl acetate and dodecyl acetate, the volume of AD's were compared with the volume of alarm pheromone present in droplets, as measured in §4.2.1. Droplet volume was measured by video recording known-age larvae producing AD's. The actual radius of the droplets was then determined, and the volume of a sphere with the same radius calculated.

#### 4.2.2.2 Identity of additional compounds

Having found that the volume of a typical AD was not fully explained by the volume of decyl acetate and dodecyl acetate alone, an attempt was made to identify the additional compounds present in the AD. Three methods were used.

In the first method, AD's were collected as in §4.2.1.1 and analysed as in §4.2.1.2. Carbon disulphide ($CS_2$), and not n-hexane, was used to extract the sample. $CS_2$ was used, as this compound does not produce a signal in a FID. Therefore, any hydrocarbon present in the droplet, which had been masked by the n-hexane solvent peak in previous analyses, would be revealed.

In the second method, solid phase micro extraction (SPME), a solventless technique, was used. Two samples were collected. In the first sample, a single AD produced by a 4-5 d w larva II, in response to simulated predator attack, was collected onto an SPME fibre (length 2 cm, 50/30 μm DVB/Carboxen/PDMS StableFlex, Supelco, PA, USA). The second

sample was the same as the first, except that five AD's were collected onto the SPME fibre. Samples were analysed by coupled gas chromatography mass spectroscopy (GC-MS, Hewlett-Packard Series II Chromatograph – mass selective detector 5972A). The samples were injected, immediately after collection, onto a 30 m fused silica capillary column (DB-5 MS, 0.25 mm i.d., 0.25 μm film thickness, J & W Scientific, USA) via an on-column septumless injector. The capillary column was connected to the MS (in the EI mode, ionisation chamber temperature 170°C, ionisation energy 70 eV) via a heated transfer line at 280°C. Helium was used as the carrier at 1 ml $min^{-1}$. The GC temperature programme was as follows: 40°C for 1 min, then increased to 250°C at 10°C $min^{-1}$ and then to 280°C at 30°C $min^{-1}$ and held for 4 min. The injector temperature was 150°C. If, between the two samples (one AD and five AD), peaks other than those representing decyl acetate and dodecyl acetate increased in peak area by a factor of approximately five, this would indicate the presence of an additional compound in the AD's rather than as contaminants.

The third method was a specific test for water. Copper sulphate ($CuSO_4$) was used. In its anhydrous state, this compound is white, but when water is added pentahydrated cupric sulphate forms, which is blue (Mellor, 1946). To test for the presence of water in AD's, a small quantity of anhydrous $CuSO_4$ was placed onto the tip of a needle. An AD was then placed onto the anhydrous $CuSO_4$. Pure decyl acetate and dodecyl acetate were also placed onto anhydrous $CuSO_4$, to ensure that they did not turn it blue. Distilled water was used as a positive control.

### 4.2.3 Location of alarm pheromone in larvae

To test the hypothesis that the pygidial gland in larvae is the site of alarm pheromone storage, larvae were dissected into sections and the sections then extracted in n-hexane and the resulting extracts analysed by GC for the presence of alarm pheromone. The detection of alarm pheromone in sections containing the pygidial gland would provide supporting evidence that one of the functions of this gland is the storage of alarm pheromone.

Larvae are very small and difficult to handle. Due to the small size and delicate nature of larvae, this experiment could only be carried out on 4-5 d old larvae II (the oldest larval age). Wandering larvae, which are slightly older and larger (measuring approximately 1 mm long and 0.25 mm wide) than feeding larvae of the same age, were selected for dissection. Larvae were frozen prior to dissection, by placing them on dry ice. This not

only reduced the chance of alarm pheromone loss during dissection, through evaporation, but also made the larvae easy to cut, using a scalpel blade.

Dry ice was placed into a container, and a standard microscope slide, cleaned as described in §2.5.1, was placed on top of the dry ice. A rearing pot containing 4-5 d old larvae was taken and larvae still feeding removed by taking the bean pods out of the rearing pot. The remaining wandering larvae were then anaesthetised by blowing $CO_2$ into the rearing pot for approximately 15 s. Once anaesthetised, the larvae could be tipped onto the microscope slide with ease and without causing larvae to produce AD's. Under a dissection microscope, larvae were cut into four sections: the head/thorax, the anterior portion of the abdomen, the middle of the abdomen and the posterior portion of the abdomen, which contains the pygidial gland. Due to the small size of the larvae, dissection cuts could not be made at specific parts of the body. Each section was then transferred to a glass vial (§4.2.1.1) using a very thin glass tube, formed by heating a Pasteur pipette and drawing it out. A 10 µl volume of n-hexane was then added to the vial. The vials were then labelled, sealed and stored at -20°C until analysis. Five larvae were sectioned in this manner. Analysis by GC was as described in §4.2.1.2.

## 4.3 Results

### 4.3.1 Alarm pheromone quantification

The calibration curves, on which the quantification of alarm pheromone was based, were linear regressions fitted through zero. Decyl acetate had a retention time of 13.184 min under the GC conditions used. The regression equation was y=422x and $r^2$=0.96. Dodecyl acetate had a retention time of 19.816 min. The regression equation was y=396x and $r^2$=0.96.

4.3.1.1 Whole body analysis

Alarm pheromone was detected in larvae and propupae, but not in pupae and adults (figure 4.2). The total mass of alarm pheromone in larvae gradually increased from 0-1 d up to 4-5 d w, but sharply declined in propupae (5-6 d). Whilst all larvae contained alarm pheromone, or at least dodecyl acetate, only two propupae contained alarm pheromone. Pheromone mass raw data were $\log_{10}(x)$ transformed to normalise the residuals.

The gradual increase of alarm pheromone mass observed in larvae was significant ($F_{(5,23)}$ = 53.65, $P$ = <0.001). Larvae I produced 0.544 ± 0.081 ng and 0.659 ± 0.142 ng (mean ± SE) at 0-1 d and 1-2 d old, respectively. The mass produced by these ages was not significantly different from each other. Larvae II produced significantly greater amounts of alarm pheromone, compared to larvae I, with 8.539 ± 1.844 ng, 17.760 ± 3.361 ng, 19.524 ± 1.610 ng and 28.831 ± 5.567 ng being produced by larvae aged 2-3 d, 3-4 d, 4-5 d f and 4-5 d w, respectively. Whilst larvae II aged from 3-4 d to 4-5 d w contained significantly greater amounts of alarm pheromone than 2-3 d old larvae, the amounts contained within these ages did not significantly differ between these ages. Of the larval WB extracts analysed, all contained decyl acetate and dodecyl acetate, with the exception of one 1-2 d old larva I. This replicate was excluded from alarm pheromone mass statistical analysis, and the subsequent ratio analysis. Propupae, which do not produce an AD in response to attack, were excluded from statistical analysis, although two of the five individuals analysed did contain a mean of 5.376 ± 4.431 ng alarm pheromone. None of the control (n-hexane) samples were found to contain decyl acetate or dodecyl acetate.

Statistical examination of the ratio of decyl acetate:dodecyl acetate revealed a significant difference with age ($F_{(5,23)}$ = 25.64, $P$ = <0.001, figure 4.3). Larvae I contained alarm pheromone in a ratio of 0.327 ± 0.029:1 and 0.276 ± 0.080:1 (mean ± SE) at 0-1 d and 1-

2 d old, respectively. These ratios were not significantly different from each other. Larvae II contained alarm pheromone ratios of 0.449 ± 0.070:1, 0.855 ± 0.096:1, 1.019 ± 0.090:1 and 1.090 ± 0.032:1 at 2-3 d, 3-4 d, 4-5 d f and 4-5 d w old, respectively. The mean ratio produced by 2-3 d old larvae was not significantly different from that produced by larvae I, but was significantly different from the ratios produced by all other larvae II. The ratio of alarm pheromone produced by larvae II 3-4 d to 4-5 d w were not significantly different from each other.

4.3.1.2 AD analysis

Of the five AD extracts analysed at each larvae II age, four extracts, per age, contained alarm pheromone components. One AD extract at 3-4 d, and two at 4-5 d f, did not contain both components. Pheromone mass raw data were $\log_{10}(x)$ transformed to normalise the residuals. Statistical analysis of AD extracts containing both alarm pheromone components showed a significant increase in the mass of alarm pheromone produced in AD's with increasing age ($F_{(3,9)} = 15.11$, $P = <0.001$, figure 4.4). Larvae 2-3 d old produced significantly less alarm pheromone in AD's compared to that produced at 3-4 d, 4-5 d f and 4-5 d w (1.904 ± 0.418 ng compared to 8.903 ± 3.057 ng, 10.397 ± 4.913 ng and 17.860 ± 4.685 ng, respectively). There was no significant difference in the mass of alarm pheromone produced between the ages of 3-4 d and 4-5 d w. Overall, the mass of alarm pheromone present in AD extracts was significantly less than that present in WB extracts ($F_{(1,25)} = 0.73$, $P = 0.40$). No decyl acetate or dodecyl acetate were found in any of the control samples.

Statistical analysis of the ratio of alarm pheromone components revealed a significant change in the ratio with age ($F_{(3,9)} = 34.55$, $P = <0.001$, figure 4.5). Larvae II 2-3 d old produced a significantly different ratio of alarm pheromone compared to larvae 3-4 d, 4-5 d f and 4-5 d w (0.374 ± 0.062:1 compared to 0.867 ± 0.017:1, 1.309 ± 0.094:1 and 1.056 ± 0.071:1, respectively). There was no significant difference in the ratio of alarm pheromone produced between the ages of 3-4 d and 4-5 d w. Overall, there was no significant difference found between the ratio of alarm pheromone components from WB and AD extracts ($F_{(1,25)} = 0.73$, $P = 0.040$).

### 4.3.2 AD composition

#### 4.3.2.1 AD volume analysis

The volume of AD produced by known-age larvae increased significantly as time increased ($F_{(5, 36)} = 8.61$, $P = <0.001$, figure 4.6). The volumes of AD produced between the ages of 0-1 d and 4-5 d f (0.443 ± 0.152 nl, 0.254 ± 0.041 nl, 0.797 ± 0.154 nl, 0.923 ± 0.168 nl and 1.525 ± 0.287 nl, respectively), did not differ significantly. Larvae 4-5 d w had a AD volume (2.133 ± 0.666 nl) significantly greater than that of larvae 0-1 d to 3-4 d old. There was not significant difference in AD volume between 4-5 d f and 4-5 d w larvae.

Using the results from GC analysis of AD's produced by larvae 2-3 d to 4-5 d w old (§4.3.1.2), the theoretical volume of alarm pheromone was calculated. The percentage of the AD volume occupied by alarm pheromone was then determined. This revealed that 0.25%, 1.12%, 0.79% and 0.97% of the AD volume produced by larvae 2-3 d, 3-4 d, 4-5 d f and 4-5 d w old, respectively, was accounted for by the alarm pheromone. This indicates that, on average, over 99% of the AD volume produced by larvae 2-3 d to 4-5 d w old consists of an additional droplet compound(s).

#### 4.3.2.2 AD analysis

The $CS_2$ used to extract AD samples was contaminated with a substance with the same retention time as n-hexane. Although this contamination was present, there appeared to be no difference in the peak area or shape of the contaminant between the control samples and the AD samples, suggesting that there was no additional compound, detectable by FID, present that had a retention time similar to the contaminant.

In the SPME analysis, only two peaks, corresponding to decyl acetate and dodecyl acetate, increased in peak area by a factor of approximately five, indicating the absence of an additional compound detectable by MS.

AD's from larvae turned anhydrous $CuSO_4$ a vivid blue colour, indicating the presence of water. Decyl acetate and dodecyl acetate did not turn the anhydrous $CuSO_4$ blue, whilst water did.

### 4.3.3 Location of alarm pheromone in larvae

Of the five 4-5 d w post-hatching larvae sectioned, alarm pheromone was found in four of the larvae. Of these four larvae, alarm pheromone was found in the posterior abdominal

section (figure 4.7), corresponding to abdominal segments V, VI, VII and VIII - XI. In one of these larvae, alarm pheromone was also found in the middle section of the abdomen.

## 4.4 Discussion

GC analysis of WB extracts showed a significant difference in alarm pheromone mass between larvae I and larvae II. Whilst larvae I contained very small amounts of alarm pheromone (<1 ng), larvae II contained relatively large quantities of alarm pheromone (9 ng to 29 ng). There was a similar difference in the ratio of alarm pheromone produced, with larvae I producing approximately three times more dodecyl acetate than decyl acetate. In larvae II roughly equal amounts of decyl acetate and dodecyl acetate were produced. The trend of alarm pheromone production in AD's was similar to that found from WB extracts, with no significant difference in ratio, although AD extracts contained significantly less alarm pheromone when compared with WB extracts. Larvae are capable of producing two or three AD's within a few minutes. It may be that not all of the decyl acetate and dodecyl acetate present within a larva is used in the first AD produced, so that some is available for a second AD.

Teerling *et al.* (1993) reported alarm pheromone occurring in a molar ratio (decyl acetate:dodecyl acetate) of 2:1 and 1.5:1, from WB extractions of two individual larvae . The present study found that alarm pheromone in AD extracts occurred in a molar ratio ranging from 0.4:1 to 1.5:1. However, the age that produced the greatest mass of alarm pheromone, 4-5 d w, produced alarm pheromone in a molar ratio of 1.2:1. The observation that larvae I produce approximately three times the mass of dodecyl acetate compared to decyl acetate is interesting. This developmental stage is the most vulnerable to predation, due to its small size (van der Hoeven & van Rijn, 1990), and Teerling reported that dodecyl acetate affects the movement of larvae more than decyl acetate. This may suggest that larvae I devote most of their resources to the production of the more biologically active compound, dodecyl acetate, whilst larvae II, which are capable of producing greater amounts of alarm pheromone do not require selective synthesis of alarm pheromone compounds. The GC analysis also found alarm pheromone in propupae, but not in pupae and adults. The presence of alarm pheromone in two propupae, and its absence in the three other replicates, suggest that the structure synthesising the alarm pheromone either ceases to function or degenerates in the propupal stage.

Analysis of the volume of AD accounted for by alarm pheromone showed that approximately 99% of the AD volume is unexplained. SPME analysis by GC-MS did not locate additional organic compounds in AD's. The simple test for water detected water in the AD's. Water may be used as the main component of the AD as this could be easily

replaced from the diet, allowing a larva that has recently produced an AD to be able to produce another in a relatively short space of time, as is thought to occur in the vinegaroon *Mastigoproctus giganteus* (Lucas) (Schmidt *et al.*, 2000). This would also explain the absence of decyl acetate and reduced amounts of dodecyl acetate found in three of the AD's analysed. The water may also aid in the dispersal of alarm pheromone. Decyl acetate and dodecyl acetate are less dense and not very soluble in water and may cover the surface of the AD, facilitating rapid dispersal of alarm pheromone upon production of an AD. Conversely, a third compound that aids in dispersal of the alarm pheromone in the water may be present. Triglycerides, present in the cornicle secretion of aphids, disperse aphid alarm pheromone, and may have a limited defensive function (Nault & Phelan, 1984; Strong, 1967). Triglycerides, or similar compounds, present in larval *F. occidentalis* anal droplets would not have been detected under the GC-MS conditions used here.

The location of alarm pheromone production and storage is currently unknown in the *F. occidentalis*. The only species in which the location of chemical storage has been identified is the phlaeothripid *B. yuccae* (Howard *et al.*, 1983), which concentrates the γ-decalactone that composes its AD in the hind gut. From the results presented here, the location of alarm pheromone is found to be in abdominal segments V to XI. A potential candidate for alarm pheromone storage, located in this region of the abdomen along with the hind-gut, is the pygidial gland (Moritz, 1997), the function of which is unknown. This gland, present in larvae and propupae, degenerates in pharate pupae. However, there could be other, as yet unidentified, sources. Abdominal glands have also been found in other thripid species, namely *Frankliniella tenucornis* (Uzel), *Aeolothrips astutus* Priesner and *Hercinothrips femoralis* (Reuter), but have not been found in any phlaeothripids examined to date (Moritz, 1988). In these species, the pygidial gland lies ventrally under the rectum, joining it immediately under the anus, i.e. the gland is located in the terminal segments of the abdomen. Of the propupae analysed, three lacked alarm pheromone and the remaining two contained reduced amounts of alarm pheromone. This situation could be expected if the abdominal gland was the site of alarm pheromone synthesis and storage, as some of the propupae analysed may have been pharate pupae lacking the abdominal gland. Additional circumstantial evidence that the abdominal gland is involved is the fact that adult *F. occidentalis*, lacking an abdominal gland, do not produce alarm pheromone. Conversely, phlaeothripids, which lack the abdominal gland entirely and may synthesis the chemicals present in AD's in the hind-gut, produce AD's both as larvae and as adults.

The present work has, for the first time, given a detailed picture of alarm pheromone production in the *F. occidentalis*, and has identified water as being a constituent of the anal droplet. The location of alarm pheromone synthesis is tentatively proposed to be the abdominal gland described by Moritz (1997).

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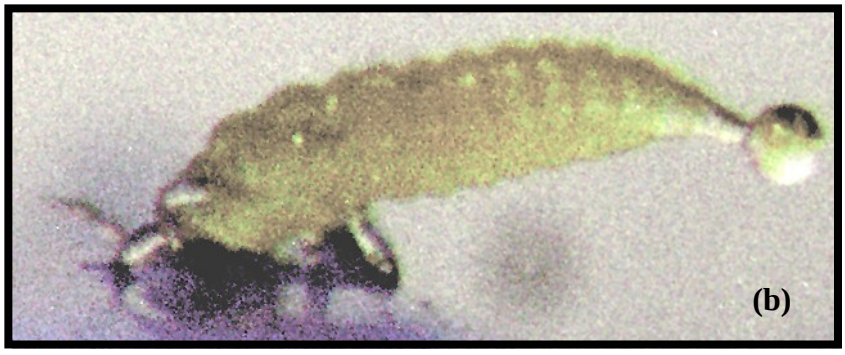


**Figure 4.1** Images of *F. occidentalis* larvae producing anal droplets (AD) in response to simulated predator attack. (a) view from above (AD highlighted by circle), (b) side view, clearly showing spherical nature of the AD. Photos: K.M. Macdonald.

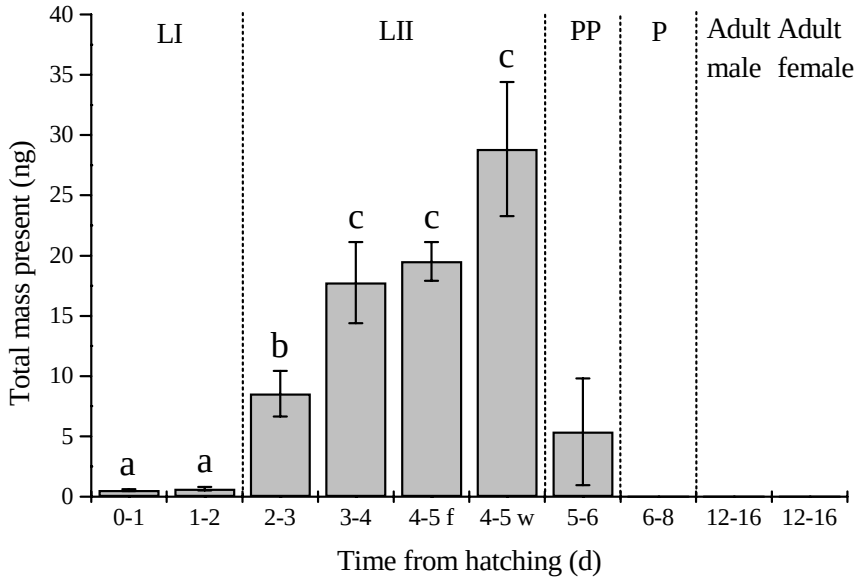


**Figure 4.2** The untransformed mean ± SE total mass of alarm pheromone present in whole body extracts of known-age *F. occidentalis*. Bars sharing the same letter are not significantly different from each other at the 95% confidence level. The significances are from statistical tests with $\log_{10}(x)$ transformed data. PP were excluded from analysis, as this stage does not produce an anal droplet in response to attack. Key: LI = larva I; LII = larva II; PP = propupa; P = pupa. f and w indicate behaviour at 4-5 d old: feeding or wandering, respectively.

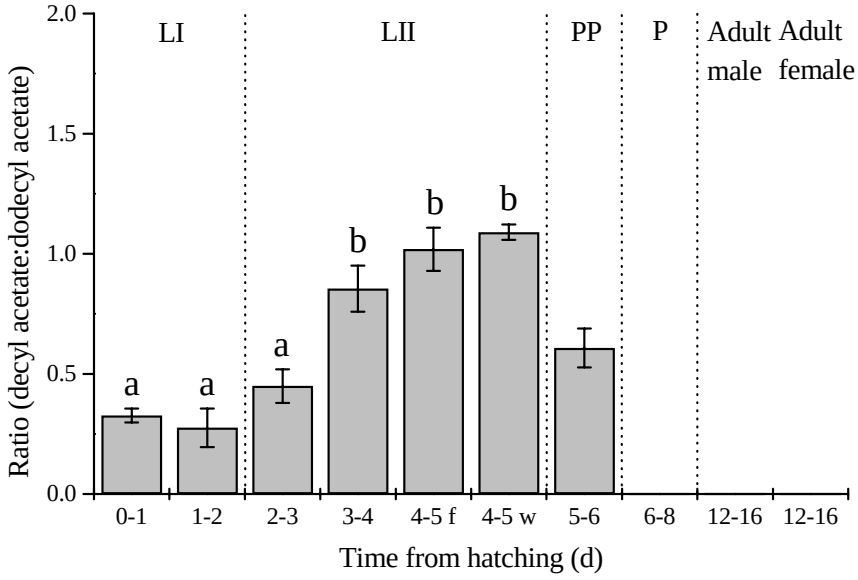


**Figure 4.3** The mean ± SE mass ratio of alarm pheromone (decyl acetate:dodecyl acetate) present in whole body extracts of known-age *F. occidentalis*. Ratio was calculated by dividing the mass of decyl acetate by the mass of dodecyl acetate. Bars sharing the same letter are not significantly different from each other at the 95% confidence level. PP were excluded from analysis, as this stage does not produce an anal droplet in response to attack. Key: LI = larva I; LII = larva II; PP = propupa; P = pupa. f and w indicate behaviour at 4-5 d old: feeding or wandering, respectively.

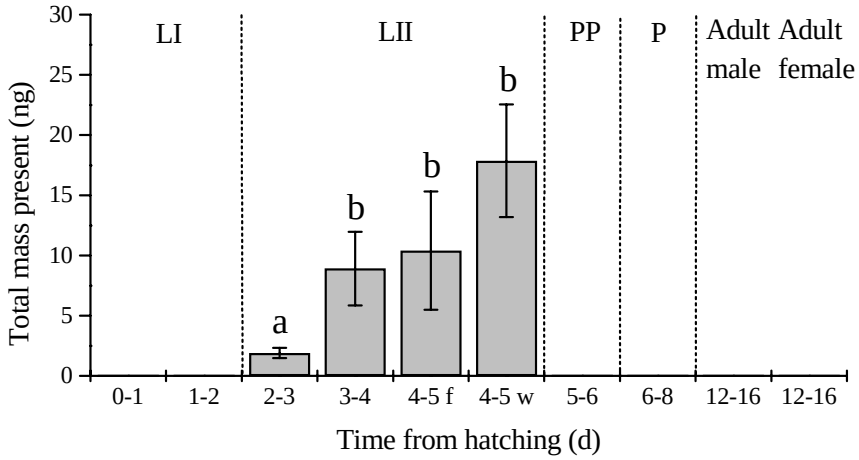


**Figure 4.4** The untransformed mean ± SE total mass of alarm pheromone present in anal droplets collected from known-age *F.* occidentalis larvae II. Bars sharing the same letter are not significantly different from each other at the 95% confidence level. The significances are from statistical tests using $\log_{10}(x)$ transformed data. Samples were not collected from LI, due to their small size. PP, P and adults do not produce anal droplets in response to predator attack. Key: LI = larva I; LII = larva II; PP = propupa; P = pupa. f and w indicate behaviour at 4-5 d old: feeding or wandering, respectively.

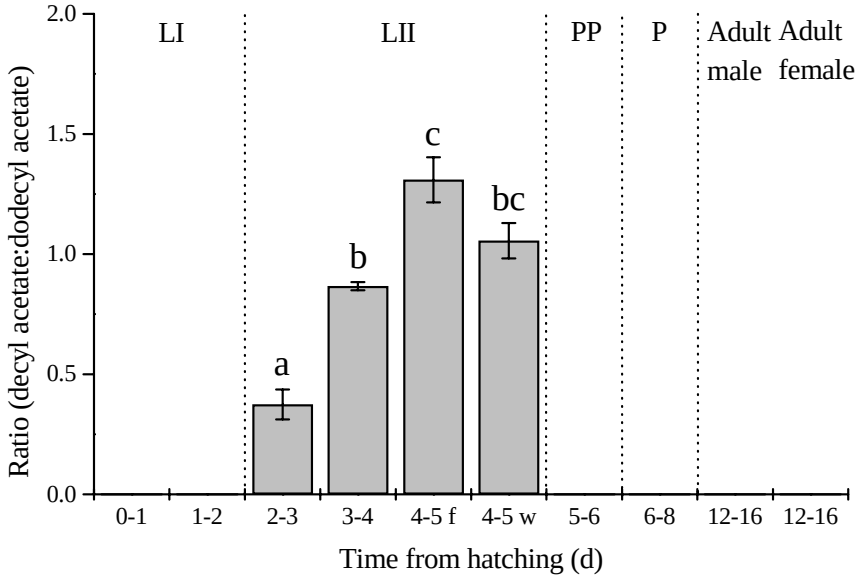


**Figure 4.5** The mean ± SE mass ratio of alarm pheromone (decyl acetate:dodecyl acetate) present in anal droplets collected from known-age *F.* occidentalis larvae II. Bars sharing the same letter are not significantly different from each other at the 95% confidence level. Samples were not collected from LI, due to their small size. PP, P and adults do not produce anal droplets in response to predator attack. Key: LI = larva I; LII = larva II; PP = propupa; P = pupa. f and w indicate behaviour at 4-5 d old: feeding or wandering, respectively.

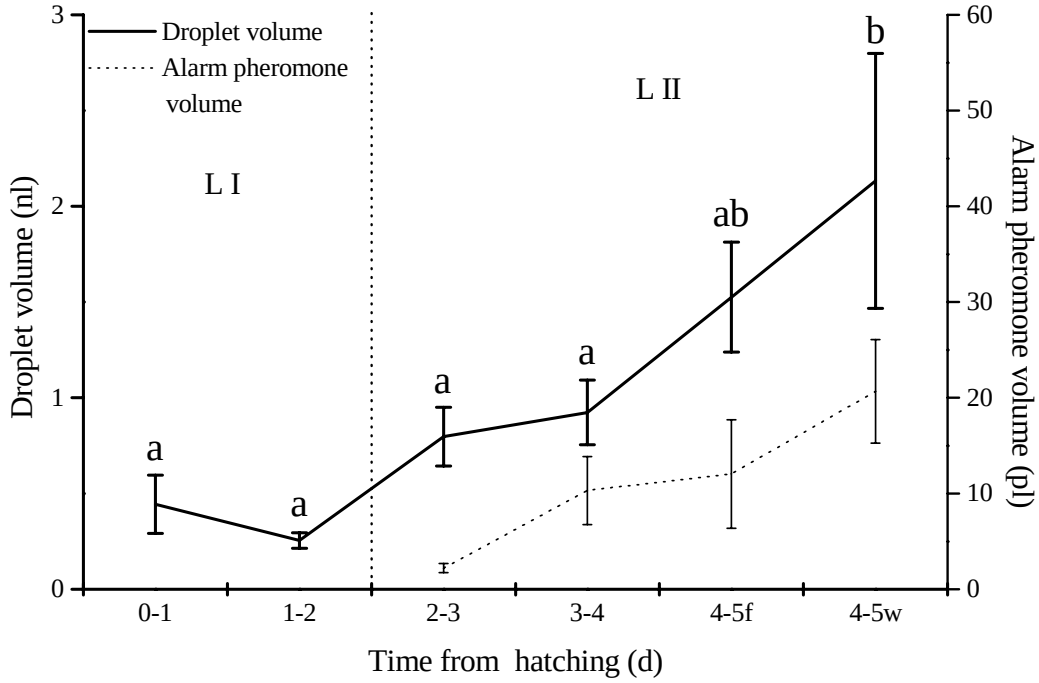


**Figure 4.6** Composition of anal droplets (AD) collected from known-age *F. occidentalis* larvae. The solid line shows the AD volume (mean ± SE) for larvae I (LI) and larvae II (LII). The dashed line indicates the theoretical volume (mean ± SE) of alarm pheromone present in LII AD's, based on the quantification of alarm pheromone in LII AD's by GC. LI AD's were not analysed due to their small size. This shows that in LII, ~1% of the total AD volume is accounted for by the alarm pheromone. Bars sharing the same letter are not significantly different from each other at the 95% confidence level.

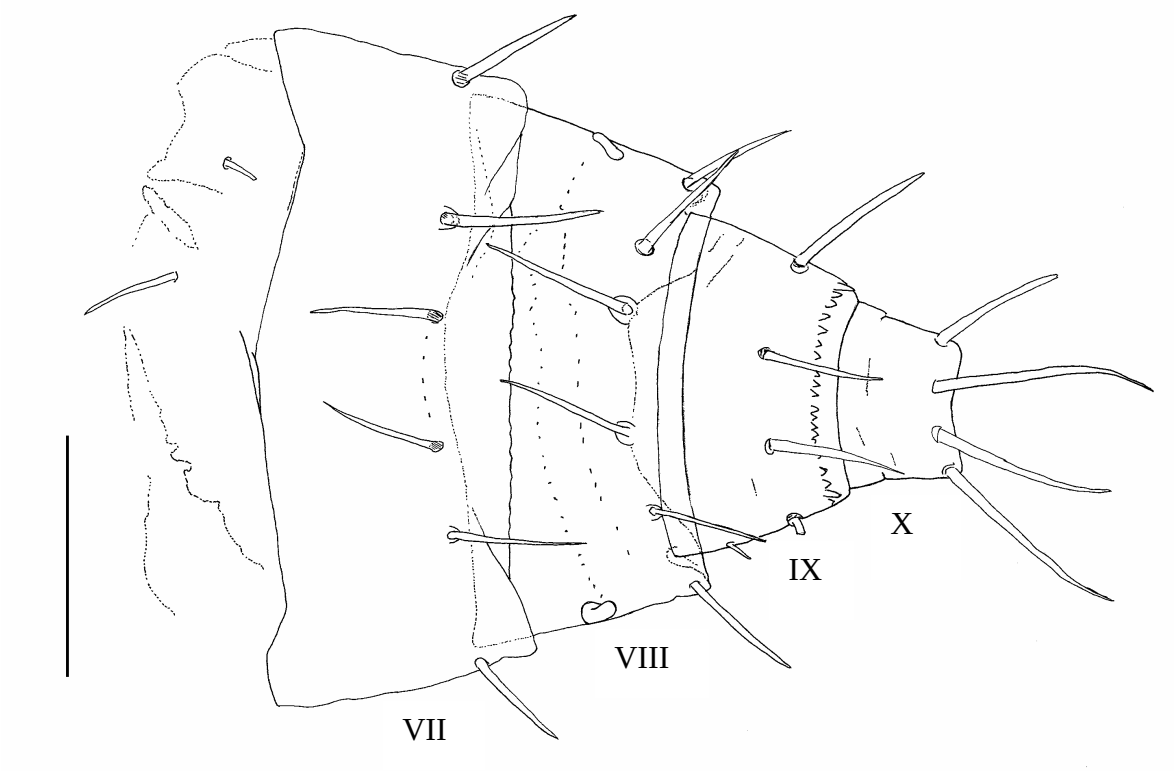


**Figure 4.7** The posterior section of a late larva II *F. occidentalis* abdomen, found to contain alarm pheromone. The pygidial gland, a potential candidate for alarm pheromone storage, is located in this region of the abdomen. Roman numerals indicate the segment number. Note that segment XI is not shown here, as it was not visible in the preparation. Bar = 0.1 mm.

# Chapter 5

# Effect on larval movement

## 5.1 Introduction

The manipulation of pest behaviour to protect a crop, or other valued resource, either by design or by accident, is crucial to effective pest management. There are a number of methods by which chemicals that modify insect behaviour can be used (reviewed by Foster & Harris, 1997). These fall into two categories: methods using chemicals that act over a short range (such as stimulants and deterrents) and methods over a long range (such as the attract-annihilate method and the disruption of behaviour using attractants and repellents).

The use of aphid alarm pheromone to protect crops has been attempted, but with variable results. Ester *et al.* (1993) reported that the aphid alarm pheromone, (*E)*-β-farnesene (EBF) showed a synergistic effect in combination with pyrethrum and that the pesticide was effective at one tenth the recommended application rate in combination with EBF. This supports the findings of El-Agamy & Haynes (1992), who found synergistic effects of EBF and malathion, although they reported that the aphids appeared to rapidly habituate, with aphids moving little after 30 min continuous exposure to EBF. This is supported by Wohlers (1981) who found that although walking aphids initially avoided EBF-contaminated plants, they also appeared to habituate readily, and eventually climbed the contaminated plants. Even though there appears to be a reduction in the alarm response of aphids to EBF after a short time, the formulation of EBF with pesticides still seems useful. Griffiths & Pickett (1987) found that the mortality of pesticide-resistant aphids to chemical and biological pesticides, which demonstrate reduced response to EBF, was significantly greater when formulated with EBF.

In contrast to the use of aphid alarm pheromone in their control, little work has been devoted to the study of the effect of *F. occidentalis* alarm pheromone on their movement.

### 5.1.1 Movement responses of insects to chemicals

The movement behaviour of insects in response to chemicals falls into three categories: taxes, kineses and releasers (Shorey, 1973; Kennedy, 1977).

When an insect exhibits taxis behaviour, the body is orientated in relation to the source of the chemicals, either away from (negative) or towards (positive). This chemotactic response can be subdivided, based on the period of time that the insect assesses the direction of the chemical source, into chemotropotaxis and chemoklinotaxis (Shorey, 1973). In chemotropotaxis, the insect uses paired sense organs, e.g. antennae, to simultaneously assess the direction of the chemical source. In negative chemotropotaxis, the insect turns away from the most stimulated side, representing the direction of the chemical, causing the insect to leave the area of the chemical. This results in insects dispersing from the source of the chemical. Positive chemotropotaxis results in insects aggregating at the chemical source. When the stimulation of the paired sense organs is equal, these directional turning responses cease.

In chemoklinotaxis only one sense organ is required or paired sense organs functioning as one. The insect assesses the direction of the chemical source by turning the body, and sense organ(s), from side to side, assessing the concentration of chemical present directionally over time. Depending on whether the response is negative or positive, the insect then turns either away or towards the chemical source.

Dethier *et al.* (1960) classified chemicals that elicit directional movements in insects away from the source as a repellent, and towards the source as an attractant.

In kinesis-mediated movement, there are no directionally orientated movements, either away from or towards a chemical. Rather insects disperse from (inverse kinesis), or aggregate towards (direct kinesis) a chemical due to changes in the rate of movement, i.e. orthokinesis, or changes in the rate of turning, i.e. klinokinesis. Dispersal from an area where a repellent is present would result orthokinetically from an increase in movement speed near the source and reduced speed when away from the source. In klinokinetic dispersal, there is no directional component to turning and so the direction of turning is random, with respect to the direction of the chemical (Kennedy, 1978). Thus, dispersal is achieved through reduced turning rate near the repellent, causing the insect to walk past the source, and increased turning away from the source, resulting in the insect remaining in an area free from the repellent. Conversely, direct klinokinesis results in the insect remaining

in the area of the chemicals, as occurs in *Neoseiulus cucmeris* (Oudemans) and *Orius tristicolor* (White), in response to *F. occidentalis* alarm pheromone (Teerling *et al.*, 1993a)

Chemicals that cause an increase in velocity, or initiate movement, are classed as locomotor stimulants, whilst those that result in a slowing down, or cessation, of movement are termed arrestants (Dethier *et al.*, 1960).

In the third category, releasers, the chemical releases a new behaviour, which sensitises the insect to a new stimulus (Shorey, 1973).

### 5.1.2 Known responses of larvae to alarm pheromone

Little is known of how alarm pheromone affects behaviour in *F. occidentalis* larvae. In aggregations on a bean pod, larvae II respond to the nearby production of an anal droplet (AD) by wagging the abdomen and walking away from the emitter, i.e. locomotion is stimulated and the larva orientates itself away from the alarm pheromone source (personal observation). This results in a wave of movement, as larvae moving away from the emitter encounter other larvae, causing them to jerk their abdomens and move. This activity may cause other larvae to produce an anal droplet and, occasionally, to drop from the bean pod.

Teerling *et al.* (1993b) reported that alarm pheromone, synthetic alarm pheromone (based on the anal droplet of a single individual) and its components caused stationary larvae to move away from the source and to drop from leaves either contaminated with or near a source of synthetic alarm pheromone. When presented with five anal droplets on a small filter paper wick, stationary larvae I and II responded significantly by moving $\geq$ 2 mm within one minute. Only larvae II moved a significantly greater distance from the wick with anal droplets compared to a wick alone. When presented with a crude whole body extract of larvae II, equivalent to five larvae II, larvae II still responded significantly, but did not move a significant distance from the source compared to larvae in the control group which did walk away. Larvae II started to respond and move significantly to crude extracts of larvae II at 0.05 larval equivalents (l.e.), but, again, not at five l.e.

In bioassays using synthetic alarm pheromone (1.5:1, decyl acetate:dodecyl acetate), larvae II responded significantly more in brighter conditions (7490 lux compared to 1712 lux). Under the brighter conditions, stationary larvae II responded significantly, by walking away, at 1 ng, but only moved a significantly greater distance at 10 ng. When tested with alarm pheromone components alone, stationary larvae II responded significantly at 1 ng to

both decyl acetate and dodecyl acetate. Whilst there was no difference in response between synthetic alarm pheromone and dodecyl acetate, larvae II responded significantly more to synthetic alarm pheromone than decyl acetate. There was no significant difference in response to synthetic alarm pheromone at doses of 1 ng, 10 ng, 100 ng, 1,000 ng, 10,000 ng and a five l.e. crude extract of larvae II None of the control larvae responded and the response at 0.1 ng was not significantly different from the control. The responses of larvae exposed to decyl acetate and dodecyl acetate did not significantly increase from 10 ng to 10,000 ng , including the crude extract. The distances moved by larvae in response to synthetic alarm pheromone increased significantly only at 10 ng and in the presence of the crude extract. Larvae exposed to decyl acetate showed no significant difference in the distance moved at any of the doses tested, although the distances moved were similar to those for 10 ng synthetic alarm pheromone and crude extract. Larvae presented with dodecyl acetate responded significantly between 10 ng and 10,000 ng. A significant number of larvae II were found to drop from leaves onto which filter paper discs with 1 μg and 10 μg synthetic alarm pheromone (molar ratio 1.5:1, decyl acetate:dodecyl acetate) had been pinned, representing approximately 50 and 500 anal droplets, respectively, from 4-5 d w larvae II. Larvae did not drop in significant numbers when 4-5 μg synthetic alarm pheromone was sprayed onto the leaves. When the leaves were in a closed container, with 10 μg synthetic alarm pheromone placed 1-2 cm from them, larvae also dropped in significant numbers.

### 5.1.3 Aims of work

The aims of this chapter were to (1) confirm that decyl acetate and dodecyl acetate affect larval movement, (2) attempt to determine the behavioural mechanism of avoidance; (3) to determine if avoidance was dose-dependent; (4) to determine if alarm pheromone ratio affected the response and (5) to determine if larvae habituate to alarm pheromone.

## 5.2 Materials and methods

### 5.2.1 Effect of dose on movement

#### 5.2.1.1 Bean leaf disc bioassay

In the first instance, the responses of larvae (4-5 d old) to decyl acetate and dodecyl acetate were examined. A bean leaf disc (BLD, 24 mm diam.) was cut from the first true leaves of a bean plant (*Phaeseolus vulgaris* L., var. 'Tendergreen') using a cork borer. Bean leaves were used as the leaf surface was smooth and unlikely to affect larval movement. This was then floated, adaxial surface uppermost, on distilled water in a clean glass Petri dish (GPD, 1.4 cm high, 5.1 cm diam.). This was then placed over a piece of white card (7 cm x 7 cm). Drawn onto the white card were five lines, spaced 6 mm apart. The BLD was then centred between the two outermost lines. When viewed from above, this arrangement allowed the observer to mentally divide the BLD into four zones (figure 5.1). The zones were assigned values of +2, +1, -1, -2. Hexane (1 µl) was placed onto the +2 zone, and 1 µl of either decyl acetate or dodecyl acetate in n-hexane (0.1 ng, 1 ng, 10 ng, 100 ng, 1000 ng, and 10000 ng $\mu l^{-1}$) placed onto the –2 zone. A larva was then placed onto the centre of the BLD and the zone it was in recorded every minute for 30 min. The side that n-hexane was placed onto the BLD was alternated between replicates. The mean zone score for each larva was then calculated. If decyl acetate or dodecyl acetate had no effect then the mean score would not be significantly different from zero. If these substances repelled the larvae, the mean score would be significantly greater than zero. A double control, consisting of 1 µl n-hexane in the +2 and -2 zones was also conducted. Four blocks of eight replicates were conducted for each substance and dose. There was no blocking between either doses or substances. Consequently, no statistical comparisons could be made between either doses or substances. A synthetic version of the alarm pheromone was not used, as the naturally occurring ratio had not been determined at the time of the experiment. Data were analysed using a two-tailed t-test of the mean, compared to zero. Larvae that became trapped in the water were excluded from analysis, even if the larva got back onto the BLD. All tests were conducted at 1000 lux and at 25-26°C.

#### 5.2.1.2 Glass rod bioassay (GRB)

In this bioassay, a thrips larva was isolated on a glass tube (length 10 cm, external diam. 1.5 mm) that was flame sealed at one end. The tube was held horizontal by a fine needle,

itself held horizontal by a retort stand, inserted down the open end of the glass tube (figure 6.2). Either 1 µl of n-hexane (control) or 1 µl synthetic alarm pheromone (1:1, 2 ng, 20 ng, 200 ng or 2000 ng $\mu l^{-1}$) was placed 3 cm from the sealed tip of the glass tube. The solvent was then allowed to evaporate before placing a 2-3 d old larva II onto the sealed tip of the glass tube, using a moistened, trimmed down brush. The time taken for the larva to exit the zone, i.e. walk past the 3 cm point where either n-hexane or synthetic alarm pheromone had been placed, was recorded. If the synthetic alarm pheromone had a repellent effect, the time taken for larvae to walk out of the zone would be significantly greater than that for larvae exposed to n-hexane alone. A complete randomised block design was used and 30 larvae per treatment bioassayed.

### 5.2.2 Ethometric analysis of larval movement on BLD

In order to gain an insight into how larvae avoid alarm pheromone, the movements of larvae on a BLD, as used in §5.2.1.1, were automatically tracked using Ethovision computerised video tracking software (Noldus Information Technology, The Netherlands).

The BLD was chosen because it allowed the larvae to repeatedly encounter alarm pheromone, and respond to it, over a short period of time. Ethovision determines the location of an object (= larva) on an arena (=BLD), by comparing successive images of the object on the arena (the experimental image) against a reference image of the arena alone. The software subtracts the reference image from the experimental image and determines the difference to be the object. This method is therefore very sensitive to any difference in the position of the arena between the experimental and reference images. Any slight difference not due to the larva (background noise) can result in Ethovision not tracking the object reliably. For this reason, the BLD was fixed onto the glass Petri dish (GPD) using a short piece of perspex rod (length 5 mm, diam. 5 mm), the ends of which were coated with a thin film of silicone grease (M494, Ambersil, UK) so that the BLD stuck to the rod, and the rod stuck to the GPD. The GPD was then filled with distilled water, up to the level of the BLD. In order to increase the contrast between the larva and the BLD, black card was placed beneath and around the sides of the GPD, reducing the amount of light reflecting off the base of the GPD and illuminating the BLD from the underside. The main light source was from a light rig and contrast was further increased by additional lighting, from above, from a cold light source. The temperature was 25-26°C.

Once the BLD and GPD were prepared, they were placed under a video camera linked to a video recorder via a monitor. Before applying the synthetic alarm pheromone and n-hexane, the video recorder was started. Once the 1µl n-hexane had evaporated, a 4-5 d w larva was placed onto the centre of the BLD and its movements recorded for 5 min, although the larva was only tracked by Ethovision for 4 min. As in §5.2.1.1, the side the n-hexane was applied to was alternated. A synthetic version of the alarm pheromone produced by 4-5 d w larvae (1.1:1, decyl acetate:dodecyl acetate)) with a total mass of 200 ng was used, as this had caused a significant avoidance effect in §5.2.1.1 and was what a larva might realistically encounter in a natural situation.

Once the larvae had been video recorded (n=35), the recordings were played back to Ethovision. In Ethovision, the circular arena was divided into two semicircles, or zones (figure 5.4). Synthetic alarm pheromone was placed into one zone and n-hexane into the other zone. The position of the larva was calculated by Ethovision five times per second to form a track of the larva's movement. The track data was then used to calculate the total time spent in each zone, the mean velocity in each zone and the mean absolute turn angle (= the change in direction of movement between consecutive samples, providing a measure of the amount of turning) for each larva. A down sampling step of three was used to eliminate body wobble, i.e. every third sample was excluded from analysis.

### 5.2.3 Effect of alarm pheromone ratio on movement

This bioassay was the same as that used in constructing the dose response curve (§5.2.1.2). The responses of 2-3 d old larvae II to different ratios of decyl acetate:dodecyl acetate were examined. The ratios used ranged from 1:0 (100% decyl acetate), 3:1, 1:1 (the naturally occurring ratio produced by 4-5 d w larvae II), 1:3 (the approximate ratio produced by 2-3 d old larvae II) and 0:1 (100% dodecyl acetate). The total mass of decyl acetate and/or dodecyl acetate was 200 ng. This mass represented approximately 11 AD's produced by 4-5 d w larvae II. As before, the control was 1 µl n-hexane.

### 5.2.4 Effect on movement of pre-exposure to alarm pheromone

The responses of larvae to alarm pheromone were tested with and without prior exposure to alarm pheromone for 24 h.

#### 5.2.4.1 Release of synthetic alarm pheromone from GC septa

In order to determine the amount of synthetic alarm pheromone released from rubber GC septa (GR-2, diam. 9.5 mm, Supelco, Poole, UK) in glass jars under standard rearing conditions, giving an indication of the level of exposure larvae received, the release rate of synthetic alarm pheromone from GC septa was measured. The septa were prepared by placing 40 µl pure synthetic alarm pheromone (1:1, decyl acetate:dodecyl acetate) onto the septa (n=3), and left for 2 h, allowing the septa to absorb all the synthetic alarm pheromone. The septa were then weighed before being placed into separate glass jars. The glass jars were then sealed with Teflon tape (Fisher Scientific Ltd., UK) and placed under standard rearing conditions. This prevented synthetic alarm pheromone escaping from the glass jars and potentially affecting thrips being reared in the same room. After 24 h, the septa were re-weighed and the lost mass of synthetic alarm pheromone calculated. This was also done for decyl acetate and dodecyl acetate, for comparison.

#### 5.2.4.2 Pre-treatment of larvae

A cohort of 1-2 d old larvae I was isolated on bean pod segments in Eppendorf tubes (§3.2.2), split into two groups, and placed into glass jars. In the first group, the glass jar contained a GC septum releasing synthetic alarm pheromone (§5.2.4.1). In the second group, the glass jar contained a GC septum with no synthetic alarm pheromone. The glass jars were then sealed by winding Teflon tape around the ground glass joint of the lid. This ensured that when the lid was placed onto the jar the seal would be airtight. The glass jars were then placed under standard rearing conditions (18:6 L:D, 25 ± 0.3°C). After 24 h the larvae, now aged 2-3 d, were bioassayed (§5.2.4.3).

#### 5.2.4.3 Bioassay

Pre-treated larvae were assessed for response to synthetic alarm pheromone using the bioassay described in §5.2.1.2. The responses of larvae from each group to n-hexane and 200 ng 1:1 (decyl acetate:dodecyl acetate) synthetic alarm pheromone were assessed. A complete randomised block design was used to enable statistical comparison not only within a group, but also between groups (n=30). Data were analysed using multiple Mann-Whitney tests.

## 5.3 Results

### 5.3.1 Effect of dose on movement

#### 5.3.1.1 Bean leaf disc bioassay

When placed onto a BLD, larvae appeared to be able to move freely around it and only a few larvae became trapped in the water. On occasion, larvae appeared to become 'stuck' on the BLD, perhaps having become impaled or stuck to a trichome. Such individuals were removed from analysis, as their movement had been restricted. In the decyl acetate and dodecyl acetate treatments, this occasionally resulted in the larva raising the abdomen, waving it about and sometimes producing a drop of fluid from the tip.

Larvae in the control group, i.e. n-hexane applied to both sides of the BLD, exhibited no preference for a particular side of the disc, having a mean score of $-0.116 \pm 0.149$, which was not significantly different from zero ($t_{30} = -0.77$, $P = 0.45$).

When decyl acetate was applied to one side of the BLD, larvae spent significantly less time on that side of the disc at doses of 100 ng, 1,000 ng and 10,000 ng, having scores significantly greater than zero ($0.585 \pm 0.084$, $t_{26} = 6.99$, $P < 0.001$; $0.403 \pm 0.111$, $t_{30} = 3.62$, $P = 0.001$; and $0.491 \pm 0.123$, $t_{29} = 3.98$, $P < 0.001$, respectively, figure 5.2a). No significant responses were found at doses of 0.1 ng, 1 ng and 10 ng ($-0.073 \pm 0.116$, $t_{31} = -0.63$, $P = 0.73$; $0.113 \pm 0.141$, $t_{30} = 0.80$, $P = 0.21$; and $0.144 \pm 0.132$, $t_{30} = 1.09$, $P = 0.14$, respectively, figure 5.2a).

Larvae appeared to be more sensitive to dodecyl acetate than decyl acetate, although this could not be proved statistically, due to the unblocked experimental design. At doses of 10 ng through to 10,000 ng, larvae had scores that were significantly greater than zero ($0.252 \pm 0.137$, $t_{30} = 1.85$, $P = 0.038$; $0.256 \pm 0.140$, $t_{29} = 1.83$, $P = 0.039$; $0.306 \pm 0.148$, $t_{29} = 2.07$, $P = 0.024$; and $0.606 \pm 0.120$, $t_{30} = 1.65$, $P = 0.055$, respectively, figure 5.2b).

#### 5.3.1.2 Glass rod bioassay

The time taken for larvae to walk out of the bioassay zone increased significantly with increasing dose (figure 5.3). Larvae took a mean ± SE of 72.8 ± 12.4 s, 135.2 ± 20.2 s, 164.4 ± 22.1 s, 188.5 ± 20.9 and 245.7 ± 18.1 s to walk past n-hexane (control, 2 ng, 20 ng, 200 ng and 2000 ng synthetic alarm pheromone, respectively. The treatment was not significant at 2 ng, but was significantly different at all other doses. Larvae took

significantly longer to walk past 2000 ng, compared to 200 ng. No larvae dropped off the glass rod during the bioassay.

### 5.3.2 Ethometric analysis of larval movement on BLD

Of the 35 replicates recorded, only 30 could be analysed successfully by Ethovision (figure 5.4). The remaining five replicates could not be analysed due to excessive background noise, which resulted in unreliable tracking of the larvae.

As indicated in §5.3.1.1, larvae spent significantly less time in the synthetic alarm pheromone zone compared to the n-hexane zone ($90.227 \pm 7.631$ s compared to $149.773 \pm 7.631$ s, respectively, $t_{30} = -5.52$, $P < 0.001$, figure 5.5). No significant differences were found in the remaining three variables examined. Velocity was almost exactly the same in both zones, being $0.132 \pm 0.008$ cm $s^{-1}$ in the synthetic alarm pheromone zone and $0.133 \pm 0.007$ cm $s^{-1}$ in the n-hexane zone (Mann-Whitney U test, W = 881.5, $P = 0.69$, , figure 5.6). Mean absolute turn angle between successive samples was also very similar, being $29.656 \pm 1.729$ degrees in the synthetic alarm pheromone zone and $28.491 \pm 2.710$ degrees in the n-hexane zone (Mann-Whitney U test, W = 938.0 $P = 0.20$, figure 5.7).

### 5.3.3 Effect of ratio on movement

Of the 240 2-3 d old larvae tested, 40 larvae per treatment, none dropped off the horizontally suspended glass rod. Larvae in the n-hexane (control) group rarely reacted, by stopping or turning around, at the point where n-hexane had been placed, 3 cm from the sealed tip of the glass rod. Conversely, larvae exposed to different ratios of alarm pheromone component(s) normally reacted by turning around and walking back down the glass rod. The distance from the source of alarm pheromone component(s) at which this occurred varied from about 2 cm to as little as a few millimetres.

The time taken by larvae to walk out of the zone, i.e. walk past the point where n-hexane or alarm pheromone component(s) had been placed, changed significantly with treatment (figure 5.8). Larvae in the control group took $56.100 \pm 7.065$ s to exit the zone, compared to $104.8 \pm 13.3$ s, $144.6 \pm 14.0$ s, $194.1 \pm 14.7$ s, $176.0 \pm 18.2$ s and $224.3 \pm 15.3$ s by larvae exposed to 200 ng alarm pheromone component(s) in ratios (decyl acetate:dodecyl acetate) of 1:0, 3:1, 1:1, 1:3 and 0:1, respectively. Larvae in the control group required significantly less time, compared to larvae in all other groups.

### 5.3.4 Effect on movement of pre-exposure to alarm pheromone

5.3.4.1 Release of synthetic alarm pheromone from GC septa

GC septa, in sealed one litre glass jars, released 0.47 ± 0.03 mg, 0.37 ± 0.09 mg and 0.33 ± 0.07 mg decyl acetate, dodecyl acetate and synthetic alarm pheromone (1:1 decyl acetate:dodecyl acetate) over a 24 h period at 25 ± 0.3°C (figure 5.9). The difference in release rate between these substances was not significantly different ($F_{(2,6)}$ = 1.08, $P$ = 0.40).

5.3.4.2 Effect on movement of pre-exposure to synthetic alarm pheromone

Overall, there was a significant difference in the times taken by larvae to walk out of the zone between larvae with and without prior exposure to alarm pheromone (figure 5.10). Larvae which had not been exposed to synthetic alarm pheromone in the 24 h preceding bioassay showed a similar response to that seen in §5.3.1.2, with larvae exposed to n-hexane (control) requiring significantly less time to walk out of the zone (31.8 ± 5.4 s) than larvae exposed to 200 ng 1:1 synthetic alarm pheromone (126.6 ± 18.2 s). In larvae pre-exposed to synthetic alarm pheromone in the preceding 24 h, there was no significant difference in the time taken for larvae exposed to n-hexane and synthetic alarm pheromone to walk out of the zone. The times taken for pre-exposed larvae (control and synthetic alarm pheromone) to exit the zone were not significantly different from that taken for larvae not pre-exposed to synthetic alarm pheromone to walk past synthetic alarm pheromone on the glass rod. No larvae dropped off the glass rod during the bioassay.

## 5.4 Discussion

The BLD bioassay to test the effect of alarm pheromone component dose on larval movement found that although 4-5 d old larvae were repelled significantly by the single components these responses were not vigorous. Single alarm pheromone component BLD bioassays found that 4-5 d old larvae were significantly repelled at masses of 100 ng and 10 ng for decyl acetate and dodecyl acetate, respectively (figure 5.2a,b). The maximum possible avoidance score for this bioassay was +2, yet the highest score recorded was +0.6, in response to 10,000 ng dodecyl acetate. It is probable that the sensitivity of this bioassay was compromised by the circular shape of the arena. Larvae tended to follow the leaf edges, so larvae walking away from alarm pheromone components would automatically be led back towards the odour source.

In order to facilitate the design of a more sensitive bioassay an attempt was made at determining the behavioural mechanism of avoidance. This was done by tracking the movement of larvae on a BLD, but this time using synthetic alarm pheromone, as this had been determined by this time. This showed a very strong avoidance of the synthetic alarm pheromone, based on the time spent in the synthetic alarm pheromone zone, but failed to reveal any significant differences in velocity and turn angle (figures 5.5 – 5.7). From observation of the tracks, it was noted that larvae appeared to avoid the synthetic alarm pheromone simply by turning away from the source and walking in a straight line, indicating that the mechanism of avoidance is likely to be negative chemotaxis (§5.1.1). The fact that this differential turning behaviour was not detected may once again be due to the shape of the arena. Larvae attempting to walk in a straight line would be forced to turn slowly, as they walked along the edge of the leaf disc, masking any difference in turning behaviour.

From these observations, a linear bioassay was developed: the glass rod bioassay. This bioassay was more sensitive than the BLD bioassay, and revealed a clear increase in the avoidance behaviour of 2-3 d old larvae, with increasing mass of synthetic alarm pheromone. When the glass rod bioassay was first attempted, 4-5 d old larvae were used. However, it was found that larvae of this age had a propensity to fall off the glass rod, regardless of treatment. This may be due to the larger size and greater velocity of 4-5 d old larvae. Conversely, 2-3 d larvae were never observed to drop off the glass rod, even at 2,000 ng synthetic alarm pheromone.

The responses of 2-3 d old larvae to different ratios of alarm pheromone components, ranging from pure decyl acetate to pure dodecyl acetate, showed that 2-3 d old larvae respond more to dodecyl acetate then decyl acetate. This mirrors the results of the dose experiment using the BLD, where larvae responded at a threshold of 10 ng for dodecyl acetate and 100 ng for decyl acetate. There was no evidence of any synergistic effect at the two physiological ratios, 1:1 and 1:3 decyl acetate:dodecyl acetate, supporting the conclusion drawn by Teerling *et al.*, (1993b). From this it would seem reasonable to use dodecyl acetate alone in future laboratory bioassays and field experiments.

In the habituation bioassay, the time taken by control larvae pre-exposed to synthetic alarm pheromone to walk past the hexane on the glass rod was significantly greater than for the non-exposed control, indicating that larval velocity was reduced by pre-exposure to synthetic alarm pheromone (figure 5.10). If larvae were habituating to their alarm pheromone one would expect pre-exposed larvae in the control group to have a similar velocity to that of control larvae not pre-exposed to synthetic alarm pheromone. As this bioassay design was based upon this assumption, the observed reduction in larval velocity meant that habituation could not easily detected. Consequently, another bioassay not reliant on larval velocity is required to test for habituation effect. These findings indicate that synthetic alarm pheromone may not be suitable as a bio irritant to increase insecticide efficacy, as has been demonstrated in some aphid species (Griffiths & Pickett, 1987). However, the bioassay used a glass rod and responses of larvae to synthetic alarm pheromone on leaves may well be different.

The observation that none of the 2-3 d old larvae used in the GRB dropped from the glass rod even at 2,000 ng and that 4-5 d old larvae did drop regularly is interesting. It may have some bearing on the alarm pheromone mediated dropping of larvae from plant leaves reported by Teerling *et al.* (1993b), who used larvae II of unknown age. If the larvae used in their experiments were close to moulting then the wandering behaviour of such larvae could have contributed to the observed dropping effect. Conversely, the mass of synthetic alarm pheromone used in the experiments presented here was far lower than those used by Teerling *et al*, (1993b).

From these experiments in can be concluded that (1) decyl acetate and dodecyl acetate do affect larval movement; (2) that the avoidance mechanism could be negative chemotaxis; (3) that the effect is dose-dependent; (4) that there is no synergism at physiological ratios, rather that dodecyl acetate has a greater affect than decyl acetate and (5) that although

habituation to alarm pheromone has not been demonstrated, pre-exposure reduces larval activity, a point that could have an important bearing if the alarm pheromone is used to manipulate larval behaviour in glasshouses.

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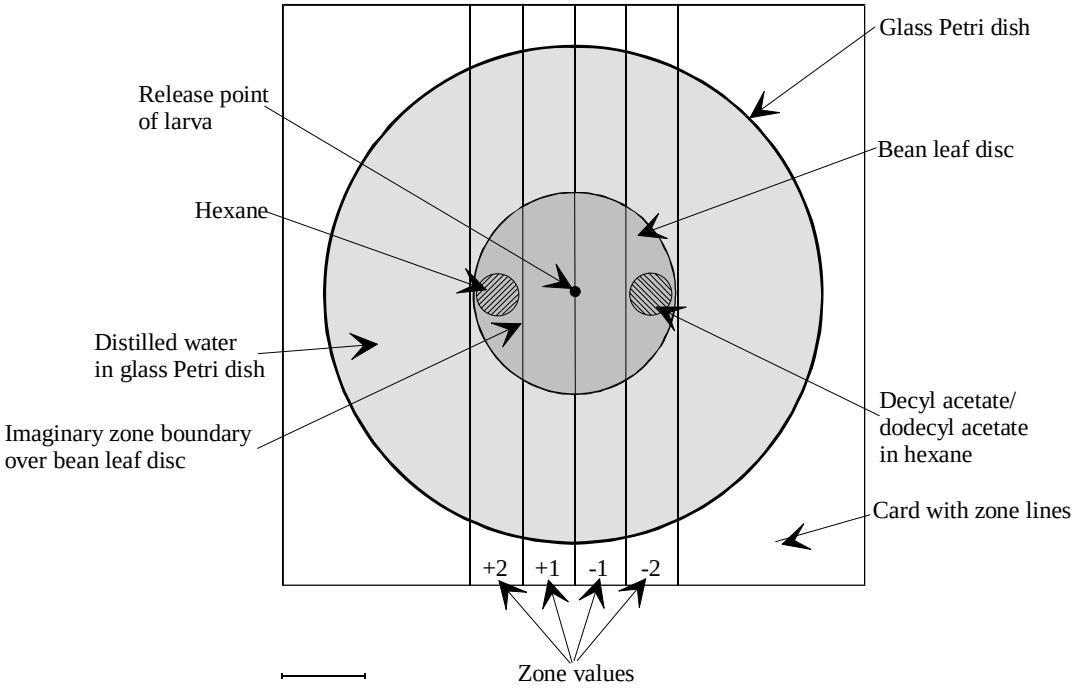


**Figure 5.1** Diagrammatic plan view of the bean leaf disc (BLD) bioassay used to examine the avoidance responses of 4-5 d old *F. occidentalis* larvae to varying amounts of alarm pheromone components, decyl acetate and dodecyl acetate. Hexane and decyl acetate, or dodecyl acetate, in hexane were applied to opposite sides of the BLD, which was floating on distilled water to restrict the larva to the BLD. The location of the larva, with respect to four zones on the BLD, was recorded once per minute for 30 min. Larvae repelled by the test substance would have a mean zone score significantly greater than zero. Non-responding larvae would have a zone score not significantly different from zero. Bar = 1 cm.

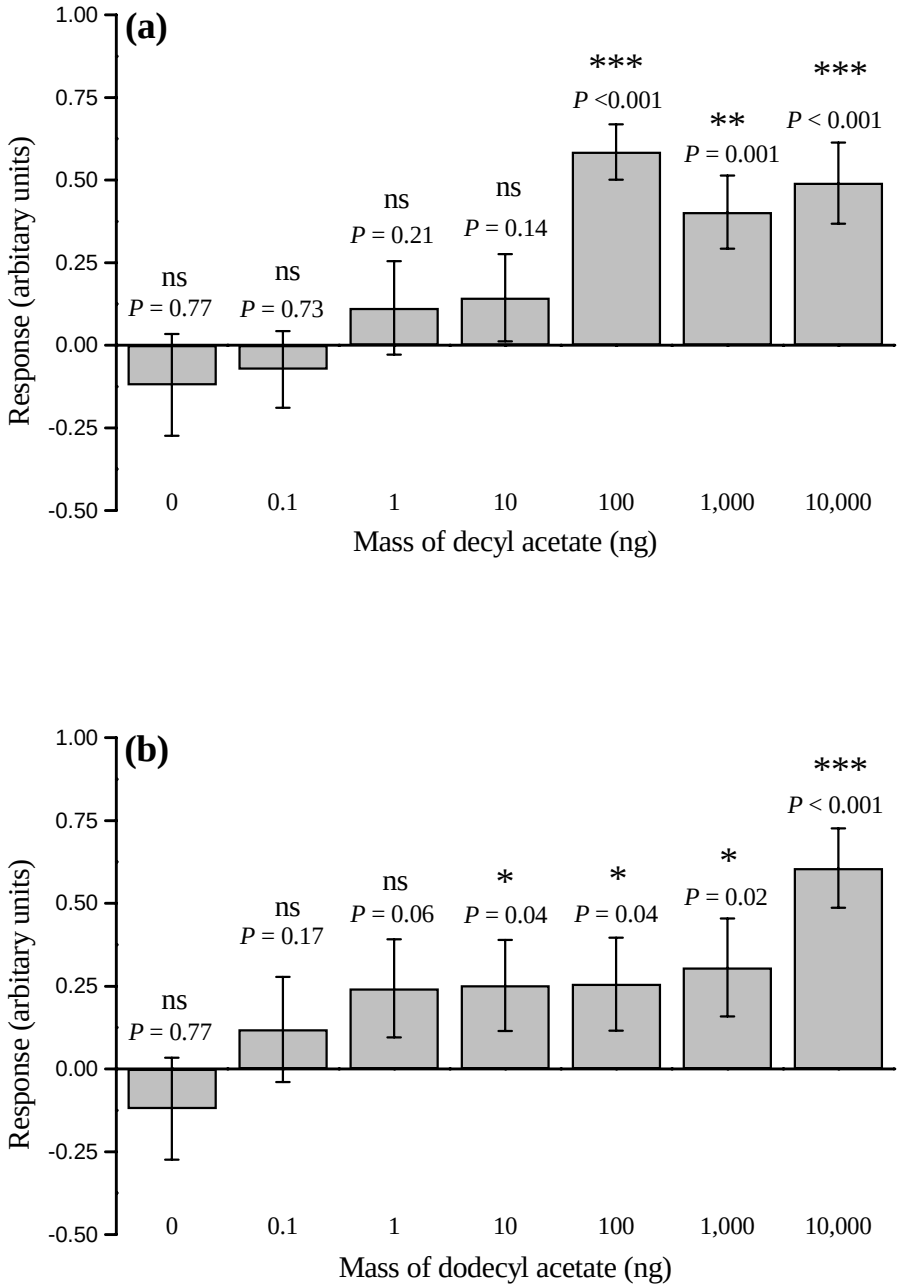


**Figure 5.2** Movement responses of 4-5 d old *F. occidentalis* larvae to varying amounts of (a) decyl acetate or (b) dodecyl acetate, using the bean leaf disc bioassay (Figure 5.1). Larvae repelled by the test substance had a response significantly greater than zero. Larvae not responding had a response score not significantly different from zero. *P*-values are for differences from zero. *P* = probability; ns = not significant.

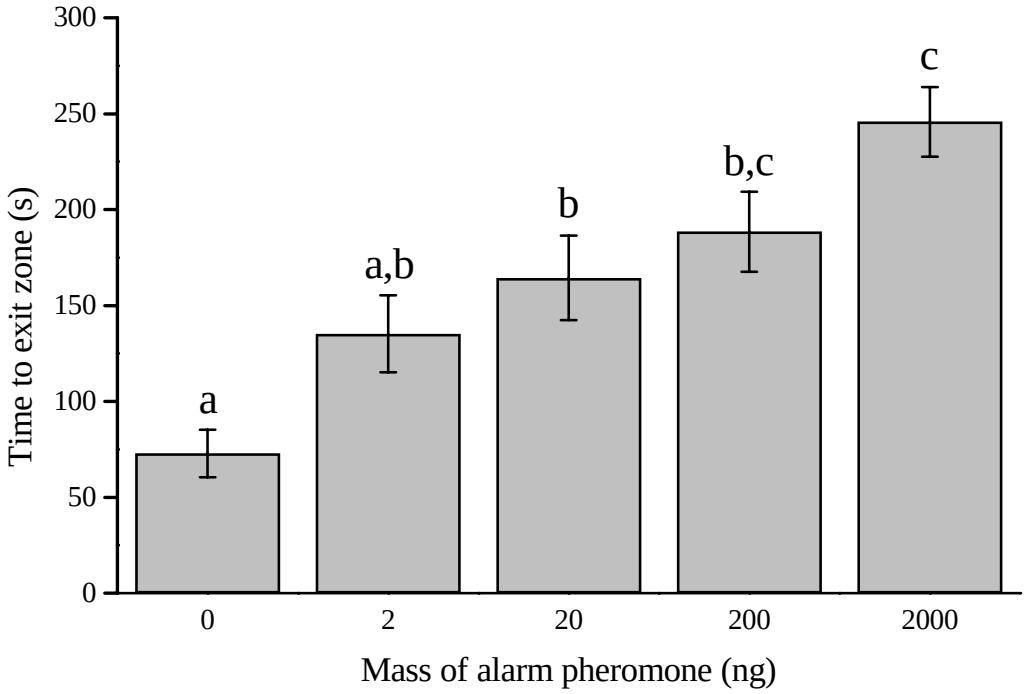


**Figure 5.3** The time taken by 2-3 d old *F. occidentalis* larvae on a glass rod to walk past varying amounts of alarm pheromone. Bars sharing the same letter are not significantly different at the 95% confidence level.

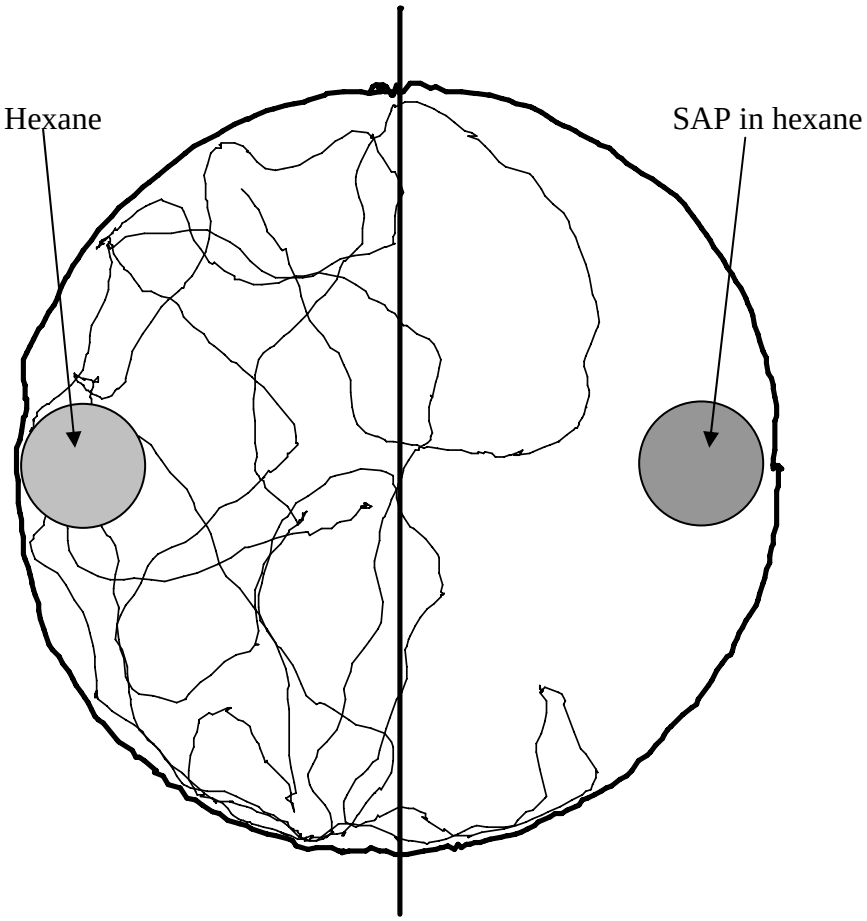


**Figure 5.4** Track of a 4-5 d old *F. occidentalis* larva on a bean leaf disc (BLD), in the presence of 200 ng synthetic alarm pheromone in hexane (SAP) and hexane control, as captured by Ethovision video tracking software. The circular line indicates the BLD boundary. This is bisected by a vertical line, to form two semicircles, or zones. SAP in hexane and hexane control were then applied to the BLD, in this example to the right and left, respectively. Once the hexane had evaporated, a larva was released near the centre of the BLD (indicated by the closed square), and its position recorded five times per second for four min. Ethovision was then used to calculate the mean time, velocity and amount of turning in each zone (figures 5.7, 5.8 and 5.9, respectively).

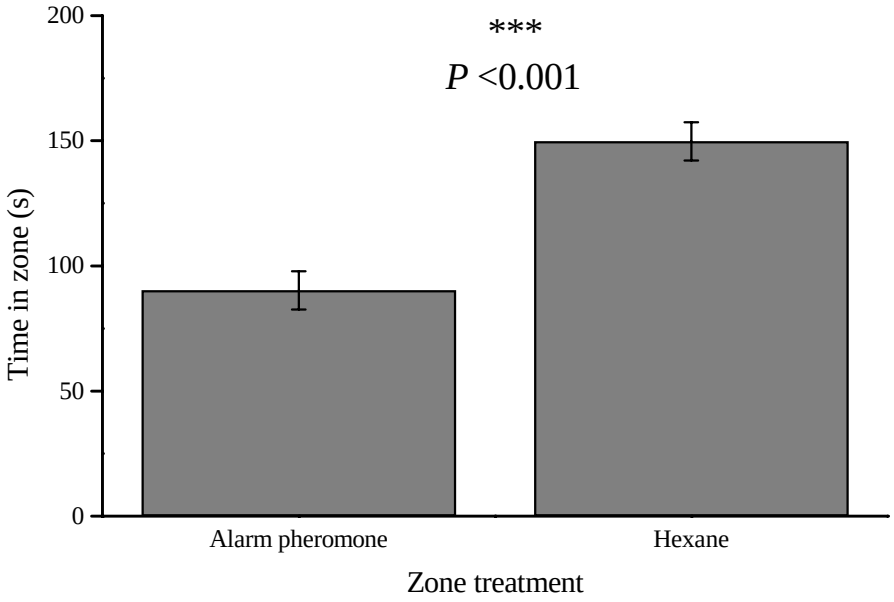


**Figure 5.5** The mean time spent by 4-5 d old *F. occidentalis* larvae in two semicircular zones, treated with either 200 ng alarm pheromone or hexane control, on a bean leaf disc, as recorded by Ethovision software over four min.

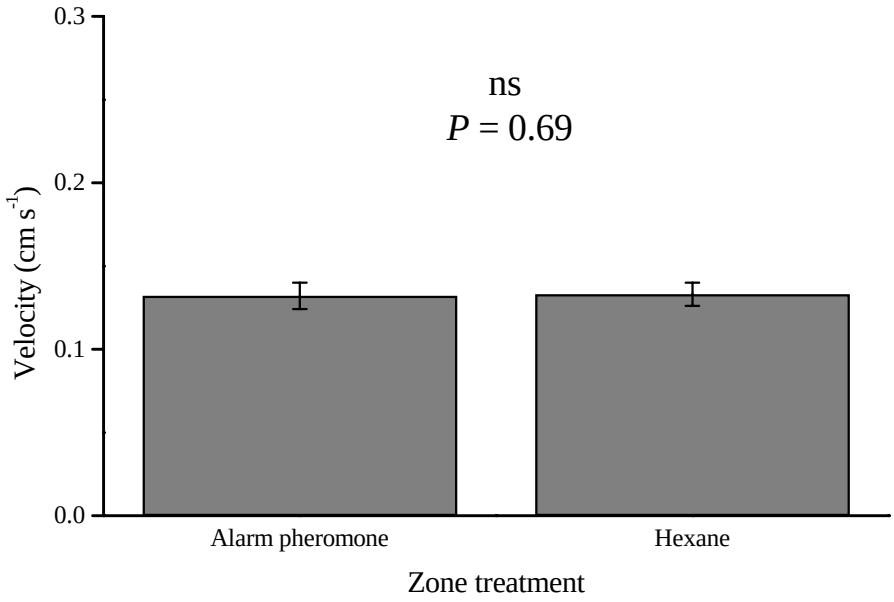


**Figure 5.6** The mean velocity of 4-5 d old *F. occidentalis* larvae in two semicircular zones, treated with either 200 ng alarm pheromone or hexane control, on a bean leaf disc, as recorded by Ethovision software over four min.

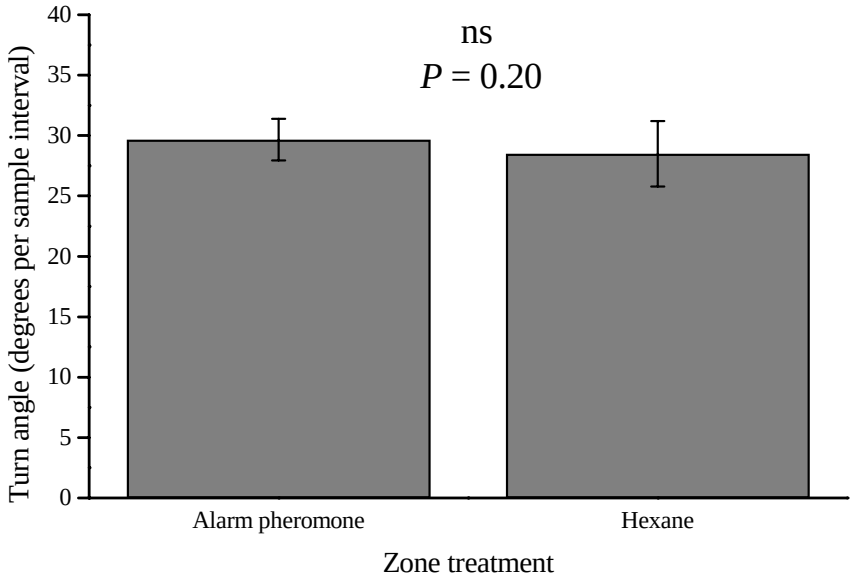


**Figure 5.7** The mean amount of turning exhibited by 4-5 d old *F. occidentalis* larvae in two semicircular zones on a bean leaf disc in the presence of 200 ng alarm pheromone in hexane and hexane control, as recorded by Ethovision software over four min. Mean amount of turning is between sample intervals of 250 ms.

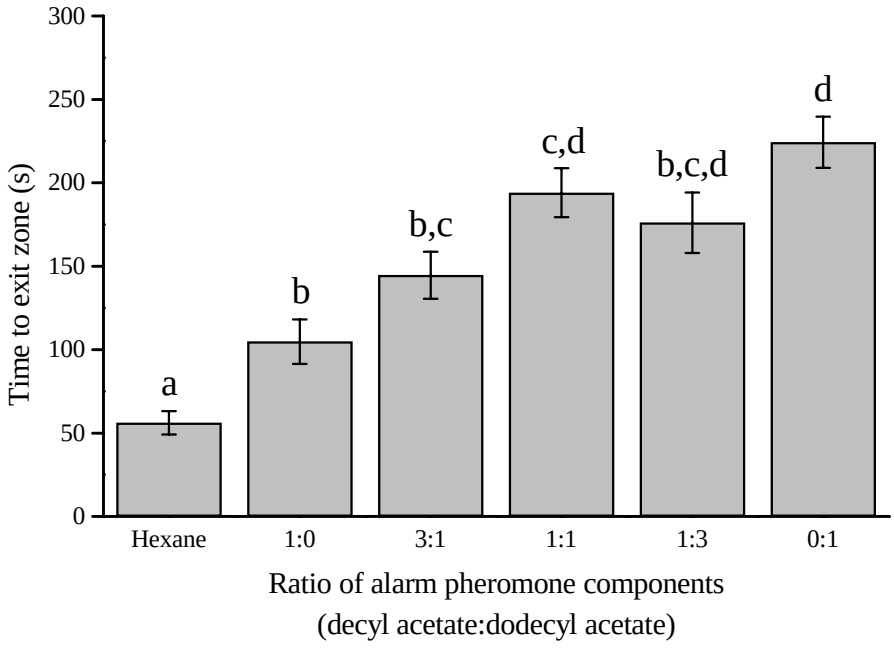


**Figure 5.8** The mean times taken by 2-3 d by *F. occidentalis* larvae to walk past differing ratios of alarm pheromone components (200 ng total mass) in the glass rod bioassay. Bars sharing the same letter are not significantly different from each other at the 95% confidence level.

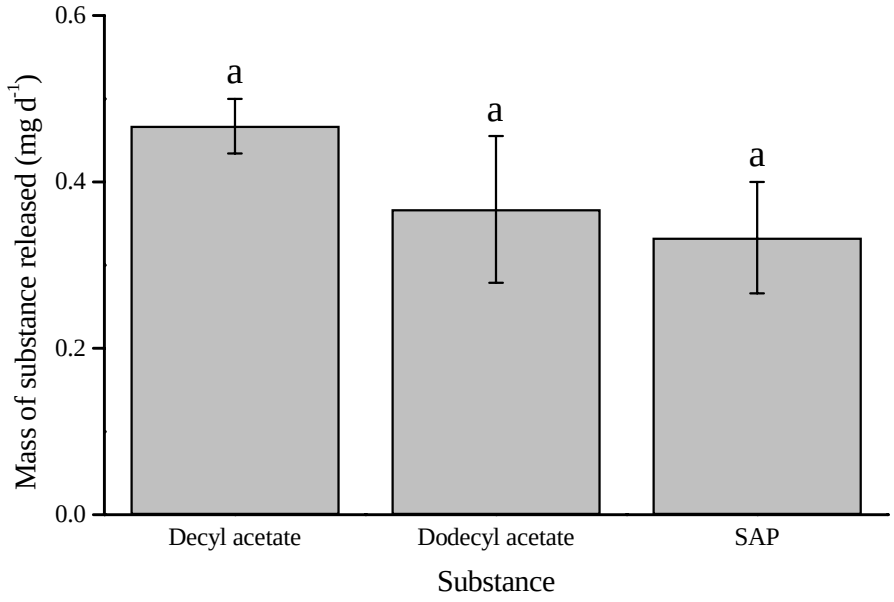


**Figure 5.9** Determination of the amount of synthetic alarm pheromone (SAP) and individual components released from rubber septa over 24 h at 25 ± 0.3°C in glass jars, giving a measure of the amount of alarm pheromone exposure experienced by *F. occidentalis* larvae in the habituation bioassay. Bars sharing the same letter are not significantly different from each other at the 95% confidence level.

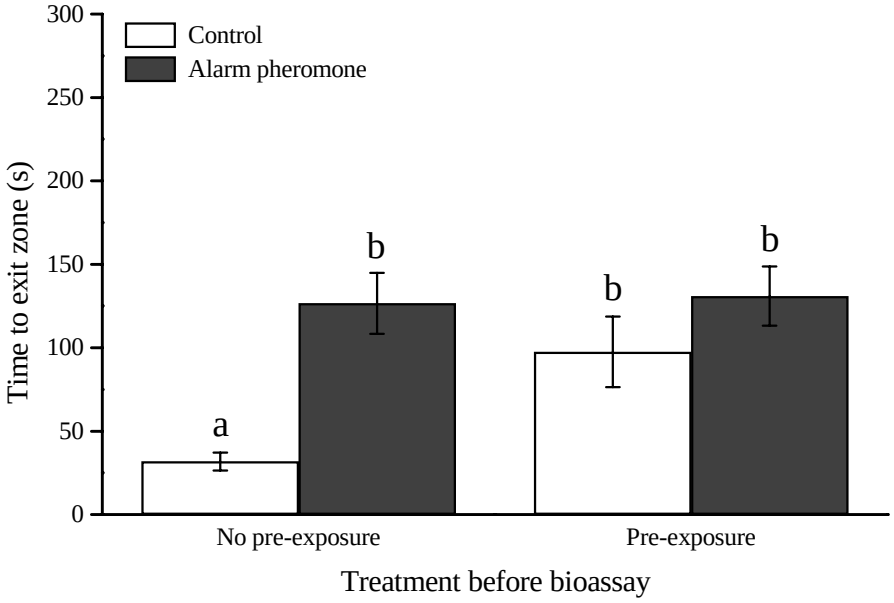


**Figure 5.10** The effect alarm pheromone pre-exposure on the time taken by 2-3 d old *F. occidentalis* to walk past 200 ng alarm pheromone in the glass rod bioassay. Bars sharing the same letter are not significantly different from each other at the 95% confidence level.

# Chapter 6

# Effect on adult movement

## 6.1 Introduction

The alarm pheromone of several aphid species has been well investigated and effects on walking, take-off and landing by adults have been demonstrated (Bowers *et al.*, 1972; Kislow & Edwards, 1972; Wohlers, 1981, 1982). However, in contrast the effects of *F. occidentalis* alarm pheromone on adult movement are poorly understood. Teerling *et al.* (1993) found that an extract of larvae II, when presented to stationary adults, repelled a significant proportion (65%), compared to the control, where only 35% were repelled. Responding adults did not move significantly greater distances when presented with larval extract, compared to control.

### 6.1.1 Possible effects of alarm pheromone on adult movement

The observation that anal droplets are only produced by larvae under threat suggests several evolutionary predictions. As alarm pheromone indicates 'danger', *F. occidentalis* perceiving this signal would be expected to move away from the source. Larvae are known to move away from the source (Teerling *et al.*, 1993), possibly retreat to refuges (Venzon *et al.*, 2000) and drop from the host plant (Teerling *et al.*, 1993) in the presence of alarm pheromone. Movement away from the pheromone would be expected to be more pronounced in adults than larvae, not only because they are more active than larvae, but because they can also fly away. The presence of alarm pheromone may also result in adults not landing in contaminated areas, to either feed or oviposit. It is known that adult females do walk away when an alarm pheromone source is presented to them (Teerling *et al.*, 1993) and that females lay fewer eggs in an area contaminated with alarm pheromone (Teerling *et al.*, 1993). However, nothing is known of how the *F. occidentalis* alarm pheromone may affect flight behaviour. The flight response of aphids to their alarm

pheromone is well known. It causes alate aphids to take-off from alarm pheromone contaminated leaves (Wohlers, 1982) and causes aphids to avoid alarm pheromone contaminated plants whilst in flight (Wohlers, 1981, 1982), so reducing the numbers of aphids landing on such plants. Western flower thrips may respond to their alarm pheromone in a similar way.

### 6.1.2 Relevance of alarm pheromone to IPM strategies

Attempts have already been made to use alarm pheromone in the control of aphids. Alarm pheromone on its own has not proved effective in controlling aphids (Pickett *et al.*, 1992), but has improved the efficacy of chemical and biological insecticides (Griffiths & Pickett, 1987; El-Agamy & Haynes, 1992) through increased aphid activity. A similar approach may be possible in *F. occidentalis* if activity levels could be increased. Manipulation of the glasshouse environment to increase flight activity is already known to improve chemical insecticide efficacy (Shipp & Zhang, 1999). Such increased activity may have additional benefits, such as increasing the exposure of *F. occidentalis* to biological controls, or through increasing trap catches. If effective, the alarm pheromone could be employed in a push-pull or stimulo-deterrent diversionary strategy (Pickett *et al.*, 1997). However, as well as having beneficial aspects, increased activity levels may also have drawbacks, such as the increased spread of plant viruses through a crop.

### 6.1.3 Aims of work

The aims of this chapter were to (1) ascertain if alarm pheromone or a single component of the alarm pheromone reduces landing rate on an attractive trap; (2) if a single component of the alarm pheromone reduces the presence of adults in cucumber flowers; (3) affects walking behaviour; (4) increases take-off rate and (5) determine if dodecyl acetate is as effective as synthetic alarm pheromone, as observed in walking bioassays with larvae (Chapter 5).

## 6.2 Materials and methods

### 6.2.1 Effect on landing

The effect of dodecyl acetate and synthetic alarm pheromone on landing rate was tested using sticky blue traps (trapping area 21x10 cm, Agralan Ltd., Swindon, UK) in commercial mature cucumber crops in glasshouses in the Humberside area. The first two experiments, conducted in October 1999, examined the landing responses to dodecyl acetate only. In September 2000, two additional experiments were conducted, in a different glasshouse, which examined the landing responses to synthetic alarm pheromone and dodecyl acetate. Blue traps were used as these are attractive to *F. occidentalis*, and it would therefore be possible to detect a reduction in trap catch. The numbers of thrips trapped was further enhanced by placing the traps at crop height (2.15 m) where most thrips would be located (Gillespie & Vernon, 1990). The traps were fixed in place with cotton twine onto wires, which supported the cucumber plants and ran the length of the row. Spacing between traps was approximately 4 m. A GC septum with synthetic alarm pheromone, dodecyl acetate or nothing (control) was affixed to one side of each trap with a pin (figure 6.1a). This released dodecyl acetate at a known rate (§6.2.1.1). Traps were arranged in a complete randomised block design, to reduce possible heterogeneous crop effects, along rows in the crop. There was one replicate of each treatment per block. In the first two experiments (1999 experiment 1, 1999 experiment 2, §6.2.1.2), only dodecyl acetate was used as a treatment, as the exact ratio of compounds produced by known-age larvae was not known at that time. Once the naturally occurring ratio was known (Chapter 4), the effect of synthetic alarm pheromone and dodecyl acetate, relative to each other, was tested to determine if a synergistic effect occurred when using synthetic alarm pheromone (2000 experiment 1, 2000 experiment 2, §6.2.1.3). In all experiments, the traps were left out for 24 h and then covered with clear plastic to prevent debris and further thrips being collected. Trapped thrips were then identified, sexed and counted using a dissection microscope. As removal of thrips from the sticky material on the trap was difficult, identification was based on general appearance and, specifically, the shading and number of antennal segments.

#### 6.2.1.1 Rate of dodecyl acetate release from a dispenser

Test substances were released from silicone rubber GC septa (GR-2, diam. 9.5 mm, Supelco, Poole, UK) to which 40 µl test substance, or nothing (control), had been applied.

The release rate of dodecyl acetate from these septa was measured prior to field trials commencing. As the naturally occurring alarm pheromone ratio was not known at that time, only dodecyl acetate was tested. However, as the molecular weights of decyl acetate and dodecyl acetate are broadly similar, it was assumed that the release rates of dodecyl acetate and synthetic alarm pheromone from GC septa would be similar.

Five GC septa were placed on separate glass Petri-dishes (diam. 5.4 cm) and 34.6 μg (40 μl) dodecyl acetate applied to the upper surface of the septa. The weight of the Petri dishes was then recorded at intervals over 96 h. During the experiment, the Petri dishes were kept in a glasshouse, so that the conditions, principally temperature and relative humidity (r.h.) that would affect release rate, would be similar to those in the field trials. The maximum and minimum temperature r.h. were recorded every 24 h.

6.2.1.2 Effect of dodecyl acetate

A total of 48 traps were placed out along rows in a mature cucumber crop. A complete randomised block design was used with one replicate of each treatment per block. There were two blocks per row and eight rows were used. Traps were collected in after 24 h. The experiment was repeated once (1999 experiment 1, 13-14.10.99, 1999 experiment 2, 14-15.10.99).

6.2.1.3 Comparison of the effects of dodecyl acetate and synthetic alarm pheromone

A total of 90 traps were placed out along rows in a mature cucumber crop. A complete randomised block design was used with one replicate of each treatment per block. There were two blocks per row and 15 rows were used. Traps were collected in after 24 h. The experiment was repeated once (2000 experiment, 1 26-27.9.00, 2000 experiment 2, 27-28.9.00).

### 6.2.2 Effects on take-off

A thrips that encounters alarm pheromone whilst on a surface can respond by either flying away, or by walking away. In order to investigate the effects of alarm pheromone on take-off rate these alternative responses had to be studied by permitting only one response to occur at a time. This was achieved by restricting a thrips to the outside of a glass rod, where it could not walk more than 3 cm away from the alarm pheromone but could fly away (§6.2.2.1), or by placing a thrips inside a glass tube where it could not fly away but could walk away from the alarm pheromone (§6.2.2.2). As these experiments were

artificial, an additional bioassay to examine the effect of alarm pheromone on take-off was performed on a bean leaf disc (§6.2.2.3), which represents a more natural substrate. All experiments were performed at 25-26°C and approximately 1000 lux using adult females of mixed-age from the chrysanthemum culture which were tested against either 200ng (total weight) 1.1:1 decyl acetate:dodecyl acetate mass ratio per 1 µl n-hexane, or 1 µl n-hexane control.

#### 6.2.2.1 Glass rod bioassay of take-off

In this bioassay, a thrips was isolated on a glass rod (length 3 cm, external diam. 1.5 mm) that was flame sealed at one end. The rod was held horizontal by a fine needle, itself held horizontal by a retort stand, inserted down the open end of the glass tube (figure 6.2). The thrips was prevented from walking off the glass rod by a barrier, formed from a glass cover slip with a small hole in its centre, which was placed onto the supporting needle before the glass tube. The barrier was held vertical against the glass tube by a glass spacer. The thrips was deterred from walking onto the glass barrier by a thin layer of silicone grease (M494, Ambersil Ltd., UK) smeared over the surface of the glass barrier facing the thrips. The silicone grease was colourless and had a very low volatile component. Either alarm pheromone or n-hexane control was placed at the open end of the glass tube. Once the n-hexane had evaporated, which took a few seconds, a thrips was placed onto the sealed end of the glass tube, using a moistened trimmed down paintbrush. The thrips was observed for 270 s and the time taken to fly away recorded. A fresh tube was used each time and each treatment replicated 30 times. If the alarm pheromone caused an increase in take-off rate relative to the control, thrips exposed to alarm pheromone would be expected to spend a significantly shorter period of time on the glass rod than on the control.

#### 6.2.2.2 Glass tube bioassay of walking

To restrict thrips movement behaviour to walking alone, the thrips were placed inside an open-ended tube (length 3 cm, internal diam. 3.5 mm), which was held horizontal by two glass supports near each end (figure 6.3). Before the thrips was introduced into the tube, using a moistened paintbrush, either 200ng alarm pheromone or 1 µl n-hexane was smeared around the inner surface of one end of the tube using the needle-tip of a microsyringe. Once the n-hexane had evaporated, a thrips was introduced into the tube through the other end of the tube. Once the thrips was in the tube, the end through which the thrips had been placed into the tube was closed with a Teflon plug. This meant that the

thrips could leave the tube only by walking past the alarm pheromone. Each thrips was observed for up to 270 s and the time taken to walk out of the tube recorded. Each treatment was replicated 30 times. If the alarm pheromone repelled the thrips, the thrips exposed to alarm pheromone would be expected to take significantly longer to walk out of the tube compared to the control.

6.2.2.3 Bean leaf disc bioassay

Thrips were placed onto leaf discs with or without alarm pheromone to test if take-off rate could be increased in the presence of alarm pheromone whilst on a natural substrate. Leaf discs (diam. 24 mm) were cut, using a cork borer of the same diameter, from the first true leaves of bean plants, *Phaseolus vulgaris* L., which had been grown in an uninfested glasshouse. The leaf disc was immediately floated on distilled water in a glass Petri dish (diam. 54 mm), adaxial side uppermost. When cutting the leaf discs, the central leaf rib was always avoided. Either 200ng alarm pheromone, in 1 µl n-hexane, or 1 µl n-hexane control, was placed onto the centre of the bean leaf disc. The ratio of alarm pheromone used was not known as accurately as later, resulting in the use of a ratio of 1:1 instead of 1.1:1. While the n-hexane evaporated, five thrips were placed into an Eppendorf tube, which was then placed into ice for 10 s. This briefly inactivated the thrips, allowing them to be tipped onto the centre of the bean leaf disc in one go. The thrips were observed for 300 s and the time at which each thrips flew off the bean leaf disc was recorded. Each treatment was replicated 36 times.

### 6.2.3 Effects on abundance in flowers

To test if dodecyl acetate reduced the abundance of *F. occidentalis* in flowers, GC septa with or without dodecyl acetate were placed just behind newly opened flowers by pinning them to black cards (0.3 mm thick, 3x3 cm) which were in turn suspended by a notch in the card (figure 6.1b) from the stem immediately behind the flower.

A total of 70 flowers were selected in a completely randomised block design of 35 pairs (blocks). Flowers within a pair were 2 – 5 m apart and 1 – 2 m above the ground. The flowers were carefully sampled into pots containing 70% alcohol 24 h after the septa were placed behind the flowers. Minimum and maximum temperature and r.h. were recorded for the 24 h period of the experiment. Thrips were identified, based on general appearance and the appearance of the antennae, counted and sexed under a dissection microscope. Ten

randomly selected males and females were mounted on glass slides for identification using a compound microscope.

## 6.3 Results

### 6.3.1 Effect on landing

6.3.1.1 Rate of dodecyl acetate release from a dispenser

Release rate of dodecyl acetate from the septa (figure 6.4) was higher in the two hours following application ($420 \pm 92$ μg septum$^{-1}$ h$^{-1}$), probably due to the presence of unabsorbed liquid dodecyl acetate on the surface of the septa. Thereafter, dodecyl acetate was released from the septa at a steadier rate of $17 \pm 6$ μg septum$^{-1}$ h$^{-1}$. This corresponds to approximately one late larva II anal droplet, which contain a total weight of 18 ng alarm pheromone (Chapter 4), being released every 4 s. When disturbed, groups of larvae can rapidly produce anal droplets (personal observation). The release rate of dodecyl acetate from septa is therefore likely to be similar to that released by larval groups which have been disturbed, for example, by a predator. It is unlikely that excessive amounts of alarm pheromone would build up around septa in a glasshouse due to the dispersing effect of air currents. Glasshouse temperature and r.h. ranged from 14-33°C and 33-95%, respectively, during the course of the experiment.

6.3.1.2 Effect of dodecyl acetate

Of the two trapping experiments conducted during 1999, only one showed a significant effect of dodecyl acetate. In both experiments, the raw data for trap catches was $\log_{10}(x+1)$ transformed and sex ratios (females to males) were $\log_{10}(x)$ to normalise the residuals.

In the first experiment (figure 6.5a), trap catches were not significantly affected by dodecyl acetate for females ($F_{(1, 23)} = 3.34$, $P = 0.081$) or males ($F_{(1, 23)} = 4.21$, $P = 0.052$). Trap catches were reduced by 14% for females and 12% for males. However, the reductions were on the margins of significance and in the predicted direction. No significant difference in the sex ratio of thrips caught on the control ($1.051 \pm 0.078$ untransformed) and dodecyl acetate ($1.002 \pm 0.060$ untransformed) ($F_{(1,23)} = 0.16$, $P = 0.69$) traps.

In the second experiment (figure 6.5b), the numbers of thrips landing on traps with dodecyl acetate was significantly lower for both females ($F_{(1, 23)} = 10.38$, $P = 0.004$) and males ($F_{(1, 23)} = 9.09$, $P = 0.006$). Trap catches were reduced by 36% for females and 18% for males. No significant difference in sex ratio was found between control ($1.693 \pm 0.199$

unstransformed) and dodecyl acetate (1.408 ± 0.147 untransformed) ($F_{(1,23)}$ = 0.19, $P$ = 0.19) traps.

During these experiments, the glasshouse conditions ranged by 14-41°C and 33-94% r.h. for experiment 1 and 15-24°C and 76-96% r.h. for experiment 2. During experiment 1 it was noted that the glasshouse vent system was open due to the high temperatures.

6.3.1.3 Comparison of the effects of dodecyl acetate and synthetic alarm pheromone

In both the trapping experiments conducted during 2000 the presence of dodecyl acetate or synthetic alarm pheromone significantly reduced landing rate in both female and male *F. occidentalis*. Raw data were $\log_{10}(x+1)$ transformed and sex ratios (females to males) were $\log_{10}(x)$ transformed to normalise the residuals.

In the first experiment, the numbers of thrips landing on traps with dodecyl acetate and synthetic alarm pheromone were significantly lower for both females ($F_{(2,53)}$ = 77.9, $P < 0.001$) and males ($F_{(2,\ 53)}$ = 37.7, $P < 0.001$). Dodecyl acetate and synthetic alarm pheromone reduced trap catches by 51% and 54% for females and 39% and 45% for males (figure 6.6a). There was no significant difference between dodecyl acetate and synthetic alarm pheromone for females ($t_{(53)}$ = 0.77, adjusted $P$ = 0.44) or males ($t_{(53)}$ = 1.39, adjusted $P$ = 0.17). Analysis of sex ratio between the control and treatment traps revealed that the ratio of females to males was significantly lower in the treatments (0.493 ± 0.028 untransformed) compared to the control (0.588 ± 0.036) ($F_{(1,54)}$ = 8.58, $P$ = 0.005).

The result was similar in the second experiment (figure 6.6b), with the dodecyl acetate and synthetic alarm pheromone significantly reducing the numbers of females ($F_{(2,\ 57)}$ = 11.68, $P < 0.001$) and males ($F_{(2,\ 57)}$ = 6.36, $P$ = 0.003) landing on traps. Reductions in trap catch due to dodecyl acetate and synthetic alarm pheromone were similar to that in experiment 1, with females being reduced by 47% and 42% and males by 37% and 36%. As before, there was no significant difference between the effects of dodecyl acetate and synthetic alarm pheromone for females ($t_{(57)}$ = 0.23, adjusted $P$ = 0.82) and males ($t_{(57)}$ = 0.27, adjusted $P$ = 0.79). However, sex ratio analysis showed no significant difference between the control (0.597 ± 0.046 untransformed) and treatment (0.519 ± 0.033 untransformed) ($F_{(1,57)}$ = 2.74, $P$ = 0.10) traps.

During these experiments, the glasshouse conditions ranged from 15-33°C and 41-95% r.h. for experiment 1 and 15-38°C and 39-95% RH for experiment 2.

### 6.3.2 Effects on take-off

#### 6.3.2.1 Glass rod bioassay of take-off

The presence of synthetic alarm pheromone caused an increase in rate of take-off in mixed-age adult females restricted on a glass rod (Mann-Whitney U-test, $P < 0.001$, table 6.1). Females were observed to take-off from any point along the length of the rod, not just near where the synthetic alarm pheromone had been placed.

#### 6.3.2.2 Glass tube bioassay of walking

As mixed-age adult females took longer to walk past the synthetic alarm pheromone, they took longer to walk out of the glass tube compared to the hexane control (table 6.2). This difference was significant (Mann-Whitney U-test, $P < 0.001$).

#### 6.3.2.3 Bean leaf disc bioassay

The presence of synthetic alarm pheromone on a bean leaf disc did not significantly increase take-off rate in mixed-age adult females (Mann-Whitney U-test, $P = 0.82$, table 6.3).

### 6.3.3 Effects on abundance in flowers

Fewer thrips were found in the flowers, than were caught on traps (figure 6.7). The number of female *F. occidentalis* in flowers was significantly reduced by 61%, in the presence of dodecyl acetate ($F_{(1, 34)} = 7.1$, $P = 0.012$). As for the trap experiments, the data had to be $\log_{10}(x+1)$ transformed prior to parametric analysis. Dodecyl acetate did not have a significant effect on the numbers of males in flowers (Wilcoxon matched-pairs signed-ranks test, $P = 0.83$). The numbers of males present was very low (<0.5 males flower$^{-1}$) and the data had to be analysed non-parametrically. All of the thrips mounted on glass slides for identification by compound microscope were found to be *F. occidentalis*.

## 6.4 Discussion

Both dodecyl acetate and synthetic alarm pheromone reduced the landing rate of male and female *F. occidentalis*, at doses similar to what might be expected from a disturbed group of *F. occidentalis* late larvae II. The lack of reduced *F. occidentalis* landing rate to dodecyl acetate observed in 1999 experiment 1 (figure 6.5a) was possibly due to increased ventilation of the glasshouse, as the vents were open at the time. This may have resulted in less volatilised dodecyl acetate around the traps. In all other glasshouse trapping experiments, the vents were closed. The absence of a significant difference in the landing rates of adult *F. occidentalis* exposed to dodecyl acetate and synthetic alarm pheromone are in agreement with effects on larvae, reported by Teerling *et al.* (1993). This suggests that dodecyl acetate is as repellent as synthetic alarm pheromone, and that dodecyl acetate would be sufficient for use in *F. occidentalis* IPM, e.g. as part of a stimulo-deterrent diversionary strategy (SDSS) (Pickett *et al.*, 1997). The reduction in trap catch of 36-54% observed in the two experiments conducted in 2000 was similar to that observed in *Myzus persicae* (Sulzer), whose landing rate is reduced by 35% in response to (*E*)-β-farnesene (Wohlers, 1981). The actual reduction of *F. occidentalis* on traps with dodecyl acetate or synthetic alarm pheromone may have been greater if a less attractive trap colour had been used, i.e. dodecyl acetate or synthetic alarm pheromone may repel more *F. occidentalis* than observed and consequently may be more effective in SDSS than would be inferred from the results presented here.

In one experiment, females were repelled more than males. Males are not found as often as females on foliage or in flowers and are trapped more at low population densities than females, although at high densities this is reversed (Higgins, 1992). It is possible that the observed sex ratio difference is due to there being more males than females in flight at the time of the experiment. However, in Portuguese glasshouses, no difference in the flight activity of male and female *F. occidentalis* has been found (Mateus *et al.*, 1996). The higher repellency of dodecyl acetate and synthetic alarm pheromone to females could be the result of females attempting to find areas of the crop free from alarm pheromone where they could lay eggs. Teerling *et al.* (1993) demonstrated that females in choice and no-choice oviposition experiments laid fewer eggs on bean pods contaminated with alarm pheromone. The effect was strongest in the choice experiment, suggesting that females were selective in where they laid their eggs and that the presence of alarm pheromone did not suppress overall oviposition.

Synthetic alarm pheromone caused a significant increase in the take-off rate of mixed-age adult female *F. occidentalis* when applied to a glass rod, but not when applied to a bean leaf disc (BLD). The lack of increased take-off rate on a BLD may be the result of *F. occidentalis* being attracted to this substrate, i.e. *F. occidentalis* are able to feed and oviposit on a BLD, but not on a glass rod. Additionally, the shape of the glass rod may have more conducive to thrips take-off. However, the increased take-off rate from a glass rod was only marginally significant. In a crop, thrips on a leaf that encounter alarm pheromone can either take-off, or walk away from the source. From the walking experiment, which showed that thrips responded strongly by walking away, it seems logical that thrips in a crop would almost certainly walk away from an alarm pheromone source, rather than take-off. However, the effect of higher doses and different conditions, e.g. different light intensities, needs to be determined. The aphid alarm pheromone has been demonstrated to improve pick-up of biological and chemical insecticides by increasing aphid activity (Griffiths & Pickett, 1987). A similar approach may be possible in the *F. occidentalis*, if adult activity is increased. The repellent effect of dodecyl acetate and synthetic alarm pheromone on adults in either flight, or walking, suggests that these substances may be used in SDSS.

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**Table 6.1** The median time (95% CI) spent by mixed-age adult female *F. occidentalis* on a glass rod with and without synthetic alarm pheromone (200 ng, 1.1:1, decyl acetate:dodecyl acetate) before taking off. Times were compared using a Mann-Whitney U-test.

| | Time spent on glass rod (s) | | |
|---|---|---|---|
| | Control | Synthetic alarm pheromone | Significance |
| Median (95% CI) | 230 (67, 270) | 62 (23, 133) | $P = 0.03$* |
| n | 30 | 30 | |

**Table 6.2** The median time (95% CI) spent by mixed-age adult female *F. occidentalis* in a glass tube with and without synthetic alarm pheromone (200 ng, 1.1:1, decyl acetate:dodecyl acetate) before exiting the glass tube. Times were compared using a Mann-Whitney U-test.

| | Time spent in glass tube (s) | | |
|---|---|---|---|
| | Control | Synthetic alarm pheromone | Significance |
| Median (95% CI) | 24 (15, 58) | 181 (46, 265) | $P < 0.001$*** |
| n | 30 | 30 | |

**Table 6.3** The median time (95% CI) spent by mixed-age adult female *F. occidentalis* on a bean leaf disc with and without synthetic alarm pheromone (200ng, 1:1, decyl acetate:dodecyl acetate) before take off. Times were compared using a Mann-Whitney U-test.

| | Time spent on bean leaf disc (s) | | |
|---|---|---|---|
| | Control | Synthetic alarm pheromone | Significance |
| Median (95% CI) | 216 (196, 228) | 225 (184, 246) | $P$=0.83 ns |
| n | 36 | 36 | |

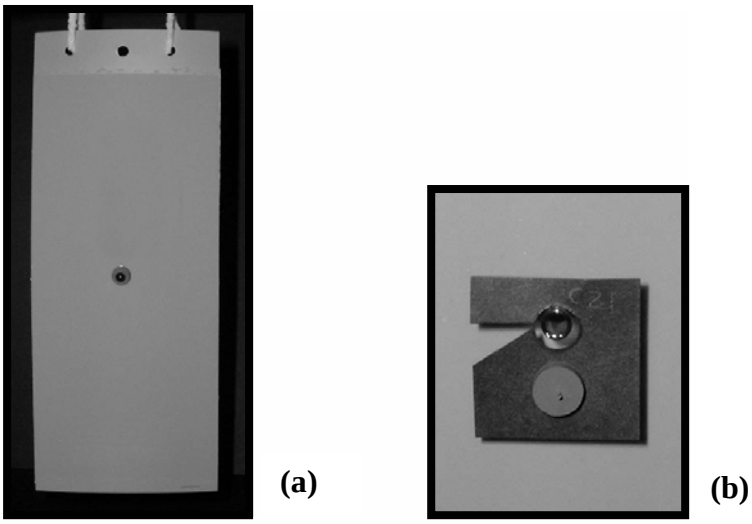


**Figure 6.1** **(a)** Blue sticky trap used to assess the effect of dodecyl acetate and synthetic alarm pheromone on *F. occidentalis* in cucumber crop field experiments. The pheromone-dispensing GC septum is fixed to the centre of the trap with a map pin (height = 24.5 cm). **(b)** Card and dodecyl acetate dispensing GC septum assembly used to release dodecyl acetate behind cucumber flowers in the flower experiment (side = 3 cm).

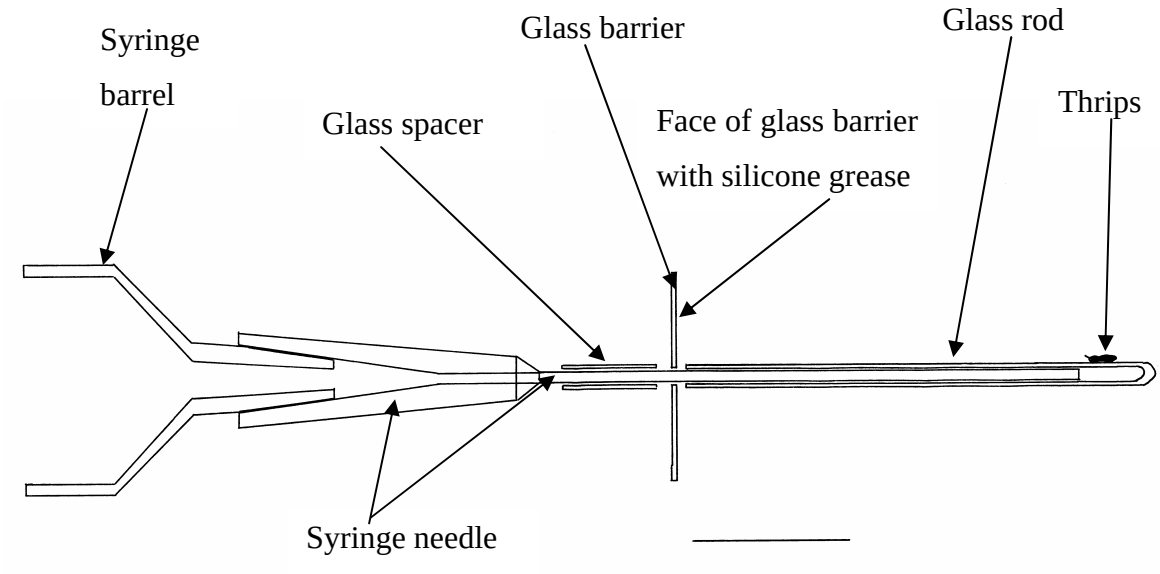


**Figure 6.2** **D**iagram of glass rod bioassay used to test the effect of synthetic alarm pheromone on adult female *F. occidentalis* take-off rate (bar = 1cm).

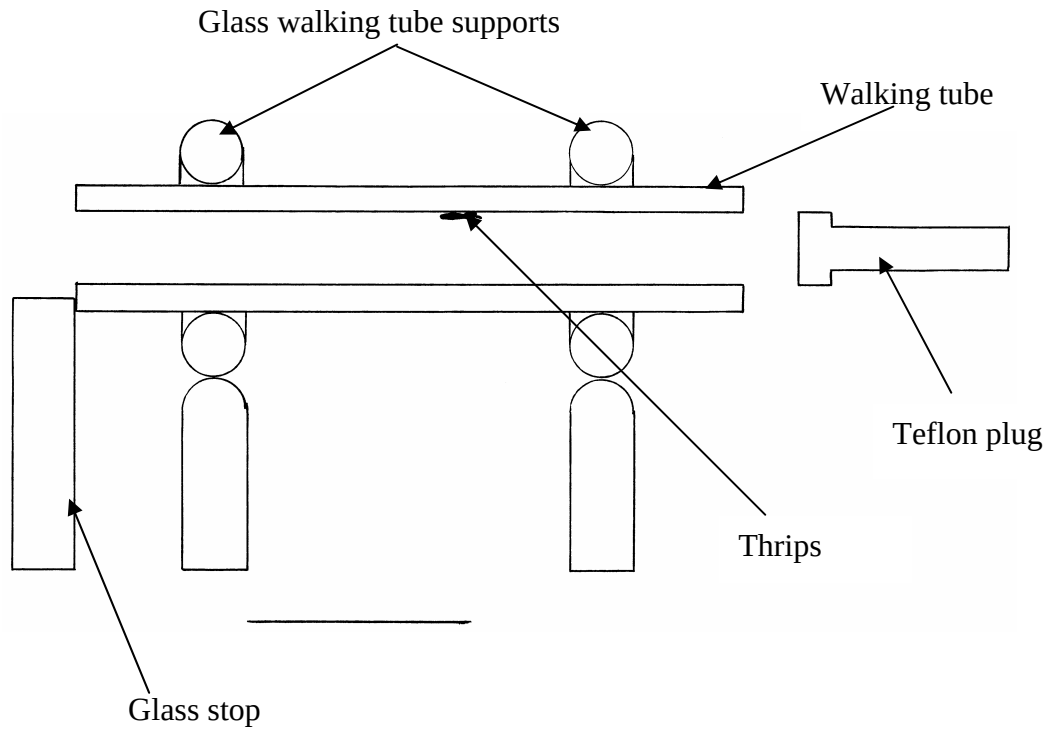


**Figure 6.3** Cross section view of the glass tube bioassay used to test the walking response of mixed-age adult female *F. occidentalis* to synthetic alarm pheromone (bar = 1 cm).

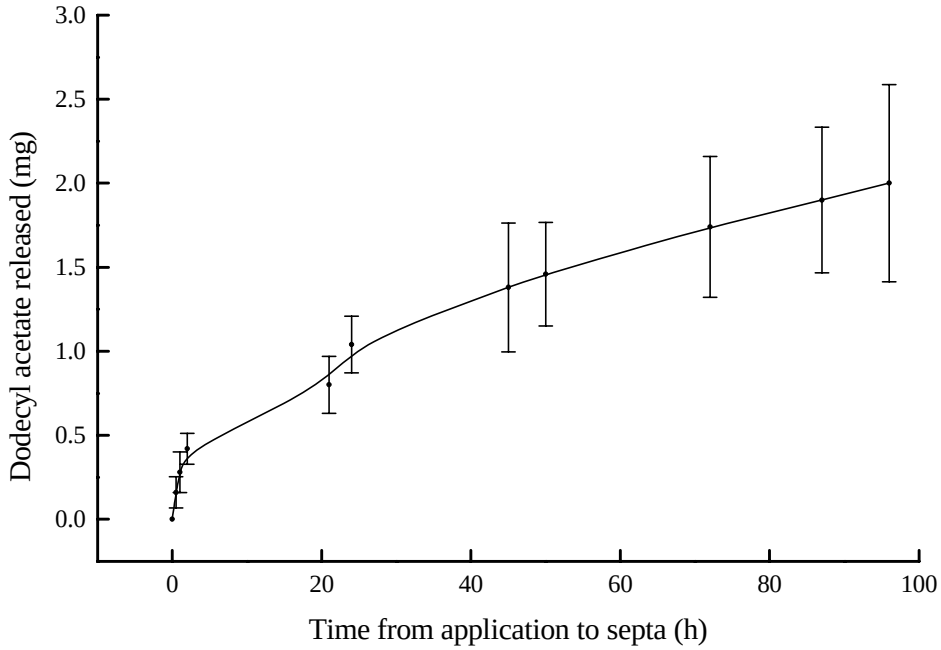


**Figure 6.4** Release rate of dodecyl acetate from GC septa in a glasshouse.

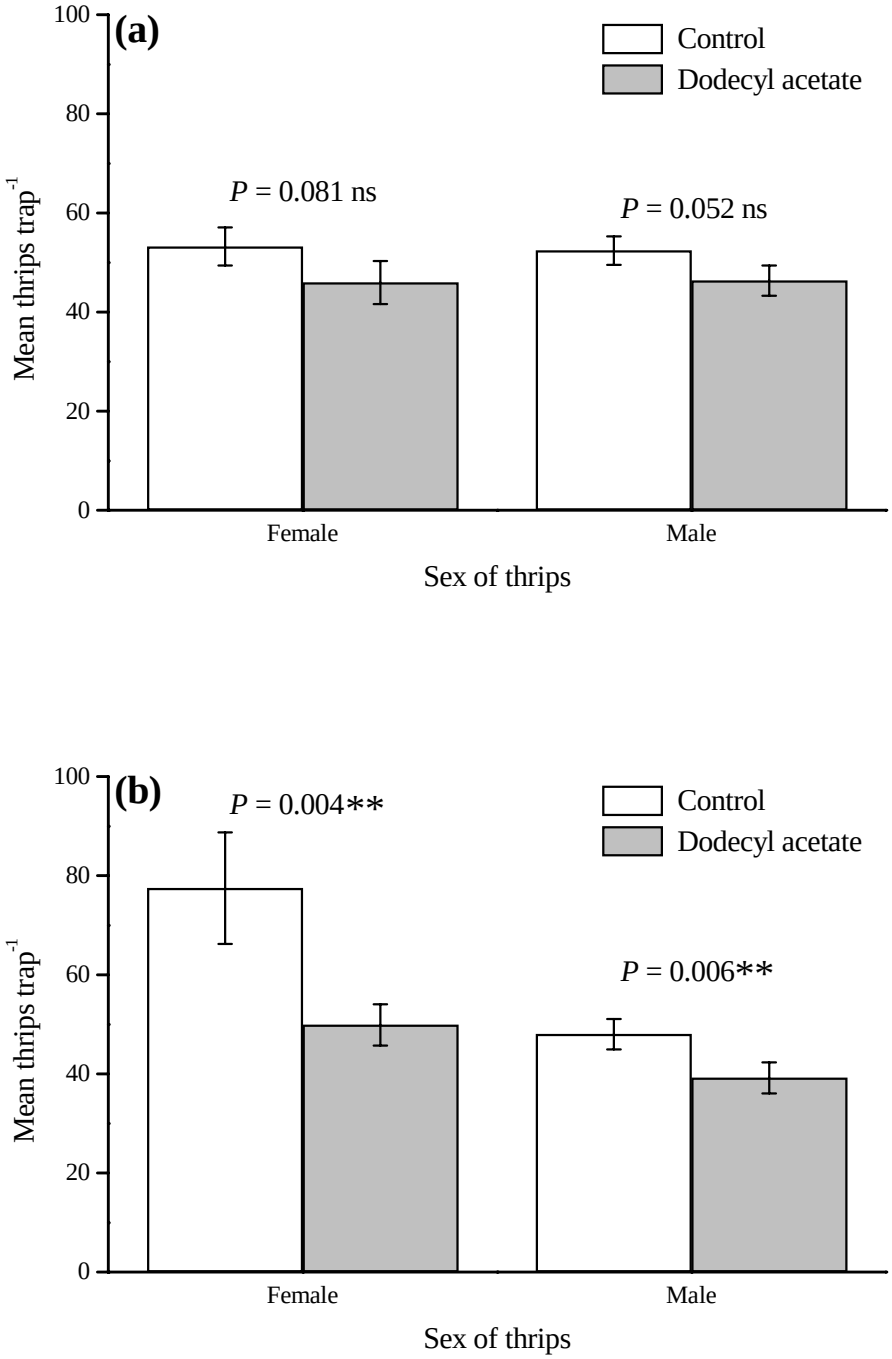


**Figure 6.5** The effect of dodecyl acetate on the landing rate of adult female and male *F. occidentalis* on blue sticky traps in a cucumber glasshouse over 24 h. (a) = 1999 experiment 1, (b) = 1999 experiment 2. The untransformed mean ± SE is shown. Means are compared within each sex. Significances are from statistical tests with $\log_{10}(x+1)$ transformed data.

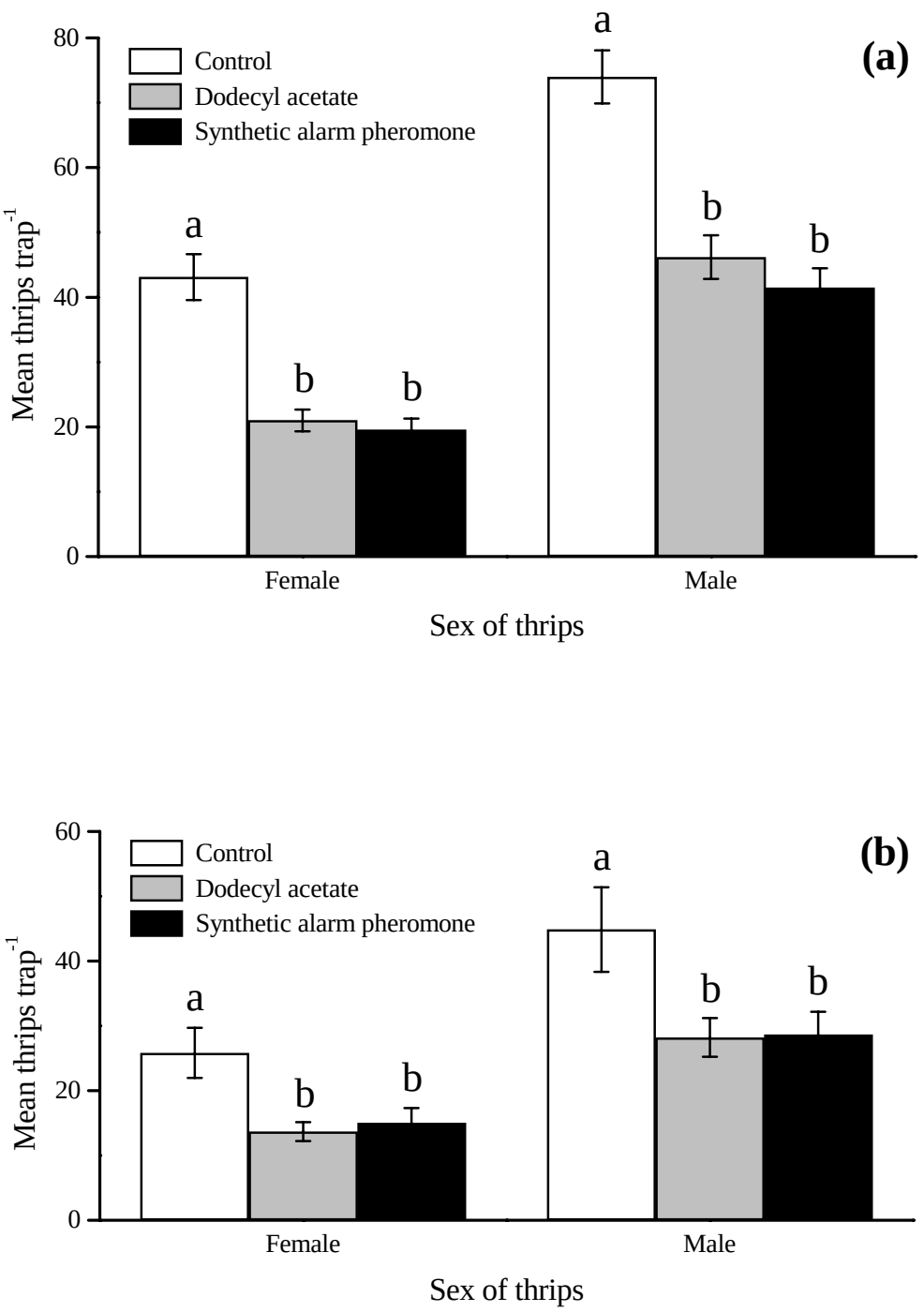


**Figure 6.6** The effect of dodecyl acetate and synthetic alarm pheromone on the landing rate of adult female and male *F. occidentalis* on blue sticky traps in a cucumber glasshouse over 24 h. (a) = 2000 experiment 1, (b) = 2000 experiment 2. The untransformed means ± SE are shown. Within each sex, bars sharing the same letter are not significantly different from each other at the 95% confidence level. The significances are from statistical tests with $\log_{10}(x+1)$ transformed data.

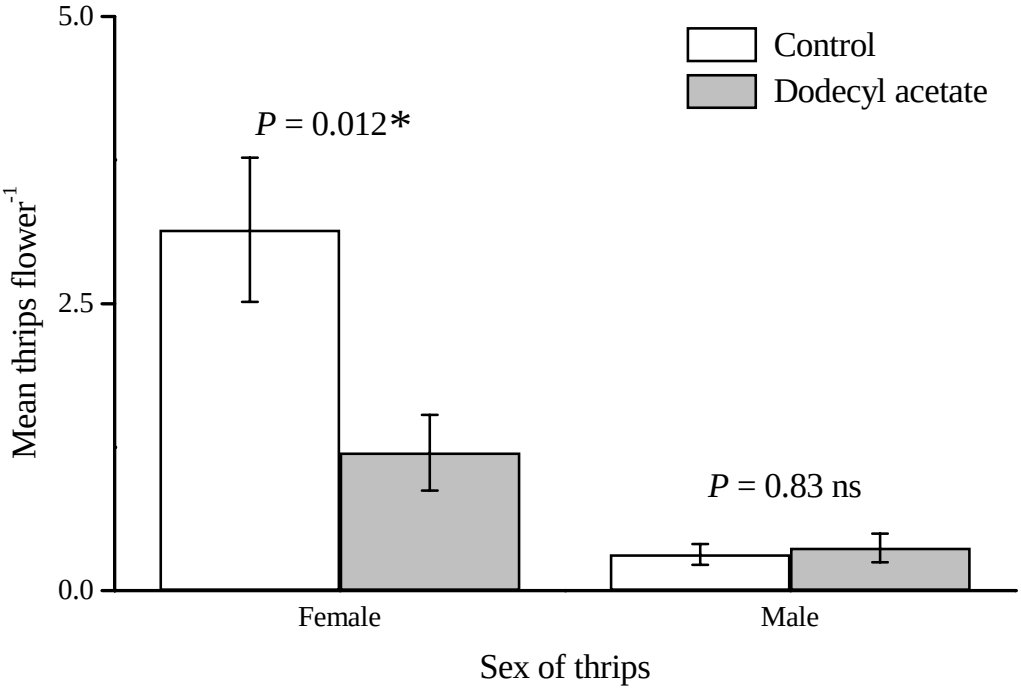


**Figure 6.7** The effect of dodecyl acetate on the abundance of female and male *F. occidentalis* in cucumber flowers over 24 h. Means are compared within each sex. The untransformed means ± SE are shown. The significances are from statistical tests with $\log_{10}(x+1)$ transformed data.

# Chapter 7

# General discussion

## 7.1 Principal findings

The overall aim of the thesis was to determine if the *F. occidentalis* alarm pheromone reported by Teerling *et al.* (1993b) could be used to enhance measures used in the control of this important pest of protected crops in the UK. To achieve this, four specific aims were set: (1) to analyse the chemistry of pheromone production in greater detail; (2) to analyse the effects of alarm pheromone on larval movement; (3) to assess if larvae habituated readily to alarm pheromone and (4) to analyse the effect of alarm pheromone on adult movement.

### 7.1.1 Chemical analysis

Detailed chemical examination of pheromone production showed that propupae, as well as larvae contain alarm pheromone, but only larvae (both male and female) produce it in the form of an anal droplet (AD) in response to simulated predator attack. The mass and ratio of alarm pheromone changed with larval age. No additional compounds were found using GC-MS. Triglycerides, which are a constituent of aphid cornicle secretions and are thought to aid alarm pheromone dispersal function (Strong, 1967), could have a similar role in *F. occidentalis*. However, the presence of such a compound would not have been detected by the GC-MS set-up used here (§4.2.2.2). The presence of water in Ads was confirmed and the site of alarm pheromone production was tentatively proposed as being the pygidial gland in larvae. The calculation of the naturally occurring amounts and ratios of alarm pheromone enabled future behavioural bioassays to be conducted at physiologically accurate levels.

### 7.1.2 Effect on larvae

Behavioural bioassays on larvae found that larval avoidance response increases with increasing mass of alarm pheromone and that dodecyl acetate has a greater effect than decyl acetate, confirmed the findings of Teerling *et al.* (1993b). No evidence of synergism at physiological ratios was found. However, the dropping effect of larvae exposed to alarm pheromone reported by Teerling *et al.* (1993b) was not observed in bioassays. Ethometric analysis of larval movement in response to alarm pheromone was inconclusive, but suggested that the mechanism of avoidance is negative chemotaxis, again supporting the observations of Teerling *et al*. (1993b).

### 7.1.3 Effect of pre-exposure on larvae

Prolonged exposure of larvae to a high dose of alarm pheromone, as may happen in the use of alarm pheromone as a bio irritant to improve insecticide efficacy, was found to decrease pre-exposed control larvae velocity. The bioassay required that the velocity of pre-exposed control larvae be unaffected in order to detect changes in the response of pre-exposed larvae challenged with alarm pheromone. Consequently, habituation to alarm pheromone could not be demonstrated. Further exploration of the habituation potential of *F. occidentalis* to alarm pheromone should therefore avoid bioassays where larval velocity is critical.

### 7.1.4 Effect on adults

Laboratory bioassays revealed that alarm pheromone induced adult females to take-off, although the effect was weak, and repelled walking females. Field scale experiments also showed for that both male and female thrips avoid landing on attractive traps baited with synthetic alarm pheromone. As was the case in larvae, there was no difference in the landing response of adults to synthetic alarm pheromone or dodecyl acetate. This effect was also found in cucumber flowers baited with dodecyl acetate, where the combined effects of increased take-off and reduced landing could be monitored, for adult females, but not adult males. This demonstrates for the first time that the movement of adult *F. occidentalis* in commercial crops can be altered using synthetic alarm pheromone or dodecyl acetate.

## 7.2 Evolutionary aspects of the alarm pheromone

If the sole purpose of a larval AD was for alarm and to alert conspecifics to the presence of danger, as suggested by Teerling *et al.* (1993b), then one might conclude that this was an altruistic act and that the producer of the anal droplet was actually increasing its inclusive fitness. In other words, the attacked larva emits an AD containing chemicals that do not affect the behaviour of the predator, i.e. are not repellent, but which are detected by nearby *F. occidentalis* and release a specific behaviour in these individuals, i.e. negative chemotaxis. This premise assumes that the conspecifics to which the attacked larva is signalling are closely related, and that they would respond appropriately to increase their survival chances. This occurs in many aphid species (Nault & Phelan, 1984; Pickett *et al*, 1992). It is uncertain how related nearby thrips are likely to be, but since thrips are haplodiploid relatedness between female siblings will be higher than for diploid insects (0.75 compared to 0.5, respectively).

Aphids, like *F. occidentalis* larvae, often aggregate at suitable feeding sites. Unlike *F. occidentalis* larvae, the aphids may often be clones. If one individual is attacked, it responds by producing a cornicle droplet which, in many species, is composed of alarm pheromone dispersed in triglycerides (Strong, 1967). Whilst the triglycerides may have a defensive function, the release of alarm pheromone causes conspecific aphids to walk away or drop from the host plant (Bowers *et al.*, 1972), which results in an increase in indirect fitness to the attacked aphid. Aphids vigorously avoid alarm pheromone, and may respond to the alarm pheromone as much as 30 mm away from its source (Nault *et al.*, 1973). However, in *F. occidentalis*, the production of an anal droplet appears to be in defence, i.e. the attacked larva attempts to place the AD onto the attacker, suggesting that the alarm pheromone is also functioning as an allomone. Two predators of *F. occidentalis* have also been shown to use the alarm pheromone as a kairomone (Teerling *et al.*, 1993a), so the production of an AD by larvae is a 'last resort' and may explain why few larvae produce AD's upon detecting alarm pheromone. The weak avoidance response of conspecifics to the presence of decyl acetate and dodecyl acetate is pheromonal, and may be an adaptive response to danger, as such larvae walk away from the source and adults take-off more, land less and lay fewer eggs in the presence of these compounds. From this viewpoint, the release of decyl acetate and dodecyl acetate by larvae under attack may primarily have been purely defensive but has evolved into a multi-functional alarm pheromone.

## 7.3 Effects on oviposition

Teerling *et al.* (1993b) demonstrated in choice and no-choice bioassays, that the presence of alarm pheromone reduced egg laying in *F. occidentalis*. Whilst this effect has been confirmed (Kirk *et al.*, 1999), I found that the results were variable and inconsistent, even though a large number of experiments were conducted. These results are not reported here, as no clear conclusions could be drawn. However, these experiments did indicate that host plant volatiles might be important in combination with the alarm pheromone in causing the reduction in oviposition. A disturbance effect was also noticed in these experiments. Within the first hour of the experiment, females often laid an unusually high number of eggs and this appeared to be restricted to females exposed to alarm pheromone. These effects are worth further exploration.

## 7.4 Potential of alarm pheromone in IPM

Teerling *et al*. (1993b) concluded that the *F. occidentalis* alarm pheromone would be of little use in the control of this species on its own, but might be of use when integrated with other control measures. Whilst the effect of alarm pheromone on individual behaviours is weak, the overall effect on all behaviours still needs to be investigated. It is possible that the alarm pheromone could be used to increase the pick-up rate of chemical and biological insecticides through increased activity levels. Although alarm pheromone was not demonstrated to increase activity levels in the present work, it is possible that this was a result of using too simple a bioassay. The repellent nature of the alarm pheromone on adults could also be applied to push-pull strategies being developed elsewhere (Pickett *et al.*, 1997). A common plant volatile, (*E*)-β-farnesene, which also serves as an alarm pheromone to many aphid species, has been shown to attract *F. occidentalis* (Pow *et al.*, 1999). This could be used to pull thrips onto a trap plant, whilst being pushed from the crop by alarm pheromone, or dodecyl acetate. Teerling *et al*. (1993a) demonstrated that the predatory bug, *Orius tristicolor* (White), used the *F. occidentalis* pheromone as a kairomone to locate thrips larvae. These predators are highly mobile and hot-spots of *F. occidentalis* activity could be 'marked' with alarm pheromone to pull predators into that area of the crop. The presence of alarm pheromone in a crop could also reduce the developmental rate of larvae. Venzon *et al*. (2000) demonstrated that the odours of a predatory bug fed on *F. occidentalis* larvae caused antipredator behaviour in *F. occidentalis* larvae. Such larvae moved into spider mite webbing, where resource

competition with the spider mites resulted in reduced developmental rate. This behaviour may have been the result of alarm pheromone consumed by the bugs effectively alerting thrips larvae to the presence of predators. However, much further work is required before any of these strategies can be employed effectively.

## 7.5 Future work

Although the use of simple bioassays and field experiments has furthered the understanding of the effects that the *F. occidentalis* alarm pheromone has on their behaviour, it has at the same time shown that a few simple bioassays are insufficient to fully explore the total range of behavioural changes that these chemicals induce in *F. occidentalis*.

Further work is required in three main areas. Firstly, the effect of alarm pheromone on developmental and reproductive dynamics needs to be explored. If alarm pheromone is released in a glasshouse, will it reduce developmental rate in larvae and so increase the chance of biological control succeeding? Secondly, how will biological control agents react in such a situation? Little is known about the mechanisms used by predators to locate alarm pheromone sources, and a firm understanding of these responses will be required to enhance biological control. Thirdly, a better understanding of how alarm pheromone affects the activity of larvae and adults in a crop situation is needed if the use of alarm pheromone in increasing insecticide efficacy is to be realised, as has been done in aphids (Griffiths & Pickett, 1980).

The *F. occidentalis* alarm pheromone produces a wide range of behavioural responses in not only the larvae and adults of this species, but also in species used in their control. What initially appear to be simple responses to this alarm pheromone on the part of *F. occidentalis* turn out to be a complex range of responses that require further study.

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# Appendix 1 Chemicals identified from thrips

| Species | Instar | Extract | Compound |
|---|---|---|---|
| **Family Thripidae** | | | |
| *Frankliniella occidentalis* (Pergande)[14] | L | WB, AD | **Decyl acetate** $C_{10}$<br>**Dodecyl acetate** $C_{12}$ |
| *Heliothrips haemorrhoidalis* (Bouché)[15] | L | AD | Coumaran (2,3-dihydrobenzofuran)<br>3-methoxyacetophenone<br>alkanes ($C_{14}$-$C_{17}$) |
| **Family Phlaeothripidae, Sub-family Phlaeothripinae** | | | |
| *Arrhenothrips ramakrishnae* Hood[1] | A? | - | Perillene (*p*-mentha-1,3,8-triene)<br>Rose furan (3-methyl-2-(3-methyl-but-2-enyl)-furan)<br>Phenol<br>Phenyl acetaldehyde |
| *Bagnalliella yuccae* (Hinds)[5] | L, A | WB | γ-decalactone (L 120ng, A 270ng) |
| *Dinothrips* sp.[1] | - | - | 3-methylbutanoic acid<br>Decanoic acid |
| *Euryaplothrips crassus* Ramakrishna & Marghabandhu[1] | A? | - | 4-octadec-9-enolide<br>Phenol<br>Phenyl acetaldehyde<br>Decanoic acid<br>Dodecanoic acid |
| *Gynaikothrips ficorum* (Marchal)[6] | L, A | - | **Tetradecyl acetate** $C_{14}$<br>**Hexadecyl acetate** $C_{16}$ (A 100ng)<br>Pentadecane (A 100ng)<br>Tridecane<br>Tetradecane<br>Heptadecane |
| *Gynaikothrips uzeli* (Zimmermann)[12] | A? | WB | **Tetradecyl acetate** $C_{14}$ 2%<br>**Hexadecyl acetate** $C_{16}$ 24%<br>**Octadecyl acetate** $C_{18}$ 2%<br>Pentadecane 43%<br>β-acaridial (2(*E*)-(4-methyl-3-pentenylidene)-butanedial) 16%<br>Tridecane<br>Tetradecane<br>*Cis*-8-heptadecene<br>Heptadecane |
| *Haplothrips leucanthemi* (Schrank)[2] | L, A | WB, AD | Mellein (3,4-dihydro-8-hydroxy-3-methylisocoumarin) 25ng |
| *Holothrips hagai* Okajima[7] | A? | WB | Pentadecane 59%<br>Heptadecadiene 12%<br>3-butanoyl-4-hydroxy-6-methyl-2*H*-pyran-2-one<br>*Z*-8-heptadecene<br>Tridecane<br>Tetradecane |

| Species | Instar | Extract | Compound |
|---|---|---|---|
| *Holothrips japonicus* Okajima[7] | A? | WB | Heptadecadiene 30%<br>Pentadecane 24%<br>*Z*-8-heptadecene 22%<br>3-butanoyl-4-hydroxy-6-methyl-2*H*-pyran-2-one<br>Tridecane<br>Tetradecane<br>*Z*-7-pentadecene |
| *Hoplothrips japonicus* Karny[3] | L, A | WB | *E*-3-dodecanoic acid 96%<br>*Z*-5-dodecanoic acid 4% |
| *Leeuwenia pasanii* (Mukaigawa) (=*Varshneyia pasaniae* Mukaigawa)[10-12] | L, A | WB | **Tetradecyl acetate** $C_{14}$ 15%<br>**Hexadecyl acetate** $C_{16}$ 3%<br>Tridecane 66%<br>Perillene (*p*-mentha-1,3,8-triene)<br>Pentadecane 13%<br>Dodecane<br>Tetradecane<br>8-heptadecene<br>2-methylbutyric acid<br>β-acaridial (2(*E*)-(4-methyl-3-pentenylidene)-butanedial) |
| *Liothrips piperinus* Priesner[10] | A? | WB | Perillene (*p*-mentha-1,3,8-triene) 23%<br>Tridecane 53%<br>Pentadecane 24%<br>Dodecane<br>Tetradecane<br>Pentadecene |
| *Liothrips kuwanai* (Moulton)[10, 12] | A? | WB | **Tetradecyl acetate** $C_{14}$ 6%<br>**Hexadecyl acetate** $C_{16}$ 5%<br>**9-hexadecenyl acetate** $C_{16}$ 1%<br>Tridecane 32%<br>Perillene (*p*-mentha-1,3,8-triene) 8%<br>Pentadecane 16%<br>*Z*-7-pentadecene 6%<br>Dodecane<br>Tetradecane<br>*Z*-8-heptadecene<br>β-acaridial (2(*E*)-(4-methyl-3-pentenylidene)-butanedial) |

| Species | Instar | Extract | Compound |
|---|---|---|---|
| *Ponticulothrips diospyrosi* Haga & Okajima[8] | A? | WB | *Z*-5-tetradecenoic acid<br>Tetradecadienoic acid<br>2-methyljuglone (plumbagin, 5-hydroxy-2-methyl-1,4-napthaquinone) 17%<br>7-methyljuglone (5-hydroxy-7-methyl-1,4-napthoquinone) 8%<br>Unknowns 10% |
| *Ponticulothrips* sp.[8] | A? | WB | 2-methyljuglone (plumbagin, 5-hydroxy-2-methyl-1,4-napthaquinone) 22%<br>7-methyljuglone (5-hydroxy-7-methyl-1,4-napthoquinone) 12%<br>*Z*-5-tetradecenoic acid<br>Tetradecadienoic acid<br>Unknowns 57% |
| *Schedothrips* sp.[1] | A? | - | Perillene (*p*-mentha-1,3,8-triene) |
| *Teuchothrips longus* Schmutz[1] | A? | - | Perillene (*p*-mentha-1,3,8-triene) |
| *Thlibothrips isunoki* Okajima[4] | A? | WB | β-myrcene |
| **Family Phlaeothripsidae, Sub-family Idolothripinae** | | | |
| *Bactrothrips brevitubus* Takahashi[9] | A | WB, E | Juglone (5-hydroxy-1,4-napthoquinone)<br>*E*-4-decenoic acid 48%<br>*Z*-5-dodecenoic acid<br>*E*-3-dodecenoic acid<br>Decanoic acid<br>Dodecanoic acid |
| *Bactrothrips carbonarius* Haga & Okijima[13] | A | WB, AD | Juglone (5-hydroxy-1,4-napthoquinone)<br>(*Z*)-5-tetradecenoic acid*<br>(*E*)-5-tetradecanoic acid<br>5,8-tetradecadienoic acid |
| *Bactrothrips flectroventris* Haga & Okajima[13] | A | WB, AD | Juglone (5-hydroxy-1,4-napthoquinone)<br>*Z*-5-dodecenoic acid*<br>Decanoic acid<br>*E*-4-decenoic acid<br>Decadienoic acid<br>Dodecadienoic acid<br>*E*-3-dodecenoic acid<br>(*E*)-5-tetradecanoic acid<br>5,8-tetradecadienoic acid |

| Species | Instar | Extract | Compound |
|---|---|---|---|
| *Bactrothrips honoris* Bagnall[13] | A | WB, AD | Juglone (5-hydroxy-1,4-napthoquinone)<br>*E*-4-decenoic acid*<br>Decanoic acid<br>Decadienoic acid<br>(*E*)-3-dodecenoic acid<br>(*Z*)-5-dodecenoic acid |
| *Bactrothrips montanus* Haga & Okajima[13] | A | WB, AD | Juglone (5-hydroxy-1,4-napthoquinone)<br>(*Z*)-5-tetradecenoic acid*<br>5,8-tetradecadienoic acid |
| *Bactrothrips pictipes* Haga & Okajima[13] | A | WB, AD | Juglone (5-hydroxy-1,4-napthoquinone)<br>(*Z*)-5-tetradecenoic acid 46% of acids<br>Decanoic acid 18%<br>(*E*)-4-decenoic acid 17%<br>5,8-tetradecadienoic acid 16%<br>(*Z*)-5-dodecenoic acid 3%<br>(*E*)-5-tetradecenoic acid 1%<br>Decadienoic acid<br>(*E*)-3-dodecenoic acid |
| *Bactrothrips quadrituberculatus* Bagnall[13] | A | WB, AD | Juglone (5-hydroxy-1,4-napthoquinone)<br>(*Z*)-5-tetradecenoic acid*<br>(*E*)-5-tetradecenoic acid<br>5,8-tetradecadienoic acid |
| *Elaphrothrips antennalis* Bagnall[13] | A | WB, AD | Juglone (5-hydroxy-1,4-napthoquinone)<br>(*Z*)-5-tetradecenoic acid*<br>5,8-tetradecadienoic acid |
| *Elaphrothrips tuberculatus* (Hood)[1,2] | A? | - | Juglone (5-hydroxy-1,4-napthoquinone)<br>(*Z*)-5-tetradecenoic acid<br>5,8-tetradecadienoic acid<br>Dodecanoic acid |
| *Holurothrips morikawai* Kurosawa[13] | A | WB, AD | Juglone (5-hydroxy-1,4-napthoquinone)<br>Decanoic acid*<br>(*E*)-4-decenoic acid*<br>(*Z*)-3-decenoic acid<br>(*Z*)-4-decenoic acid<br>Decadienoic acid |

| Species | Instar | Extract | Compound |
|---|---|---|---|
| *Machatothrips artocarpi* Moulton[13] | A | WB, AD | Juglone (5-hydroxy-1,4-napthoquinone)<br>Dodecadienoic acid*<br>(*E*)-3-dodecenoic acid |
| *Mecynothrips pugilator* Bagnall[13] | A | WB, AD | Juglone (5-hydroxy-1,4-napthoquinone)<br>(*Z*)-5-tetradecenoic acid*<br>5,8-tetradecadienoic acid |
| *Mecynothrips simplex* Karny[13] | A | WB, AD | Juglone (5-hydroxy-1,4-napthoquinone)<br>Decanoic acid*<br>(*E*)-4-decenoic acid |
| *Mychiothrips fruticola* Haga & Okajima[13] | A | WB, AD? | 5,8-tetradecenoic acid<br>(*Z*)-5-tetradecenoic acid |
| *Neatractothrips macrurus* Okajima[13] | A | WB, AD | Juglone (5-hydroxy-1,4-napthoquinone)<br>Decanoic acid*<br>(*E*)-4-decenoic acid? |
| *Nesothrips lativentris* Karny[13] | A | WB, AD | Juglone (5-hydroxy-1,4-napthoquinone)<br>Decanoic acid*<br>(*E*)-4-decenoic acid |
| *Ophthalmothrips miscanthicola* Haga[13] | A | WB, AD | Juglone (5-hydroxy-1,4-napthoquinone)<br>5,8-tetradecenoic acid |

Chemicals identified from thrips. L = larva; A = adult; WB = whole body extract; AD = anal droplet; E = entrained odour; * = main acid component; ? = not specified, but inferred; ([1]Blum (1991); [2]Blum *et al*. (1992); [3]Haga *et al*. (1989); [4]Haga *et al*. (1990); [5]Howard *et al*. (1983); [6]Howard *et al*. (1987); [7]Suzuki *et al*. (1993); [8]Suzuki *et al*. (1995), [9]Suzuki *et al*. (1990); [10]Suzuki *et al*. (1988); [11]Suzuki *et al*. (1986); [12]Suzuki *et al*. (1989); [13]Suzuki *et al*. (2000); [14]Teerling *et al*. (1993); [15]Zabaras *et al*. (1999)).

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## Appendix 2 Chrysanthemum propagation protocol

Chrysanthemums (*Dendranthema grandiflora* Tzvelev, variety Yellow Princess Anne, Rydale Nurseries, Carlisle) were propagated at Keele University, in glasshouses.

Chrysanthemums were initially grown in large pots (27 cm diam., 21 cm high) at one plant per pot. Cuttings were taken from these plants ('mother plants') and induced to flower through manipulation of the light regime. The mother plants were kept under ambient light conditions and new mother plants were started every four weeks.

Cuttings were taken from the mother plants weekly. These cuttings were approximately 7.5 cm long and had three pairs of leaves, with the smallest leaves being no less than 3 cm long. The cuttings were then placed into a small pot (14 cm diam., 9 cm high). Each pot contained five cuttings evenly spaced around the edge of the pot. The pots were then placed under a mister unit for two weeks to allow the cuttings to root.

After removal from the mister unit, the growing points of the cuttings were removed, leaving two pairs of leaves, in order to encourage the development of vegetative lateral buds, increasing the potential number of flowers. These plants were then left to grow for four weeks under ambient lighting conditions before being placed into a short day length (8:16 L:D) to initiate flower bud formation. During this time it was important to maintain the temperature at 15°C or below during the night to encourage bud initiation.

After flower bud initiation, the plants were taken out of short daylength and kept under a 12 h daylength. During March/April and September/October the plants were given a 2 h night break, i.e. lights were switched on during the night for 2 h. Between November and February, the night break was 3 h long. No night breaks were required between May and August. At this time the plants were sprayed every 10 d with a growth retardant (B-nine, 85% daminozide, as recommended for the variety, Hortichem, UK) to dwarf the plants. This continued until flowering occurred.

Whitefly was controlled by releasing *Encarsia formosa* Gahan (Encar-f, Syngenta Bioline Production Ltd., UK). All plants were grown in multi-purpose compost.