The aggregation pheromone of the western flower thrips

Ian A. N. Dublon B.Sc. (Hons)

Project supervisors:

Dr J.G.C. Hamilton and Dr W.D.J. Kirk

January 2004- January 2009

(Sabbatical: *August 2006-July 2007*)

Thrips Research Group, Centre for Applied Entomology and Parasitology,

School of Life Sciences, Huxley Building,

Keele University,

Keele, Staffordshire, ST5 5BG, UK.

Submitted for the fulfilment of the requirements of the degree of Doctor of Philosophy.

Abstract

The western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) is a polyphagous insect pest and virus vector with a ubiquitous distribution. Its control necessitates an IPM strategy and as part of this there is a potential for exploiting the male-produced aggregation pheromone. Neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate have previously been identified as male-produced compounds with the former being shown to increase trap catch in glasshouse trials. Headspace solid phase microextraction (HS-SPME) was combined with gas chromatography-flame ionisation detection (GC-FID) to estimate the production rate of this compound (0.1-0.3 ng male^{-1} h^{-1}). The rate of production is notably lower than in some larger insects. GC-MS was used in an attempt to identify minor produced male headspace compounds though no additional compounds were consistently detectable in male only entrainments. Neryl (*S*)-2-methylbutanoate elicits a biological contact response in females when manually applied to filter discs. When filter paper discs were exposed to males for around 24 h, a substance was left behind on the discs that elicited a biological response when presented anew to females. This response was transferable to new filter discs using solvent extraction and its strong effect on females could not be replicated by application of neryl (*S*)-2-methylbutanoate in isolation. This suggests the presence of an additional compound entirely, or that a synergist (perhaps (*R*)-lavandulyl acetate) is required in combination with neryl (*S*)-2-methylbutanoate. It was not possible to identify any components from filter disc extract using GC-MS suggesting that production of this compound occurs at very low levels. In plastic house trials (*R*)-lavandulyl acetate unexpectedly reduced trap catch. Neryl (*S*)-2-methylbutanoate increased trap catch though the racemic mixture did not. Experiments that varied the visual stimulus of the lure indicated that a visual stimulus was needed for the lure to work.

Keele
Graduate School

Submission of Thesis for a Research Degree

Degree for which thesis being submitted: Doctor of Philosophy by research (Ph.D.)

Title of thesis: The aggregation pheromone of the western flower thrips

Date of submission: 20/02/09

Original registration date: 05/01/04

(Date of submission must comply with Regulation 2D)

Name of Candidate: Ian Adam Nicholas Dublon

Research Institute: Institute of Science Technology in Medicine (iSTM)

Name of Lead Supervisor: James Gordon Campbell Hamilton

I certify that:

(a) I understand that the decision to submit this thesis is entirely my own decision

(b) The thesis being submitted for examination is my own account of my own research

(c) My research has been conducted ethically

(d) The data and results presented are the genuine data and results actually obtained by me during the conduct of the research

(e) Where I have drawn on the work, ideas and results of others this has been appropriately acknowledged in the thesis

(f) Where any collaboration has taken place with other researchers, I have clearly stated in the thesis my own personal share in the investigation

(g) The greater portion of the work described in the thesis has been undertaken subsequent to my registration for the higher degree for which I am submitting for examination

(h) Where part of the work described in the thesis has previously been incorporated in another thesis submitted by me for a higher degree (if any), this has been identified and acknowledged in the thesis

(i) The thesis submitted is within the required word limit as specified in the Regulations

Total words in submitted thesis (including the text and footnotes, but excluding references and appendices): 45,458

Signature of Candidate: .....

Cc: Lead Supervisor

Director of Postgraduate Research

Contents

List of Figures

List of Tables

Acknowledgements

I would like to thank:

◇ my supervisors, Dr Gordon Hamilton and Dr William Kirk;

◇ my advisor Professor Richard Ward;

◇ my Family for everything;

◇ Keele University School of Life Sciences for providing a fees-waiver bursary;

◇ my exceptional CAEP, COMB, ISTM, Keele and rest of the world friends;

◇ my past Keele thrips lab colleagues: Ann O'Leary, Neil Holmes and Vladimir Krumov;

◇ min kära vän, M. Therése Eriksson (i mångt och mycket);

◇ The life science and neuroscience academics within the department who have been inspiring and generally very decent and tolerant/patient[1] of my singing to myself;

◇ Dr Willem Jan de Kogel (WUR, Wageningen, Netherlands);

◇ Dr Krishna Kumari Bandi (LICT, Hyderabad, India);

◇ Jude Bennison (ADAS, Boxworth, UK);

◇ Dr Melanie M. Davidson (CFR, Christchurch, New Zealand);

◇ Professor David Hall (NRI, Greenwich, UK);

◇ Dr Neil Herbert (Grace Davison Discovery Sciences, UK);

◇ International Flavors & Fragrances, UK and Spain;

◇ Keele University School of Life Sciences technical and purchasing staff, especially Jayne Bromley, Brenda Bould, Nigel Bowers, Alan Dudley, Ron Knapper, Ian Wright, Lisa Preston and Christine Bain;

◇ Keele University School of Life Sciences administrative staff (especially Lisa Cartlidge and Debbie Goodall);

◇ Keele University School of Life Sciences domestic staff (especially Kath, Eleanor and Terry);

◇ Keele University Graduate school staff, who were great to work with during my sabbatical;

◇ Mrs Sheila Brown at the Student Financial Support Office.

[1] delete as appropriate

List of abbreviations

ANOVA Analysis of Variance

CAR Carboxen

CCD Charge-Coupled Device

c.v. coefficient of variation

DDT Dichlorodiphenyltrichloroethane

DVB Divinylbenzene

EI Electron Impact

f: F-number

FEP Fluorinated Ethylene Propylene

GC Gas Chromatography

GC-EAG Gas Chromatography - Electroantennography

GC-FID Gas Chromatography - Flame Ionisation Detection

GC-MS Gas Chromatography - Mass Spectrometry

GLM General Linear Model

HS Headspace

HS-SPME Headspace Solid Phase Microextraction

INSV *Impatiens Necrotic Spot Virus*

IPM Integrated Pest Management

MSDS Material Safety Data Sheet

MW Molecular Weight

m/z mass to charge ratio

NIST National Institute of Standards and Technology (USA)

PDMS Polydimethylsiloxane

ppm parts per million

PTFE Polytetrafluoroethylene

r_m intrinsic rate of population increase

*R*f Retention factor

SEM Standard Error of the Mean

SEM Scanning Electron Microscopy

SPME Solid Phase Microextraction

TEM Transmission Electron Microscopy

TIC Total Ion Chromatogram

TLC Thin Layer Chromatography

t_R Retention time

TSWV *Tomato Spotted Wilt Virus*

UV Ultraviolet

UVA Ultraviolet A

Species cited and authorities used

Thysanoptera

Thysanoptera authorities verified using Mound, L. (2008) *Thysanoptera (Thrips) of the World - a checklist.*

URL: `http://www.ento.csiro.au/thysanoptera/worldthrips.html`

Aeolothrips fasciatus Linnaeus

Apelaunothrips consimilis Ananthakrishnan

Arrhenothrips ramakrishnae Hood

Bactrothrips brevitubus Takahashi

Bagnalliella yuccae Hinds

Callococcithrips fuscipennis Moulton

Cycadothrips chadwicki Mound

Dolichothrips sp. Karny

Ecacanthothrips inarmatus Kurosawa

Elaphrothrips (=*Ethirothrips*) *antennalis* Bagnall

Elaphrothrips tuberculatus Hood

Eugynothrips (Loepothrips) intorquens Karny

Euryaplothrips crassus Ramakrishna and Margabandhu

Frankliniella conspicua Moulton

Frankliniella intonsa Trybom

Frankliniella nubila Treherne

Frankliniella occidentalis Pergande

Frankliniella schultzei Trybom

Frankliniella tritici Fitch

Frankliniella tritici var. *maculata* Priesner

Frankliniella umbrosa Moulton

Gynaikothrips ficorum Marchal

Gynaikothrips uzeli Zimmermann

Haplothrips aculeatus Fabricius

Haplothrips kurdjumovi Karny

Haplothrips leucanthemi Schrank

Heliothrips haemorrhoidalis Bouché

Holothrips hagai Okajima

Holothrips japonicus Okajima

Holothrips yuasai Kurosawa

Hoplothrips japonicus Karny

Leewenia pasanii Mukaigawa

Liothrips kuwanai Moulton

Liothrips piperinus Priesner

Liothrips wasabiae Haga and Okajima

Megalurothrips distalis Karny

Mychiothrips fruticola Haga and Okajima

Oidanothrips frontalis Bagnall

Pezothrips kellyanus Bagnall

Ponticulothrips diospyrosi Haga and Okajima

Psalidothrips lewisi Bagnall

Psalidothrips simplus Haga

Schedothrips sp. Ananthakrishnan

Taeniothrips dianthi Priesner

Teuchothrips longus Schmutz

Thlibothrips isunoki Okajima

Thrips angusticeps Uzel

Thrips coloratus Schmutz

Thrips flavus Schrank

Thrips hawaiiensis Morgan

Thrips imaginis Bagnall

Thrips major Uzel

Thrips nigropilosus Uzel

Thrips obscuratus Crawford

Thrips palmi Karny

Thrips simplex Morison

Thrips tabaci Lindeman

Xylaplothrips inquilinus Ananthakrishnan

Non-Thysanoptera

Dendranthema (Chrysanthemum) grandiflora Tzvelev

Chapter 1

General introduction

1.1 Introduction

The western flower thrips, *Frankliniella occidentalis* (Pergande), is a polyphagous insect pest distributed within the Americas, Europe, Africa, Asia and Australasia. Its distribution has increased greatly since the 1960s, reaching the UK in the 1980's as a result of international movement of horticultural material. The rate of spread within Europe was rapid and measurable; *F. occidentalis* spread from its original 1983 Netherlands outbreak at a rate of 229± 20 km year^{-1} (Kirk & Terry, 2003).

F. occidentalis, like several other thrips, acts as a vector for a variety of plant viruses including pollen-borne viruses and tospoviruses. The role of thrips as virus vectors is well documented, for example *Thrips tabaci* (Lindeman) was identified as a vector for *Tomato spotted wilt virus* in 1927 (Pittman, 1927). *F. occidentalis* is also able to act as a vector for bacteria and fungi (Ullman *et al.*, 1997) and, like many other phytophagous thrips species can also cause mechanical feeding and oviposition injury to plants. Direct damage to plant structures can also occur when *F. occidentalis*

ingests sap. This process can cause serious cosmetic damage to ornamentals (Figure 1.3), fruit and vegetable plants (Lewis, 1997).

The risk of large economic losses due to infestation of protected and field crops by *F. occidentalis* and other species, requires the use of control measures. Pesticide application and/or integrated pest management (IPM) is usually necessary. The seriousness of the problem is highlighted by the fact that direct loss of ornamental UK plants in 1999 from *F. occidentalis* accounted for an annual cost of between £12.5-25m (Sampson, 2000). It is difficult to gain a more recent figure but the reported losses in 2000 were atypically high because the pest was new. However, crop infestation from pest thrips species remains a serious economic concern with recent assessments of potential crop damage by *Thrips palmi* in the period, 2004-2014 should it arrive in the UK being £16.9-19.6m (MacLeod *et al.*, 2004).

Traditionally *F. occidentalis* infestations have been controlled with persistent chlorinated hydrocarbons including DDT and aldrin, with lower persistence organophosphates and carbamates replacing these treatments (Ullman *et al.*, 1997). Recent changes in European legislation have meant that certain pesticides have been proscribed in member states, and many more will follow (Brown, 2004). As a result of this, and as a result of increased incidence of *F. occidentalis* resistance to the remaining pesticides, there has been a greater impetus to implement additional or alternative control methods. Pesticides derived from alternative sources (spinosad from the soil actinomycete *Saccharopolyspora spinosa*) have been developed along with thrips biological control agents. The use of predatory mites e.g. *Amblyseius (Neoseiulus) cucumeris* and heteropteran bugs e.g. *Orius laevigatus* is now commonplace. Control using Steinernematidae and Heterorhabditae nematodes is also being developed although this technology is relatively new having been developed in the late 1990s (Chyzik *et al.*, 1996). Nematodes, including those containing the bacterial symbiont *Photorhabdus* spp., are known to increase mortality rates in thrips species (L. Gerritsen, personal

communication, Wageningen, 2004). Current trials are being undertaken with alternative nematode species in the UK (J. Bennison, personal communication, Warwick HRI 2004).

Behavioural studies of *F. occidentalis* have led to the investigation and identification of specific volatile chemicals used in communication. Alarm pheromones have been identified for *F. occidentalis* (Teerling *et al.*, 1993), and it is thought that these could be utilised as part of an IPM strategy, potentially disrupting feeding patterns and mating practices (MacDonald *et al.*, 2002). The addition of alarm pheromone to certain insecticides has been shown to increase *F. occidentalis* larval movement and therefore mortality (Cook *et al.*, 2002). An *F. occidentalis* aggregation pheromone component has been identified (Hamilton *et al.*, 2005) and if synthetically produced it could be used for monitoring and mass trapping purposes (de Kogel & van Deventer, 2003; Kirk & Hamilton, 2004). Since the commencement of this study in January 2004 two male-produced compounds have been identified and one is commercially available as Thripline$_{ams}$ (Syngenta Bioline, Essex, UK), a pheromonal lure intended for *F. occidentalis* population monitoring.

1.2 *Frankliniella occidentalis*: classification, reproductive biology and morphology

Classification

The western flower thrips belongs to the insect order Thysanoptera (Suborder Terebrantia). The Terebrantia can be further divided into eight families, with *F. occidentalis* belonging to the family Thripidae (Mound, 2002). Terebrantian families also include Adiheterothripidae, Aelothripidae, Fauriellidae, Heterothripidae, Melanthripi-

dae, Merothripidae, Thripidae and Uzelothripidae. The Thripidae feed primarily on flowers or leaves of higher plants and comprise of at least 1700 species and include most terebrantian pest species (Mound, 1997).

The suborder Tubulifera contains the family Phlaeothripidae. One of the two subfamilies, the Phlaeothripinae, contains crop pests (Mound, 1997).

The western flower thrips is remarkably variable in its phenotypic appearance and in particular, its colour and therefore it has previously been erroneously identified as *F. conspicua* Moulton, *F. nubila* Treherne, *F. tritici* var. *maculata* Priesner and *F. umbrosa* Moulton. These terms are now accepted as junior synonyms for *F. occidentalis* (Nakahara, 1997). Individuals within the *Frankliniella* genus are particularly difficult to identify and when this is combined with colour variations and size differences it is possible to mistake identification. Characteristic *F. occidentalis* head setae sizes and positions are shown in Figure 1.4. See section 1.2 below.

Reproductive biology

Reproduction in terebrantian thrips has been studied in detail. Male *F. occidentalis* fight for control of territories where reproductive females are likely to land (Terry & Gardner, 1990). Males mate and can aggregate, forming swarms on artificial surfaces and flower heads (Terry & Schneider, 1993). It is likely that males choose an aggregation site based on either a landmark site and/or a site that is a possible feeding or oviposition site. Flower heads may be preferential swarming sites as it has been shown that females fed on pollen have a higher reproductive fitness (Terry & Gardner, 1990).

During this aggregation behaviour and the fighting behaviour that also often occurs, males release specific chemicals. It was postulated and later confirmed that one of these compounds acts as an aggregation pheromone that attracts other males and females (Kirk & Hamilton, 2004, Hamilton *et al.*, 2005). Aggressive interactions be-

tween males are escalated at low male densities (Terry, 1995) and this may affect the level of pheromone release. It has also been observed that only certain males are likely to fight; fighting males generally have wider abdomens, longer legs and wings, and a greater weight than passive males (Terry & Dyreson, 1996). The aggregation itself may also attract additional females (Terry & Gardner, 1990). Mating normally occurs when a female enters the aggregation and both parties separate after copulation has occurred (Terry & Schneider, 1993). Females generally mate with the first male they encounter within the aggregation (Terry & Dyreson, 1996).

F. occidentalis has an arrhenotokous parthenogenetic life cycle. Males are derived from unfertilised eggs and are haploid (Moritz, 1997). If they are removed from populations this will result in population control over several generations as unmated females will produce only males and female numbers will decrease. Removing both males and females will reduce the population in the shortest time. *F. occidentalis* has six developmental stages (egg, larva I, larva II, pro-pupa, pupa, adult) and the duration of the life cycle is affected by temperature. It has been reported that between 15 and 28°C, developmental rate is linearly related to temperature (van Rijn *et al.*, 1995) though there is some debate as to developmental rate linearity outside of this range (McDonald *et al.*, 1998). The *F. occidentalis* life cycle is accepted as being holometabolic (Lewis, 1973), although it is somewhat atypical; all thrips have two larval instar stages with the Terebrantia having two further pre-adult instars whilst the Phlaeothripidae have three such instars (Mound & Walker, 1982a). Both larval instars resemble smaller versions of the adult but lack wings and defined genital appendages.

Morphology

The initial egg stage is smooth and pale in colour. Terebrantian eggs are laid directly into the plant via an incision made by the ovipositor (Kirk, 1985). They also possess an anterior operculum which is removed by the emerging first instar larva's

saw-shaped oviruptor (Kirk, 1985).

During embryogenesis the size of the egg increases thus causing further structural damage to the surrounding plant cells (Moritz, 1995). Organ systems are formed and most become visible and physiologically active (Moritz, 1997).

Post emergence, the first and second instar larvae increase in size rapidly with feeding. Ocelli are absent from all larval stages but both larval instars I and II possess "punch and suck" mouth-parts (see 1.2 below) (Moritz, 1997). Differentiating between late larval instar I and early larval instar II stages requires the use of a compound microscope. When viewed in ventral aspect larval instar I stages have one pair of setae on each of the the sternites on abdominal segments IV-VIII whist larval instar II stages have three pairs (Speyer & Parr, 1941).

Most terebrantian adult thrips are approximately 1-2 mm in length and this is thought to be an important factor with respect to pest status; it is relatively easy to not detect individual thrips as they are able to hide within the plant's external structures (Moritz, 1997).

F. occidentalis is variable in its phenotypic appearance. Colours of adult females include off white, yellowish, orange, and dark brown to almost black. Adult males are all pale (Kirk & Terry, 2003) and smaller than females. Temperature is likely to affect colour variation as it does in *T. tabaci*, where temperature during the pupal stage determines the darkness of the adult body colour (Murai, 2000; Murai & Toda, 2002). Additionally, during the larval stage temperature may also affect adult *F. occidentalis* body size, as is the case in *T. tabaci* (Murai & Toda, 2002).

Wild-type field *F. occidentalis* appears to be considerably more variable in phenotype than glasshouse forms and this can compound difficulties in identification (Kirk & Terry, 2003). Two monophyletic groups of *F. occidentalis* have been identified in Cal-

ifornia and it appears that these two dark and bicoloured populations are genetically distinct. Sequence data from mitochondrial genes COI and 16S and the nuclear gene ITS were compared in this study (O'Donnell, 2007).

Mouthparts

F. occidentalis, like other thripids, possesses "punch and suck" mouthparts. The mouth cone is ventrally directed and the mandible pierces the plant material. The maxillary stylets join together forming a tube which enters the plant tissue through or next to the hole made by the mandible, thus enabling the uptake of food (Kirk, 1997).

Sensory receptors

Thrips respond to a wide range of stimuli, including temperature, visual stimuli and chemical stimuli. It has also been postulated that take off rate is affected by thrips density and changes in air pressure (O'Leary, 2005). Typically insect antennae have olfactory functions; the muscle arrangements permit a wide range of movement, nerve channels lead directly into association centres in the midbrain and a great variety of hairs, sensilla and pore plates cover the flagellum (Metcalf & Metcalf, 1992). In the Terebrantia, Adiheterothripae possess cone-like sensory areas on antennal segments III and IV whilst thripidae possess simple or forked sense cones on antennal segments III and IV. Heterothripidae have sensory areas in bands at the apex of antennal segments III and IV (Mound and Kibby, 1998). The tubuliferan phlaeothripid *Bagnalliella yuccae* also possesses sense cone chemoreceptors on antennal segments III and IV (Slifer & Sekhon, 1974). The use of SEM and TEM suggests that *F. occidentalis* antennae feature specialised olfactory organs, gustatory organs and mechano-sensory organs (Walbank, 1996).

Chemical emission structures

Male *F. occidentalis* like several other thripids have distinctive pore plates on abdominal sternites III-VII (Figures 1.1 and 1.2). It has been suggested that these are implicated

in chemical release (Moritz, 1997; Kirk & Hamilton, 2004; El-Ghariani & Kirk, 2008). *F. occidentalis* larva II emits alarm pheromone in anal secretions (Teerling *et al.*, 1993). This has also been shown to occur in larva I. It is unclear where in the abdomen this substance is produced (MacDonald, 2003).

1.3 *F. occidentalis*, distribution and ecology

F. occidentalis, like certain other members of the Thripidae, is a polyphagous virus vector. *Frankliniella* and *Thrips* genera have also been suggested to act as major pollinators in a limited number of crops (Kirk, 1997).

Population distribution

F. occidentalis spread from the west coast of California since the late 1960's where it was previously restricted to western North America and Mexico (Kirk & Terry, 2003). *F. occidentalis*, although occasionally found in some eastern states of the USA failed to become established. It is thought that interspecific competition with *F. tritici*, the eastern flower thrips, may have played a role in this (Kirk, 2002). Interestingly *F. occidentalis* was present in New Zealand from as early as 1934 (Mound & Walker, 1982b).

F. occidentalis distribution in the late 1970's increased throughout the United States entering Canada and reaching Europe via the Netherlands. It is thought that the export of horticultural material was responsible for this (Kirk, 2002). *F. occidentalis* was first discovered in the United Kingdom in 1986 on a Cambridge chrysanthemum crop (Baker *et al.*, 1993). Initially the UK government tried to contain outbreaks by implementing phytosanitary measures, including destruction of infected crops and glasshouse sterilisation. By 1989 after a failed attempt to prevent further outbreaks, *F. occidentalis* became classed as established on the British mainland and containment

measures were dropped (Baker *et al.*, 1993).

Ecology

Several biotypes of *F. occidentalis* exist. Whilst some of these strains are relatively innocuous (e.g. the New Zealand tree lupin biotype), some are pesticide resistant (e.g. the New Zealand 1992 outbreak biotype) and thus alternative control methods are necessary (Martin & Workman, 1994).

F. occidentalis, when compared with another pest species, the onion thrips *T. tabaci*, has a marginally lower intrinsic rate of population increase (r_m) at 25°C. This suggests that additional factors help account for the greater pest status of *F. occidentalis* (van Rijn *et al.*, 1995).

The ability of *F. occidentalis* to utilise multiple hosts often allows it to remain reproductively active throughout the year. In American near-orchard habitats it is able to achieve this by alternating between hosts that produce leaves and flowers at different periods and seasons (Cockfield *et al.*, 2003).

In climates with cold winters, *F. occidentalis* survival is restricted by low outdoor temperatures. *F. occidentalis* individuals are however, capable of surviving under glass (McDonald *et al.*, 1997). Cold hardiness is also affected by rearing temperature (Tsumuki *et al.*, 2007) and exposure to cold extremes may reduce female reproductive output (McDonald *et al.*, 1997).

Phytophagy and vector capacity

Feeding *F. occidentalis* ingest sap and this directly damages plant structures, often causing marked discolouration (Figure 1.3) (Lewis, 1997). As mentioned previously, in-plant oviposition by females also causes further damage to plant structures as the egg increases in size. Resulting scar tissue on the plant may cause abnormal growth or in the case of ornamental crops may cause significant cosmetic damage. An abnormal

curling in cucumber known as 'pigtail' is possibly caused in this way (Jacobson, 1997). In some cases injury may not be immediately apparent as effects may be insidious or delayed (Childers & Achor, 1995).

F. occidentalis act as a vector for a variety of plant viruses (including tospoviruses from the Bunyaviridae family and pollen-borne viruses), bacteria and fungi (Ullman *et al.*, 1997). *F. occidentalis* is among nine species out of around 5500 identified thrips species that are known to act as a vector of the *Tospovirus* genus, which is considered to be reliant on thrips for their existence in nature (Mound, 2002). There are, as of 2007, thirteen identified thrips species capable of acting as bunyavirus vectors (Ullman, 2007). Tospoviruses such as *Tomato spotted wilt virus* (TSWV) (Figure 1.5) and *Impatiens necrotic spot virus* (INSV) rely exclusively on thrips feeding for transmission. The *F. occidentalis* first larval instar acquires TSWV within seconds of feeding and the subsequent adult remains infective for its entire life. The thrips need to take up the virus as a larva I if they are to transmit the infection when feeding and in subsequent life stages (Moritz, 2002).

Pollen-borne virus transmission by thrips is not fully understood. It is thought that infected pollen is moved between plants and that thrips facilitate transmission through mechanical wounding of the plant during feeding, where they may also deposit pollen containing virus particles (Ullman *et al.*, 1997). *T. tabaci* transmits *Prunus necrotic ringspot virus* (PNRSV) in this way (Greber *et al.*, 1991).

Economic costs associated with F. occidentalis

In one reported study 20.5% of cuttings and 12% of ornamental plants used for international trade were infested with *F. occidentalis* (Frey, 1993). *F. occidentalis* feeds on more than 500 plant species in over 50 families worldwide (Moritz, 2002). In the UK, as of 1989, *F. occidentalis* had been found to be present on 68 plant genera (Baker *et al.*, 1993). In the UK during 1999 direct loss of ornamental plants accounted for an

annual cost of between £12.5-25 m (Sampson, 2000). In the Netherlands glasshouse crops lost to TSWV in 1998 accounted for around £12.5 m at 2001 exchange rates (Roosjen *et al.*, 1998). As mentioned previously, obtaining current figures for thrips damage is difficult partly because of commercial secrecy but it is accepted that it is less than late 1990s levels.

1.4 Chemical communication and insects

Semiochemical is the generic term for an inter or intraspecific signalling substance from an organism (Mori, 1989). Allelochemicals relay interspecific communication whilst pheromones relay intraspecific communication (Whittaker & Feeny, 1971). Allelochemicals can be differentiated further into allomones and kairomones where allomones provide an adaptive advantage to the emitter whilst kairomones provide an adaptive advantage to the receiver (Whittaker & Feeny, 1971). Certain semiochemicals can act as both allomones *and* kairomones depending on the organism that releases or receives the signal.

1.5 Allelochemicals

Secondary plant compounds are non-essential for the plant's metabolism and include an array of organic volatile semiochemical compounds that are used as allomones by the plant. Plants are able to produce these compounds using one or more of four organ types; osmophores, glandular trichomes of stems and leaves, ducted oil glands and oil cells of leaves and fruits (Metcalf & Metcalf, 1992). Plant allomone attractants are received by the insect (see section 1.2 for information on thrips chemoreception) and this causes attraction and hence increases pollination if the insect carries pollen from

one plant to another.

Plant-derived allomones can act as antifeedants, causing the insect to be dissuaded from eating the plant, thus benefiting the plant as the emitter. Botanical pesticides and toxins, whilst not necessarily being construed as allomones (according to Whittaker & Feeny, 1971) are of significant importance in this regard. This has resulted in the commercial development of plant derived insecticides and the development of anti-feedants. Qualitative toxins and quantitative digestibility reducing botanicals all serve to minimise the damage caused to crops by insects. Plant derived essential oils also include volatile insecticidal allomones that are produced in very small quantities by a variety of plants.

1.5.1 Kairomones

Plant kairomones can be used as part of an IPM strategy to attract specific insects from main crops and onto side fodder crops or traps. It has been shown that synthetic kairomone analogues can be synthesised and deployed in lures and traps. If used in conjunction with feeding deterrents, kairomones can form part of a 'push-pull' or stimulo-deterrent strategy (Pyke *et al.*, 1987), with the kairomone pulling the insect to the desired crop (for a review see Cook *et al.*, 2007). The attractiveness of plant kairomones can be assessed using laboratory and field bioassays. It has been shown using a Y-tube bioassay that adult female *F. occidentalis* were attracted by benzenoids (benzaldehyde and *p*- and *o*-anisaldehyde (4-methoxybenzaldehyde)), monoterpenes (geraniol, nerol, linalool and (+)-citronellol), sesquiterpene ((*E*)-β-farnesene) and phenylpropanoids (eugenol and 3-phenylpropionaldehyde). The attractiveness of these volatiles is concentration dependant with excess potentially having a repulsive effect (Koschier *et al.*, 2000). Glasshouse trials involving *p*-anisaldehyde and *F. occidentalis* have been undertaken in New Zealand. Yellow and black traps with *p*-anisaldehyde

and yellow water traps with *p*-anisaldehyde caught significantly more adults than those without *p*-anisaldehyde (Teulon *et al.*, 1999).

The electrophysiological response to a specific kairomone can also be observed using coupled Gas Chromatography Electroanntennography (GC-EAG) (Pow *et al.*, 1998), with either whole antennae or single antennal cells. GC-EAG studies with *F. occidentalis* female antennae and *p*-anisaldehyde (in dichloromethane) have shown electrophysiological activity in the thrips antennae with corresponding *p*-anisaldehyde fractions (R. van Tol, personal communication, Wageningen 2004; W.J. de Kogel, personal communication, Keele 2005). This process provides evidence of a response but does not necessarily prove that the specific semiochemical will alter insect behaviour, so it cannot prove that the compound is bioactive.

The kairomone methyl anthranilate is a strong attractant for additional flower thrips. Male and female *Thrips hawaiiensis*, *T.coloratus*, *T. flavus* and *Megalurothrips distalis* are attracted and display greater attraction than to the *p*-anisaldehyde attractant (Imai *et al.*, 2001). Kairomone specificity was demonstrated as *T. tabaci* is not attracted to methyl anthranilate (Murai *et al.*, 2000).

1.5.2 Allomones

Specific allomones attract specific pollinators, with the insect responding to specific cues. This has been demonstrated in cycads where three individual species in the genus *Macrozamia* were found to release three different combinations of volatiles. *M. machinii* cone volatiles consisted of over 80% linalool with less than 9% of β-myrcene and attracted the specific pollinator *Tranes* weevil, whilst *M. lucida* and *M. macleayi* cone volatiles consisted of up to 97% β-myrcene with no linalool and attracted the specific Aeolothripidae thysanopteran pollinator *Cycadothrips chadwicki*. Pollinator

movement was greatest at cone thermogenesis and peak odour emission. This varied according to the pollinator species (Terry *et al.*, 2004).

Allomone antifeedants can deter insects from feeding or ovipositing on commercially important crops, effectively forcing them onto alternative crops. The lepidopteran large white butterfly *Pieris brassicae* is deterred from feeding by the antifeedant extracts found in peppermint *Mentha piperita* (Endersby & Morgan, 1991). *F. occidentalis* is repelled by rosemary leaves and volatiles (Bennison *et al.*, 2002). The pirate bug *Orius laevigatus*, a commercially available biological thrips predator is also repelled by rosemary and this reduces the overall benefits of this antifeedant (Bennison *et al.*, 2002).

Thrips can often be controlled with botanical and synthetic insecticides. *F. occidentalis* like several other thrips is afforded some protection as a result of its thigmotropic behaviour; it is often able to avoid contact with insecticides as it can be protected by the structures inside the host flowers. In-plant tissue egg laying also reduces the risk of contact from insecticides (Tommasini & Maini, 2002).

The majority of essential oils, are complex mixtures of monoterpenes (C_{10}) and sesquiterpenes (C_{15}) including phenolpropenes and cinnamates (Isman & Machial, 2006). Trials investigating the fumigant toxicity of several undisclosed essential oils on *F. occidentalis*, *T. tabaci* and *T. simplex* have been undertaken in the Netherlands. Increased mortality was shown in all the species at high concentrations although *F. occidentalis* was the least sensitive (W.J. de Kogel, personal communication, Wageningen 2004). The effect of essential oil application on natural predators and parasitoids of thrips is unknown. There is also a potential effect on crop fragrance.

There is no specific synthetic thrips insecticide so broad spectrum, low persistence synthetic organophosphate or carbamate based insecticides such as dichlorvos are often applied (Jacobson, 1997). Dichlorvos has since been withdrawn in the UK due to fears

that it may be a genotoxic carcinogen (Anonymous, 2002). As a result of this and additional withdrawals, alternative control strategies need to be utilised. Increasingly this situation is reflected in national crop protection strategies where biocontrol is given more precedence (van Lenteren, 2004).

The spinosad insecticide is a recently developed insecticide derived from fermented *Saccharpolyspora spinosa*. It has been shown to control *F. occidentalis* in laboratory and field studies. It was initially thought that spinosad had no effect on biological thrips predators such as *Orius* spp., making it highly suitable for use in conjunction with biological control IPM methods. It now appears that spinosad may reduce *Orius* spp. populations (Ludwig & Oetting, 2001).

1.5.3 Insect-produced allomones

Insects produce a wide range of defensive secretions against natural predators. These compounds vary in their complexity and function and can be classed accordingly. Most allomones appear to be synthesised anew within the insect though this is unclear in the case of some polyunsaturated acids and sterols (Morgan, 2004).

Defensive allomones often contain mixtures of simple hydrocarbons, carboxylic acids, alcohols, aldehydes, ketones, esters (including cyclic esters such as lactones and quinones), phenols, steroids and additional organic compounds (Blum, 1978; Morgan, 2004).

1.5.4 Thysanopteran-produced allomones

F. occidentalis and the greenhouse thrips, *Heliothrips haemorrhoidalis* are the only two thripid species in which the constituents of the defensive anal droplets have been

identified (Zabaras *et al.*, 1999) (Table 1.1). It is likely that more thripidae release defensive anal droplets but the composition of these has, as yet not been chemically analysed. It is clear that *Thrips tabaci* larvae produce defensive anal droplets against predatory mites (Bakker & Sabelis, 1989).

Many phlaeothripids (Idolothripine and Phaeothripinae) produce anal droplet defensive secretions. Initially it was shown in 1983 that *Bagnalliella yuccae* produces an anal secretion containing the lactone (cyclic ester) γ-decalactone (Howard *et al.* 1983). Further studies on additional Phlaeothripinae species have revealed the presence of several long-chain hydrocarbons, esters, acids, terpenes, and aromatic compounds (tables 1.2, 1.3). Multiple compounds are often produced and in some instances it is possible to identify the genus according to the defensive secretion produced (Suzuki *et al.* 2004). It is thought that these allomones provide highly effective repellents against a variety of thrips-predating ant species (Blum, 1991).

It is unclear where thysanopteran defensive allomones are produced. Semiochemical producing glands are varied in nature across the insects and exhibit differences in morphology often related to the different functions assigned to them (Percy-Cunningham & MacDonald, 1987). Glands may be unicellular and thus very simple in structure or may form an internal structure distinct from but connected to a reservoir (Percy-Cunningham & MacDonald, 1987). In the case of Phlaeothripidae the secretory reservoir appears to be the hindgut but as yet no secretory cells have been found (Howard *et al.* 1983; 1987; Tschuch *et al.* 2008).

1.6 Intraspecific chemicals

Pheromones, like allomones, as secondary metabolites are often classed as natural products. These compounds vary greatly in chemical structure but are manufactured via

a limited number of biosynthetic pathways (Morgan, 2004). Such pathways can provide indications of the types of compounds that may be produced and the type of compounds to expect in subsequent analyses.

Pheromones can be used for population monitoring, mass trapping and mating disruption and identification rates have increased since the first pheromonal isolation in 1959 for the lepidopteran silkworm moth *Bombyx mori* by Butenandt and co-workers (Morgan, 2004). Many more pheromones across a wide range of insect orders have been identified subsequently (Mori, 1989). Successful isolation and synthesis of pheromones allows them to be used in IPM. The 2004 BioPesticides handbook details some fifty-five semiochemicals that are commercially available for insect biocontrol and shows the commercial importance of this emerging semiochemical market (Copping, 2004).

1.6.1 Multicomponent pheromones and isomeric enantiomers

Insects generally use multicomponent pheromones (as is the case with *F. occidentalis* alarm pheromone) (Silverstein & Young, 1976). The importance of multicomponent pheromones was demonstrated by Klun *et al* (1973).

Covalent bonded carbon to carbon atoms create right and left geometric (*trans* or (*E*) and *cis* or (*Z*)) isomers. It is commonplace for insects to differentiate between these forms. Optical isomerism may also arise where there is a chiral carbon centre. In such cases two optically active enantiomers (*R* and *S*) are created and once again the insect is likely to be able to distinguish between the two enantiomers. A racemic mixture of both enantiomers in equal proportions may also be bioactive as has recently been found to be the case in the apple leaf midge, *Dasineura mali* (Cross & Hall, 2009).

Differentiating between *R* and *S* enantiomers when ascertaining pheromone structure can be challenging (Howse *et al.*, 1998). It is normal for natural pheromone

volatiles to be available in very small amounts and it is therefore often impossible to determine the absolute configuration using conventional methods such as degradation to simple compounds or X-ray crystallography. It is however possible to start with a compound of known absolute configuration and thus generate the target molecule where the absolute configuration is also known. The chiroptical properties of this synthetic molecule can then be compared with those of the natural pheromone. If obtaining optical data on the natural pheromone is unachievable, pure enantiomers can be synthesised and assessed for biological activity in an appropriate bioassay. Normally the bioactive enantiomer is the natural pheromone (Mori, 1989). Chiral pheromones are common in insects and this enables fewer compounds with identical chemical composition to have very different intraspecific signalling roles. Standard GC columns are able to separate geometric but not chiral isomers.

Chiral multicomponent sex pheromones have recently been identified for the nettle caterpillars, *Darna trima* and *D. bradleyi*. These species are important lepidopteran pests in South East Asian oil palm plantations where they cause defoliation of palm trees. GC-EAG analysis of pheromone gland extracts revealed two antennally active compounds which were subsequently identified using GC-MS. *D. trima* females produce 2-methylbutyl (*E*)-7,9-decadienoate and (*E*)-2-hexenyl (*E*)-7,9-decadienoate, whilst *D. bradleyi* produce methyl (*E*)-7,9-decadienoate and isobutyl (*E*)-7,9-decadienoate (Sasaerila *et al.*, 2000). Field trials have shown that the *S* enantiomer of 2-methylbutyl (*E*)-7,9-decadienoate in combination with (*E*)-2-hexenyl (*E*)-7,9-decadienoate acts as an essential synergist for *D. trima* attraction, whereas the *R* enantiomer failed to increase trap rates.

1.6.2 Alarm pheromones

An alarm pheromone may induce searching, approach, recruitment, alerting, attack or repellence in an insect and are often concentration dependant (Howse *et al.*, 1998). Alarm pheromones are ubiquitous in social insects where secretions recruit other individuals into colony defence. A fair amount of attention has been focussed on ant alarm pheromones. In 1971, 13 ants were recorded as having known or probable alarm pheromones (Wilson, 1971). The chemistry of these has subsequently been confirmed in several Myrmicinae such as the common elbowed red ant, *Myrmica scabrinodis* (Hymenoptera: Formicidae) which contains a mixture of simple alcohols (3-hexanol, 3-heptanol, 3-octanol, 6-methyl-3-octanol, 3-nonanol, 3-decanol and 3-undecanol) and their corresponding ketones (Morgan *et al*, 1979; Morgan, 2004).

Alarm pheromones also feature in hemipterans. The sesquiterpene, (E)-β-farnesene, is known to be an alarm pheromone for aphid species from over 30 genera including the cotton aphid *Aphis gossypii* (Hemiptera: Aphididae). For a review see (Byers, 2005).

The *F. occidentalis* alarm pheromone, identified in 1993 using Gas Chromatograph Mass Spectroscopy (GC-MS) contains a mixture of decyl acetate and dodecyl acetate (Figures 1.6 and 1.7) (Teerling *et al*, 1993) at a molar ratio of 1.5:1 (MacDonald *et al.*, 2002). MacDonald, 2003 provided a detailed account of the chemical ecology of this alarm pheromone.

1.6.3 Sex and aggregation pheromones

Sex pheromones may control a variety of behaviours including alerting, upwind flight, landing, orientation and copulation (Howse *et al.*, 1998). Sex pheromones attract the opposite sex exclusively whereas aggregation pheromones attract both males and

females of the same species.

Due to relative ease of rearing and pest status, sex pheromones have been elucidated for many Lepidoptera. Females of many species release volatiles that attract conspecific males. These are generally straight chain hydrocarbons (C_{12}-C_{18}) with either an alcohol, ester, aldehyde or acetate at one end and have up to three double bonds with alternative isomeric (E) or (Z) enantiomers (Morgan, 2004).

Minute quantities of sex pheromone are often produced by female Lepidoptera. In the case of the male leafminer *Cameraria ohridella*, a major pest of European *Aesculus hippocastanum* horse-chestnut trees, males are able to detect picogram quantities of ($8E$, $10Z$)-tetradeca-8,10-dienal released from virgin females (Svatoš *et al.*, 1999).

Several aggregation pheromones have been identified in nitidulid Coleoptera (Ginzel *et al.*, 2007) (see section 3.1.1), and it has been suggested that they are also present in the sand fly *Phlebotomus papatasi* (Psychodidae) (Schlein, *et al.*, 1984).

As mentioned in section 1.2, adult male *F. occidentalis* like other thripids possesses pore plates on abdominal sternites III-VII. The ultrastructure of sternal glands in the Thripidae has been examined using *T. hawaiiensis* (Bode, 1978; Sudo & Tsutsumi, 2002), *T. validus* (Bode, 1978), *F. intonsa* (Sudo & Tsutsumi, 2002) and most recently *F. occidentalis* (El-Ghariani & Kirk, 2008). The phlaeothripine *Psalidothrips simplus* has also been examined (Sudo & Tsutsumi, 2002). Thripidae possess secretory cells, a cuticular ridge and secretory ductules with a wide subcuticular space filled with secretion. The phlaeothripine species studied lacked both a cuticular ridge and a secretory ductule.

Epidermal gland cells can be classed into three groups according to whether the surface cuticle is secreted by the gland cell and on the mode of secretion (Noirot & Quennedy, 1974). *Thrips validus* has been shown to fit into Class 1, where the gland

cell is covered by the cuticle and lacks ducts formed by other cells with the secretion passing through the cuticle without passing through any other cells (Bode, 1978). *F. occidentalis* sternal glands have not been studied in sufficient detail to place them into a class under this classification. The general structure appears to be similar to the structure found in *T. validus* glands (El-Ghariani & Kirk, 2008).

Evidence for the existence of a sex pheromone of *F. occidentalis* based on Y-tube olfactometer experiments was published (Hamilton & Kirk, 2003; de Kogel & van Deventer, 2003; Kirk & Hamilton 2004) and expanded upon in 2005 (Hamilton *et al.*, 2005) (see also, Chapter 3). Kirk & Hamilton (2004), using Solid Phase Micro Extraction (SPME) and GC-MS analysis, noted two key compounds appearing in only male headspace volatiles during odour entrainments (see the two main retention peaks X and Y in Figure 1.8).

It was considered likely that one or both of these volatiles would have a pheromonal effect on other *F. occidentalis* individuals and it was hoped that these compounds could be run in a Y-tube bioassay with them being likely to attract both males and females (Kirk, personal communication, Keele 2004).

A generic chemical structure based on these compounds was patented by Keele University with respect to their application in monitoring and potentially controlling pest thrips species (Hamilton & Kirk, 2003). Since the commencement of this study, two major headspace compounds were subsequently identified as neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate (Chapter 3) (Hamilton *et al.*, 2005). Neryl (*S*)-2-methylbutanoate acts as an aggregation pheromone as it attracts both males and female western flower thrips. The function of (*R*)-lavandulyl acetate is, as yet, unclear.

1.7 Applications of pheromones for *F. occidentalis* control

It is important to examine the efficacy of pheromones when used as part of an IPM strategy. It is clear that several problems occur when pheromones are used for mass trapping and/or mating disruption; there may be a lack of attraction to the attractant, a lack of highly efficient traps or conversely the trap may be easily saturated with caught individuals. The need for many traps will also increase financial outlay (Howse *et al.*, 1998). These factors have to be considered before such a system may be implemented.

Long-term testing is needed to conclusively demonstrate efficacy of pheromones when used in mass trapping. In a twenty year study on the bark beetle *Ips duplicatus* in a forest island, mass trapping was successful with a synthetic aggregation pheromone blend of ipsdienol and (*E*)-myrcenol which significantly reduced tree mortality (Schlyter *et al.*, 2003).

The development and application of new pheromones is hindered by the high cost of development and the need for costly governmental registration when used for non-monitoring purposes. Whilst pheromones are not commonly used for insect control in UK they can be very successful in controlling insect populations. In the USA the lepidopteran cotton pest, the pink bollworm *Pectinophora gossypiella* was controlled using pheromones. Mating disruption via confusion is achieved by the release of the synthetic pheromone Gossyplure. This is a two component, highly persistent sex pheromone comprised of a 1:1 mixture of (*Z*,*Z*) and (*Z*,*E*)-7,11-hexadecadienyl acetate (Howse *et al.*, 1998). Whilst the use of pheromones in controlling cotton pests as part of an IPM strategy has proven effective (Tamhankar *et al.*, 2000) advances in plant genetics present additional strategies for insect control (Qaim & Zilberman, 2003).

In principle, the isolation of alarm pheromone is of great significance with respect

to using semiochemicals to control thrips. If this pheromone was released using a controlled release system it may increase insect activity. This is desirable if the insect needs to be coated with insecticide in order to increase the efficacy of the insecticide. This has been successfully demonstrated on *F. occidentalis* larval stages using maldison combined with dodecyl acetate, where larval mortality was significantly increased. Notably, combining dodecyl acetate and fipronil in a similar way proved to be unsuccessful in increasing larval mortality (Cook *et al.*, 2002). The pheromone also induces movement of both larvae and adults away from its source, reduces oviposition and causes some larvae II to drop from leaves. Notably the response by *F. occidentalis* to its alarm pheromone is relatively weak and this necessitates that it is used in conjunction with other IPM strategies (Teerling *et al*, 1993).

The isolation of *F. occidentalis* aggregation pheromone is also of great significance. The pheromone specifically needs to attract females due to the thrips parthenogenic life cycle. Disrupting mating by confusion would only result in more males being produced by unmated females but not serve to decrease population levels on the crop. A successful pheromone could be used for monitoring and mass trapping purposes.

1.8 Aims

F. occidentalis is a major crop pest with a ubiquitous distribution. An IPM strategy is necessary to control its populations. As a result of increasing insecticide resistance and pesticide withdrawals this strategy should utilise semiochemical and biological control agents in addition to conventional treatments. The use of semiochemicals (specifically the development and application of an aggregation pheromone) provide an exciting and novel way of controlling *F. occidentalis* and potentially other *Frankliniella* and *Thrips* species and their populations.

The overall aim of this PhD project is to investigate the aggregation pheromone ecology of *F. occidentalis*. The proposed aims of the work are to:

1) Examine the component, neryl (*S*)-2-methylbutanoate, that is thought to have aggregation pheromone properties with respect to *F. occidentalis*, with a view to determining how this compound could be used to monitor and control the pest in protected crops;
2) To develop new bioassays to investigate the properties of male produced compounds;
3) To undertake studies to reveal the dynamics of sex pheromone release from *F. occidentalis* males;
4) To attempt to identify the minor headspace compounds produced by *F. occidentalis* males;
5) To analyse field trial data in a trial that uses components thought to have aggregation pheromone properties with respect to *F. occidentalis*.

Species	Main anal secretions:	Additional components:
Panchaetothripinae		
Heliothrips haemorrhoidalis	Coumaran (2,3-dihydrobenzofuran)	simple hydrocarbons (C_{14}-C_{17}), 3-methoxyacetophenone (Zabaras *et al.*, 1999)
Thripinae		
Frankliniella occidentalis	decyl acetate and dodecyl acetate	(Teerling *et al.*, 1993)

Table 1.1: Classes of anal secretions known in the Thripidae

Species	Main anal secretions:	Additional components:
Idolothripini		
Bactrothrips brevitubus	acid (C_{10})	acid (C_{12}) (Suzuki *et al.*, 2000), quinone (juglone) (Suzuki *et al.*, 1990; 2000)
Bactrothrips carbonarius	not stated	acids (C_{14}), quinone (juglone) (Suzuki *et al.*, 2000)
Bactrothrips flectoventris	not stated	acids (C_{10}-C_{14}), quinone (juglone) (Suzuki *et al.*, 2000)
Bactrothrips honoris	not stated	acids (C_{10}-C_{12}), quinone (juglone) (Suzuki *et al.*, 2000)
Bactrothrips montanus	not stated	acids (C_{14}), quinone (juglone) (Suzuki *et al.*, 2000)
Bactrothrips pictipes	not stated	acids (C_{10}-C_{14}), quinone (juglone) (Suzuki *et al.*, 2000)
Bactrothrips quadrituberculatus	not stated	acids (C_{14}), quinone (juglone) (Suzuki *et al.*, 2000)
Bactrothrips sp.	not stated	acids (C_{10}), quinone (juglone) (Suzuki *et al.*, 2000)
Elaphrothrips (= Ethriothrips) antennalis	not stated	acids (C_{14}), quinone (juglone) (Suzuki *et al.*, 2000)
Elaphrothrips tuberculatus	quinone (juglone)	acids (Blum *et al.*, 1987)
Holurothrips morikawai	not stated	acids (C_{10}), quinone (juglone) (Suzuki *et al.*, 2000)
Mecynothrips pugilator	not stated	acids (C_{14}), quinone (juglone) (Suzuki *et al.*, 2000)
Mecynothrips simplex	not stated	acids (C_{10}), quinone (juglone) (Suzuki *et al.*, 2000)
Mecynothrips sp.	not stated	acids (C_{10}-C_{14}), quinone (juglone) (Suzuki *et al.*, 2000)
Neatractothrips macrurus	not stated	acids (C_{10}), quinone (juglone) (Suzuki *et al.*, 2000)
Opthalmothrips miscanthicola	not stated	acids (C_{14}), quinone (juglone) (Suzuki *et al.*, 2000)
Pygothripini		
Gastrothrips sp.	no components detected	(Suzuki *et al.*, 2000)
Machantothrips artocarpi	not stated	acids (C_{12}), quinone (juglone) (Suzuki *et al.*, 2000)
Nesothrips lativentris	not stated	acids (C_{10}), quinone (juglone) (Suzuki *et al.*, 2000)

Table 1.2: Classes of anal secretions known in the Phlaeothripidae I: Idolothripinae

Species	Main anal secretions:	Additional components:
Arrhenothrips ramakrishnae	monoterpene (rose furan)	monoterpene (perillene) and aromatics (phenol and phenylacetaldehyde) (Blum *et al.*, 1988)
Bagnalliella yuccae	lactone	(Howard *et al.*, 1983, Haga *et al.*, 1989)
Dolichothrips sp.	terpene	simple hydrocarbon, terpenes, quinone (Suzuki *et al.*, 2004)
Haplothrips aculeatus	acid	acids (Suzuki *et al.*, 2004)
Haplothrips kurdjumovi	acid	acid (Suzuki *et al.*, 2004)
Haplothrips leucanthemi	mellein (isocoumarin (C_{10} lactone))	(Blum *et al.*, 1992)
Xylaplothrips inquilinus	unknown terpene	terpene, simple hydrocarbons, esters, 2-hydroxy-6-methylbenzaldehyde (HMBA) (Suzuki *et al.*, 2004)
Eugynothrips (Loepothrips) intorquens	simple hydrocarbon	simple hydrocarbons, terpenes (Suzuki *et al.*, 2004)
Euryaplothrips crassus	ester	aromatics (phenol and phenylacetaldehyde) (Blum *et al.*, 1988)
Gynaikothrips ficorum	simple hydrocarbon(s) (Howard *et al.*, 1987)	simple hydrocarbons, esters (C_{14},C_{16}), (Howard *et al.*, 1987) terpene (Suzuki *et al.*, 2004)
Gynaikothrips uzeli	simple hydrocarbon (C_{15})	simple hydrocarbons, esters, terpene (Suzuki *et al.*, 1989)
Hoplothrips japonicus	acid	acid (Haga *et al.*, 1989; Suzuki *et al.*, 2004)
Liothrips kuwanai	simple hydrocarbon	simple hydrocarbons, esters, terpenes (Suzuki *et al.*, 1989; 2004)
Liothrips piperinus	simple hydrocarbon	simple hydrocarbons, esters, terpenes (Suzuki *et al.*, 1988; 2004)
Liothrips wasabiae	terpene	terpene, simple hydrocarbons, esters (Suzuki *et al.*, 2004)
Liothrips sp.	simple hydrocarbon	simple hydrocarbons, esters, terpene (Suzuki *et al.*, 2004)
Teuchothrips longus	not stated	terpene (perillene) (Blum *et al.*, 1988)
Callococcithrips fuscipennis	simple hydrocarbon	terpenes, esters (Tschuch *et al.*, 2008)
Ponticulothrips diospyrosi	acid	acid, quinones and unkown organic compound (Suzuki *et al.*, 2004)
Ponticulothrips (Undescribed species)	Unknown organic	Unknown with M^+ 224, ester, quinones (Suzuki *et al.*, 1995; 2004)
Thlibothrips isunoki	terpene	(Haga *et al.*, 1990; Suzuki *et al.*, 2004)
Schedothrips sp.	not stated	terpene (perillene) (Blum *et al.*, 1998)
Leeuwenia pasanii	simple hydrocarbon	simple hydrocarbons, esters, acid, terpenes (Suzuki *et al.*, 1986; 1988;1989; 2004)
Ecacanthothrips inarmatus	acid	acid (Suzuki *et al.*, 2004)
Mychiothrips fruticola	acid	acid (Suzuki *et al.*, 2004)
Psalidothrips lewisi	acid	acid (Suzuki *et al.*, 2004)
Holothrips hagai	simple hydrocarbon	simple hydrocarbons, 3-butanoyl-4-hydroxy-6-methyl-2H-pyran-2-one (Suzuki *et al.*, 1993; 2004)
Holothrips japonicus	simple hydrocarbon	simple hydrocarbons, 3-butanoyl-4-hydroxy-6-methyl-2H-pyran-2-one (Suzuki *et al.*, 2004)
Holothrips yuasai	simple hydrocarbon	simple hydrocarbons, 3-butanoyl-4-hydroxy-6-methyl-2H-pyran-2-one (Suzuki *et al.*, 2004)
Oidanothrips frontalis	simple hydrocarbon	simple hydrocarbons, terpene (Suzuki *et al.*, 2004)
Apelaunothrips consimilis	acid	acid, ester, simple hydrocarbons (Suzuki *et al.*, 2004)

Table 1.3: Classes of anal secretions known in the Phlaeothripidae II: Phlaeothripinae

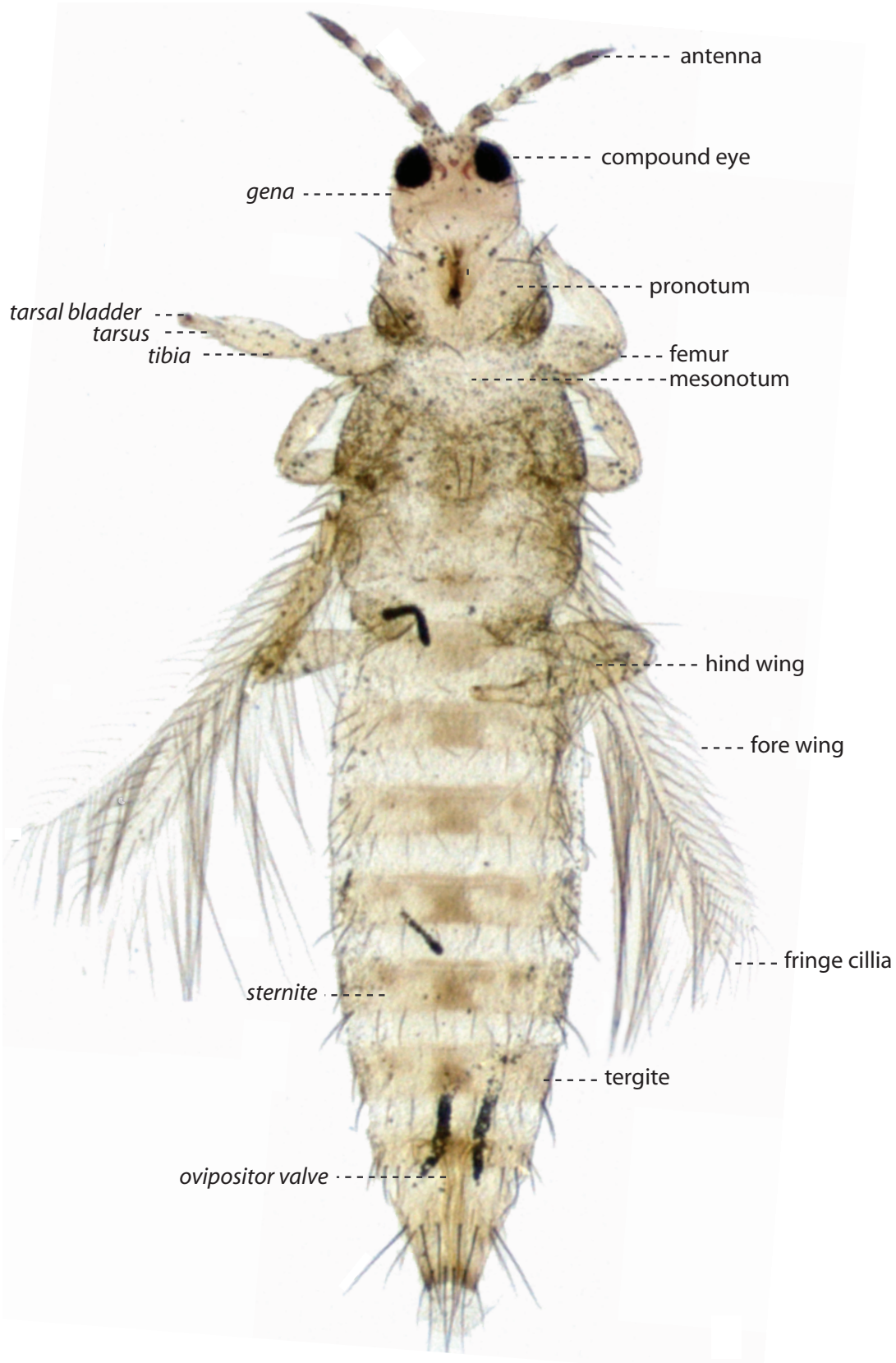

Figure 1.1: Generalised dorsal illustration of an adult female thripid annotated with key ventral (italicised) and dorsal appendages [Photo: Dublon 2005]

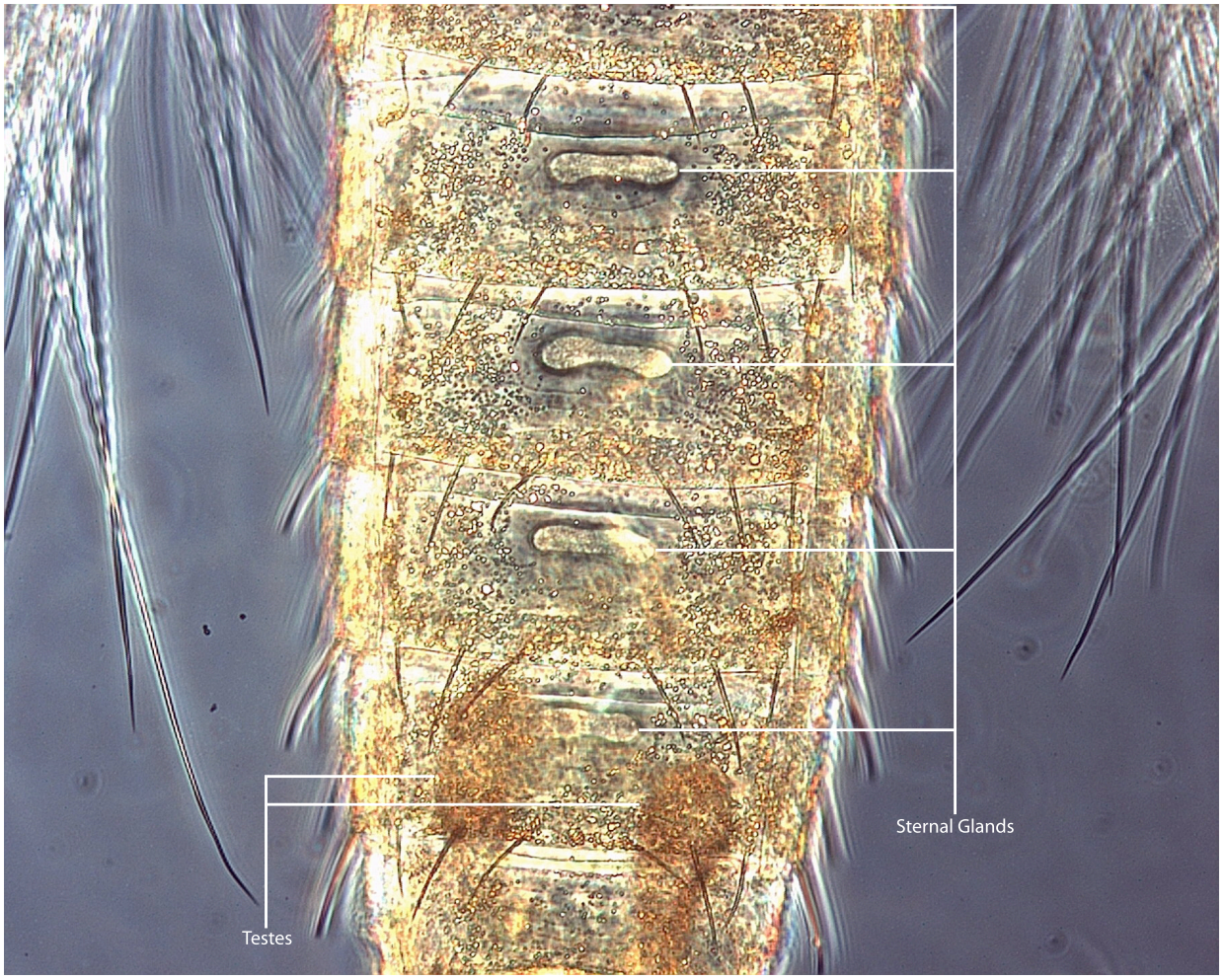

Figure 1.2: Male *F. occidentalis* sternal glands (Magnification 20 x 1.5) [Photo: Cedar Chittenden]

Figure 1.3: Feeding damage by *F. occidentalis* on chrysanthemum flowers [Photo: Dublon, 2005]

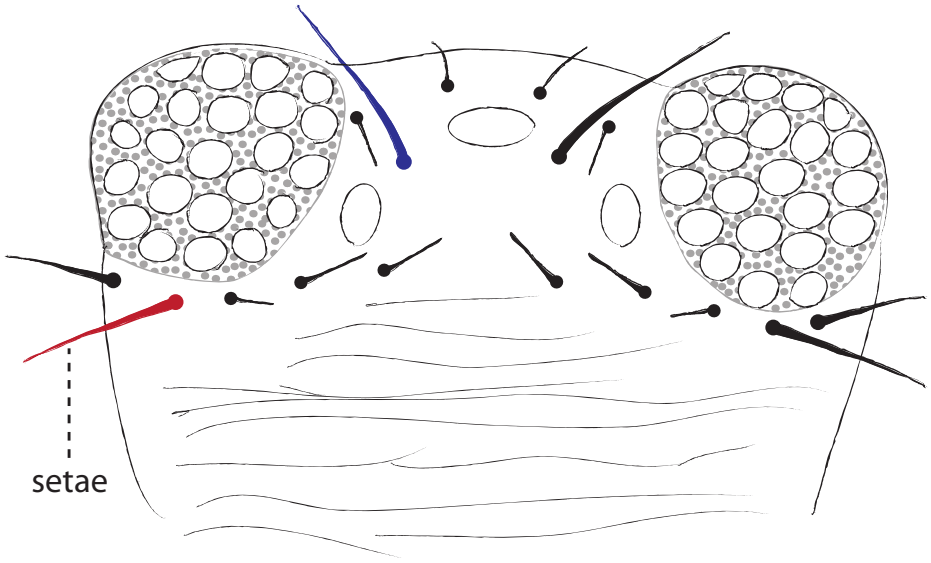

Figure 1.4: *F. occidentalis* head setae arrangements. Note the length of the the major postocular setae (red) is long and nearly as long as setae III (blue) [Redrawn after: Mound and Kibby, 1998]

Figure 1.5: *Tomato spotted wilt virus* damage on chrysanthemum leaves [Photo: Dublon, 2004]

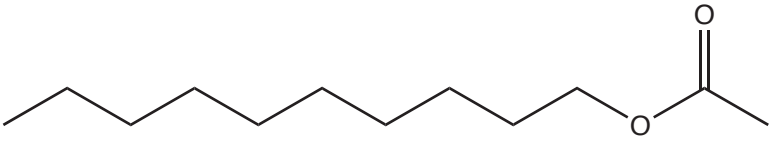

Figure 1.6: Chemical structure of decyl acetate

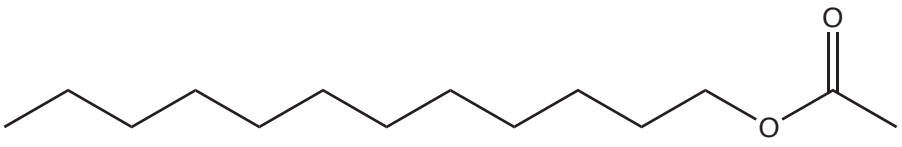

Figure 1.7: Chemical structure of dodecyl acetate

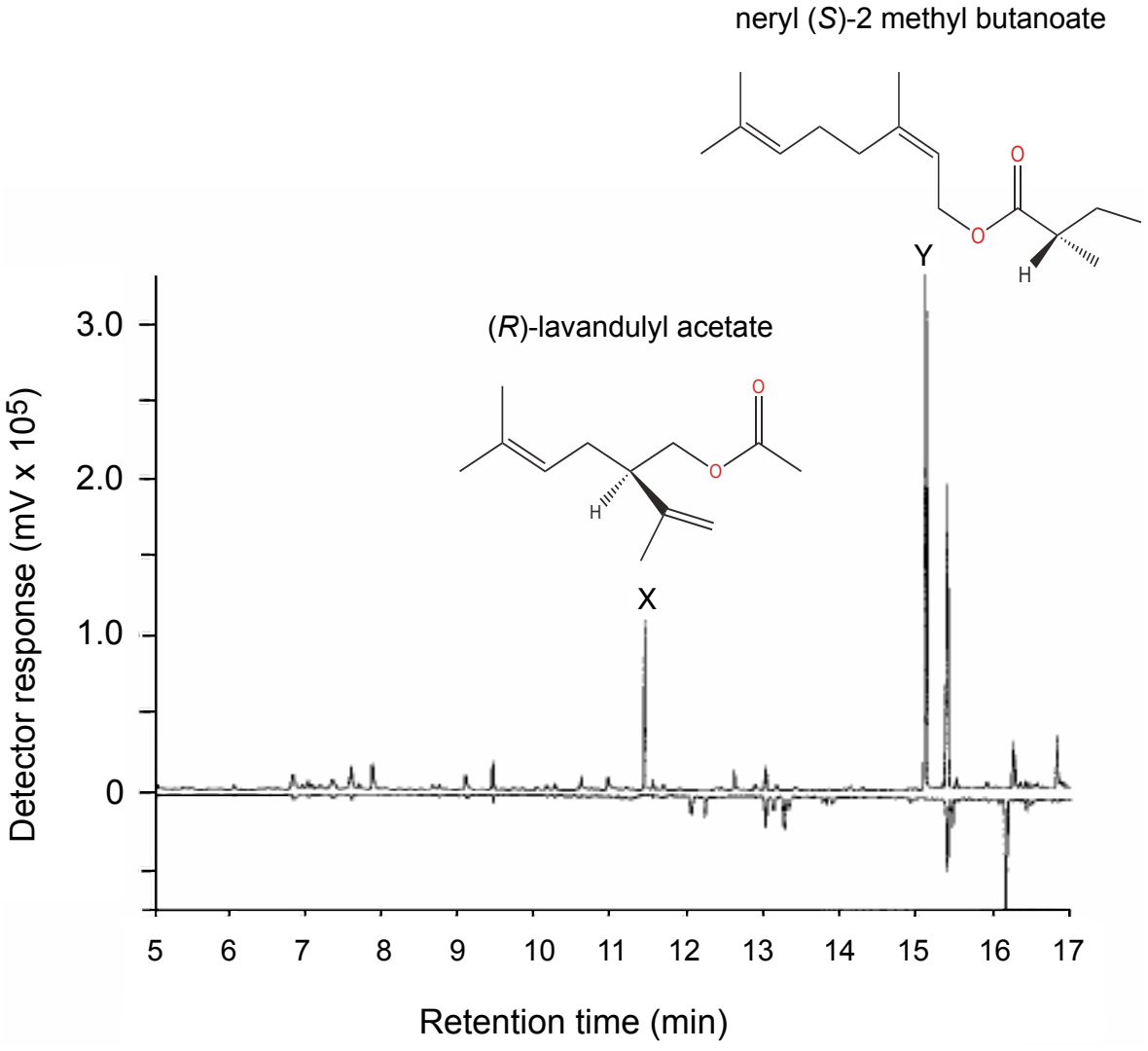

Figure 1.8: GC chromatogram from SPME fibre collections of the headspace volatiles collected from mixed-age adult males [After Kirk & Hamilton, 2004]

1.9 References

Anonymous. (2002) Ministers act against a range of insecticides containing the chemical dichlorvos. *HSE Press Release E076:02, 19th April 2002.* pp 1-5.

Baker, C.R.B., Barker, I., Bartlett, P.W. and Wright, D.W. (1993) Western flower thrips, its introduction and spread in Europe and role as a vector of tomato spotted wilt virus. In: *British Crop Protection Council Monograph, no. 54. Plant Health and the European Single Market.* Edited by: D. Ebbels. British Crop Protection Council, Farnham, UK. pp 355-360.

Bakker, F.M. and Sabelis, M.W. (1989) How larvae of *Thrips tabaci* reduce the attack success of phytoseid predators. *Entomologia Experimentalis et Applicata*, 50 47-51.

Bennison, J., Maulden, K., Dewhirst, S., Pow, E., Slatter, P. and Wadhams, L. (2002) Towards the development of a push-pull strategy for improving biological control of western flower thrips on chrysanthemum. *Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera*, 199-206.

Blum, M.S. (1978) Biochemical defenses of insects. Chapter 11. In: *Biochemistry of insects.* Edited by: M. Rockstein. Academic Press, London, UK. pp. 466-513.

Blum, M.S., Fales, H.M. and Ananthakrishnan, T.N. (1988) *Unpublished data.*

Blum, M.S. (1991) Chemical ecology of the thysanoptera. In: *Towards understanding thysanoptera. Gen. Tech. Rep. NE-147.* Edited by B.L. Parker, M. Skinner and T. Lewis, U.S. Department of Agriculture, Forest Service, Radnor Northeastern Research Station. pp 95-112.

Blum, M.S., Foottit, R. and Fales, H.M. (1992) Defensive chemistry and function of the anal exudate of the the thrips *Haplothrips leucanthemi*. *Comparative Biochemistry and Physiology - Part C*, 1 209-211.

Bode, W. (1978) Ultrastructure of the sternal glands in *Thrips validus* Uzel (Thysanoptera, Terebrantia). *Zoomorphologie*, 90 53-65.

Brown, R. (2004) Food And Environment Protection Act 1985 Control Of Pesticides Regulations 1986: Notice Of Revocation (Off-Label). Occasional Publication: Revocation Notice, Department of Environment, Food and Rural Affairs, United Kingdom. 20th January 2004.

Byers, J.A. (2005) A cost of alarm pheromone production in cotton aphids *Aphis gossypii*. *Naturwissenschaften*, 92 69-72.

Childers, C.C. and Achor, D.S. (1995) Thrips Feeding and Oviposition Injuries to Economic Plants, Subsequent Damage and Host Responses to Infection. In: *Thrips Biology and Management, NATO ASA Series A276*. Edited by: B.L. Parker, M. Skinner and T. Lewis. Plenum Press, New York, USA. pp. 31-50.

Chyzik, R., Glazer, I. and Klein, M. (1996) Virulence and efficacy of different entomopathogenic nematode species against western flower thrips (*Frankliniella occidentalis*). *Phytoparasitica*, 24 103-110.

Cockfield, S.D., Johnson, D.G., Beers, E.H. and Zack, R.S. (2003) Ecology of western flower thrips in intra- and near-orchard habitats. *Proceedings of the 77th Annual Western Orchard Pest & Disease Management Conference, 15-17th January 2003.*

Cook, D.F., Dadour, I.R. and Bailey, W.J. (2002) Addition of alarm pheromone to insecticides and the possible improvement of the control of the western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), 48 287-290.

Cook, S. M., Khan, Z.R. and Pickett, J.A. (2007) The use of push-pull strategies in integrated pest management. *Annual Review of Entomology*, 52 375-400.

Copping L.G. (2004) Semiochemicals. Chapter 4. In *The manual of biocontrol agents. Third edition of the biopesticide manual*. Edited by: L.G. Copping. British Crop Protection Council, Alton, UK. pp. 442-525.

Cross, J.V. and Hall, D.R. (2009) Exploitation of the sex pheromone of apple leaf midge *Dasineura mali* Kieffer (Diptera: Cecidomyiidae) for pest monitoring: 1. Development of lure and trap. *Crop Protection*, 28 139-144.

El-Ghariani, I.M. and Kirk, W.D.J. (2008) The structure of the male sternal glands of the western flower thrips, *Frankliniella occidentalis* (Pergande). *Acta Phytopathologica et Entomologica Hungarica*, 43 257-266.

Endersby, N.M. and Morgan, W.C. (1991) Alternatives to synthetic insecticides for use in crucifer crops. *Biological Agriculture and Horticulture*, 8 33-52.

Frey, J.E. (1993) The analysis of arthropod pest movement through trade in ornamental plants. In: *British Crop Protection Council Monograph, no. 54. Plant Health and the European Single Market*. Edited by: D. Ebbels. British Crop Protection Council, Farnham, UK. pp 157-165.

Ginzel, M. D., Bearfield, J. C., Keeling, C. I., McCormack, C. C., Blomquist, G. J. and Tittiger, C. (2007) Antennally mediated negative feedback regulation of pheromone production in the pine engraver beetle, *Ips pini*. *Naturwissenschaften*, 94 61-64.

Greber, R.S., Klose, M.J., Milne, J.R. and Teakle, D.S. (1991) Transmission of prunus necrotic ringspot virus using plum pollen and thrips. *Annals of Applied Biology*, 118 589-593.

Haga, K., Suzuki, T., Kodama, S. and Kuwahara, Y. (1989) Secretions of thrips III. Secretion acid-emitting thrips *Hoplothrips japonicus* (Thysanoptera: Phlaeothripidae). *Applied Entomology and Zoology*, 24 242-244.

Haga, K., Suzuki, T., Kodama, S. and Kuwahara, Y. (1990) Secretions of thrips VI. Identification of β-myrcene from *Thlibothrips isunoki* (Thysanoptera: Phaeothripidae). *Applied Entomology and Zoology*, 25 138-139.

Hamilton, J.G.C. and Kirk, W.D.J. (2003) Method of monitoring/controlling thrips. Priority date: 21 December 2001. International patent application published under the Patent Cooperation Treaty WO 03/055309. World Intellectual Property Organization, Geneva.

Hamilton, J.G.C., Hall, D.R., Kirk,W.D.J. (2005) Identification of a male-produced aggregation pheromone in the western flower thrips *Frankliniella occidentalis*. *Journal of Chemical Ecology*, 31 1369-1379.

Howse, P., Stevens, I. and Jones, O. (1998) *Insect Pheromones and their Use in Pest Management*. Chapman & Hall, London, UK.

Howard, D.F., Blum, M.S., and Fales, H.M. (1983) Defence in thrips: forbidding fruitiness of a lactone. *Science*, 220 335-336.

Howard, D.F., Blum, M.S., Jones, Fales, H.M., Tomalski, M.D. (1987) Defensive function and chemistry of the anal exudate of the cuban laurel thrips *Gynaikothrips ficorum* (Marchal). *Phytophaga*, 1 163-170.

Imai, T, Maekawa, M, and Murai, T. (2001) Attractiveness of methyl anthranilate and its related compounds to the flower thrips, *Thrips hawaiiensis* (Morgan), *T. coloratus* Schmutz, *T. flavus* Schrank and *Megalurothrips distalis* (Karny) (Thysanoptera: Thripidae). *Applied Entomological Zoology*, 36 475-478.

Isman, M.B. and Machial, C. M. (2006) Pesticides based on plant essential oils: from traditional practice to commercialization. Chapter 2. In: *Naturally occurring bioactive compounds*. Edited by: M. Rai and M.C. Carpinella. Elsevier B.V. pp. 29-44.

Jacobson, R. J. (1997) IPM In Glasshouses. Chapter 18. In: *Thrips as crop pests*. Edited by: T. Lewis. CAB International, Wallingford, UK. pp. 639-666.

Kirk, W.D.J. (1985) Egg-hatching in thrips (Insecta: Thysanoptera). *Journal of Zoological Society of London*, (A)207 181-190.

Kirk, W.D.J. (1997) Feeding. Chapter 4. In: *Thrips as crop pests*. Edited by: T. Lewis. CAB International, Wallingford, UK. pp. 119-174.

Kirk, W.D.J. (2002) The pest and vector from the west: *Frankliniella occidentalis*. *Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera*, 33-42.

Kirk, W.D.J. and Terry, I. (2003) The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). *Agricultural and Forest Entomology*, 5 301-310.

Kirk, W.D.J. and Hamilton, J.G.C. (2004) Evidence for a male-produced sex pheromone in the western flower thrips *Frankliniella occidentalis*. *Journal of Chemical Ecology*, 30 167-174.

Klun, J.A., Chapman, O.L., Mattes, K.C., Wojtkowski, P.W., Beroza, M. and Sonnet, P.E. (1973) Insect sex pheromones: minor amount of opposite geometrical isomer critical to attraction. *Science*, 181 661.

de Kogel, W.J. and van Deventer (2003) Intraspecific attraction in the western flower thrips, *Frankliniella occidentalis*; Indications for a male sex pheromone. *Entomologia Experimentalis et Applicata*, 107 87-89.

Koschier, E.H. de Kogel, W.J. and Visser, J.H. (2000) Assessing the attractiveness of volatile plant compounds to western flower thrips *Frankliniella occidentalis*. *Journal of Chemical Ecology*, 26 2643-2655.

Lewis, T. (1973) *Thrips. Their biology, ecology and economic importance.* Academic Press, London, UK.

Lewis, T. (1997) Pest Thrips In Perspective. Chapter 1. In: *Thrips as crop pests.* Edited by: T. Lewis. CAB International, Wallingford, UK. pp. 1-13.

Ludwig, S. and Oetting, R. (2001) Effect of spinosad on *Orius insidiosus* (Hemiptera: Anthocoridae) when used for *Frankliniella occidentalis* (Thysanoptera: Thripidae) con-

trol on greenhouse pot chrysanthemums. *Florida Entomologist*, 84 311-313.

MacDonald, K.M., Hamilton, J.G.C., Jacobson, R. and Kirk, W.D.J. (2002) Effect of alarm pheromone on landing and take-off by adult western flower thrips. *Entomologia Experimentalis et Applicata*, 103 279-282.

MacDonald, K.M. (2003) The alarm pheromone of the western flower thrips. Ph.D. thesis, Keele University, Keele, Staffordshire, UK.

McDonald, J.R., Bale, J.S. and Walters, K.F.A. (1997) Effects of sub-lethal cold stress on the western flower thrips, *Frankliniella occidentalis*. *Annals of Applied Biology*, 131 189-195.

McDonald, J.R., Bale, J.S. and Walters, K.F.A. (1998) Effect of temperature on development of the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). *European Journal of Entomology*, 95 301-306.

MacLeod, A., Head, J., Gaunt, A. (2004) An assessment of the potential economic impact of *Thrips palmi* on horticulture in England and the significance of a successful eradication campaign. *Crop Protection*, 23 601-610.

Martin, N.A., Workman, P.J. (1994) Confirmation of a pesticide resistant strain of western flower thrips in New Zealand. *Proceedings of the New Zealand Plant Protection Society, 1994*.

Metcalf, R. and Metcalf, E. (1992) *Plant kairomones in insect ecology and control*. Chapman and Hall, London, UK.

Morgan, E.D., Parry, K., Tyler, R.C. (1979) The chemical composition of the dufour gland secretion of the ant *Myrmica scrabrinodis*. *Insect Biochemistry*, 9 117-121.

Morgan, E.D. (2004) *Biosynthesis in insects*. Royal Society of Chemistry, Cambridge, UK.

Mori, K. (1989) Synthesis of optically active pheromones. *Tetrahedron*, 45 3233-3298.

Moritz, G. (1995) Morphogenetic development of some species of the order Thysanoptera (Insecta). In: *Thrips biology and management, NATO ASA Series A276*. Edited by: B.L. Parker, M. Skinner and T. Lewis. Plenum Press, New York, USA. pp. 489-504.

Moritz, G. (1997) Structure, growth and development. In: *Thrips as crop pests*. Edited by: T. Lewis. CAB International, Wallingford, UK. pp. 15-63.

Moritz, G. (2002) The biology of thrips is not the biology of their adults: a developmental view. *Thrips and tospoviruses: proceedings of the 7th international symposium on Thysanoptera*, 259-267.

Mound, L.A. and Walker, A.K. (1982a) Antennal aberration in Thysanoptera. *Entomologist's Monthly Magazine*, 118 175-176.

Mound, L.A. and Walker, A.K. (1982b) Terebrantia (Insecta: Thysanoptera). *Fauna of New Zealand*, 1 1-113.

Mound, L.A. (1997) Biological diversity. Chapter 6. In: *Thrips as crop pests*. Edited by: T. Lewis. CAB International, Wallingford, UK. pp. 197-215.

Mound, L.A., Kibby, G. (1998) *Thysanoptera: An identification guide. 2nd edition.* CAB International, Wallingford, UK.

Mound, L.A. (2002) So many thrips - so few tospoviruses? *Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera*, 15-18.

Murai, T. (2000) Effect of temperature on development and reproduction of the onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), on pollen and honey solution. *Applied Entomological Zoology*, 35 499-504.

Murai, T., Imai, T. and Maekawa, M. (2000) Methyl anthranilate as an attractant for two species and the thrips parasitoid *Ceranisus menes*. *Journal of Chemical Ecology*, 26(11) 2557-2565.

Murai, T. and Toda, S. (2002) Variation of *Thrips tabaci* in colour and size. *Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera*, 377-378.

Nakahara, S. (1997) Annotated list of the *Frankliniella* species of the world (Thysanoptera: Thripidae). *Contributions on Entomology, International*, 2 355-386.

Noirot, C. and Quennedy, A. (1974): Fine structure of insect epidermal glands. *Annual Review of Entomology*, 19 61-80.

O'Donnell C. (2007) Color morphs of western flower thrips in california: phenotypic or genetic variation? *Journal of Insect Science*, 7 28.

O'Leary, A.T. (2005) The flight behaviour of the western flower thrips. Ph.D. the-

sis, Keele University, Keele, Staffordshire, UK.

Percy-Cunningham, J.E. and MacDonald, J.A. (1987) Biology and ultrastructure of sex pheromone-producing glands. Chapter 2. In: *Pheromone biochemistry.* Edited by: G.D. Prestwich and G.J. Blomquist. Academic Press, Inc., London, UK. pp. 27-76.

Pittman, H.A. (1927) Spotted wilt on tomatoes. Preliminary note concerning the transmission of the 'spotted wilt' of tomatoes by an insect vector (*Thrips tabaci* Lind.). *Journal of the Council of Scientific and Industrial Research (Australia)* 1 74-77.

Pow, E.M., Bennison, J.A., Birkett, M.A., Luszniak, M.J., Manjunatha, M., Pickett, J.A., Segers, I.S., Wadhams, L.J., Wardlow, L.R. and Woodcock, C.M. (1998) Behavioural responses of western flower thrips (*Frankliniella occidentalis*) to host plant volatiles. *Proceedings of the 6th International Symposium on Thysanoptera, Antalya, Turkey*, 121-128.

Pyke, B., Rice, M., Sabine, B. and Zalucki, M.P. (1987) The push-pull strategy - behavioural control of *Heliothis. Aust.. Cotton Grower* May-July, 7-9.

Qaim, M and Zilberman, D. (2003) Yield Effects of Genetically Modified Crops in Developing Countries. *Science*, 299 900 - 902.

Roosjen, M, Buurma, J. and Barwegen, J. (1998) Verbetering schade-inschattingsmodel quarantaine-organismen glastuinbouw. *Verslagen en Mededelingen Plantenziektenkundige Dienst, Wageningen*, 197 1-24.

Sampson, C. (2000) WFT - the problem. In: *Abstracts from an ADAS /HDC/ HRI seminar on "Western flower thrips - control in protected ornamentals", Wellesbourne,*

UK. Edited by: ADAS/HDC/HRI. Unpublished. Cited by Kirk (2002).

Sasaerila, Y., Gries, R., Gries. G., Khaskin, G., King, S. and Teo, C.B. (2000) Decadienoates: sex pheromone components of nettle caterpillars *Darna trima* and *D. bradleyi*. *Journal of Chemical Ecology*, 26 1969-1981.

Schlein, Y., Yuval, B. and Warburg, A. (1984) Aggregation pheromone from the palps of feeding female *Phlebotomus papatasi* (Psychodidae). *Journal of Insect Physiology*, 30 153-156.

Schlyter, F., Zhang, Q.-H., Liu, G.-T. and Ji, L.-Z. (2003) A successful case of pheromone mass trapping of the bark beetle *Ips duplicatus* in a forest island, analysed by 20-year time-series data. *Integrated Pest Management Reviews*, 6 185-196.

Silverstein, R.M. and Young, J.C. (1976) Insects Generally Use Multicomponent Pheromones. In: *Pest Management with Insect Sex Attractants and Other Behavior-Controlling Chemicals: Proceedings of the American Chemical Society, ACS Symposium Series 23, Aug 1975*. Edited by: M. Beroza. American Chemical Society, Washington, D.C., USA. pp 1-8.

Slifer, E.H. and Sekhon, S.S. (1974) Sense organs on the antennae of two species of thrips (Thysanoptera, Insecta). *Journal of Morphology*, 143 445-456.

Speyer, E.R. and Parr, W.J. (1941) The external structure of some thysanopterous larvae. *The Transactions of The Royal Entomological Society of London*, 91 559-635.

Sudo, M. and Tsutsumi, T. (2002) Ultrastructures of the sternal glands in two thripine thrips and one phlaeothripine thrips (Thysanoptera: Insecta) *Proceedings of Arthropo-*

dan Embryological Society of Japan, 37 35-41.

Suzuki, T. Haga, K. and Kuwahara, Y. (1986) Anal secretion of thrips I. Identification of perillene from *Leewenia pasanii* (Thysanoptera: Phlaeothripidae). *Applied Entomology and Zoology*, 21 461-466.

Suzuki, T., Haga, K., Watanabe, K. and Kuwahara, Y. (1988) Secretions of thrips II. Secretions of three gall-inhabiting thrips (Thysanoptera: Phaeothripidae). *Applied Entomology and Zoology*, 23 291-297.

Suzuki, T., Haga, K., Watanabe, K. Leal, W.S., Kodama, S. and Kuwahara, Y. (1989) Secretions of thrips IV. Identification of β-acadridal from three gall-forming thrips (Thysanoptera: Phaeothripidae). *Applied Entomology and Zoology*, 24 222-228.

Suzuki, T., Haga, K., Kodama, S., Nemoto, T. and Kuwahara, Y. (1990) Secretions of thrips V. Secretion of idolothripine thrips, *Bactrothrips brevitubus* (Thysanoptera: Phaeothripidae). *Applied Entomology and Zoology*, 25 1-7.

Suzuki, T., Haga, K., Kodama, Izuno, M., Matsuyama, S. and Kuwahara, Y. (1993) Secretion of thrips VII. Identification of 3-butanoyl-4-hydroxy-6-methyl-2H-pyran-2-one from *Holothrips japonicus* and *H. hagai* (Thysanoptera: Phaeothripidae). *Applied Entomology and Zoology*, 28 108-112.

Suzuki, T., Haga, K., Kataoka, M., Tsutsumi, T. Nakano, Y., Matsuyama, S. and Kuwahara, Y. (1995) Secretion of thrips VIII. Secretions of the two *Ponticulothrips* species (Thysanoptera: Phaeothripidae). *Applied Entomology and Zoology*, 30 509-519.

Suzuki, T., Haga, K., Tsutsumi, T. and Matsuyama, S. (2000) Chemical analysis and

composition of acid components of anal secretions of idolothripine thrips. *Journal of Chemical Ecology*, 26 2449-2458.

Suzuki, T., Haga, K., Tsutsumi, T. and Matsuyama, S. (2004) Analysis of anal secretions from phlaeothripine thrips. *Journal of Chemical Ecology*, 30 409-423.

Svatoš, A., Kalinová, B., Hoskovec, M., Kindl, J., Hovorka, O. and Hrdý, I. (1999) Identification of a new lepidopteran sex pheromone in picogram quantities using an antennal biodetector: (8*E*,10*Z*)-tetradeca-8,10-dienal from *Cameraria ohridella*. *Tetrahedron Letters*, 40 7011-7014.

Tamhankar, A.J., Gahukar, R.T., and Rajendran, T.P. (2000) Pheromones in the management of major lepidopterous and coleopterous pests of cotton. *Integrated Pest Management Reviews*, 5 11-23.

Teerling, C.R., Pierce, H.D., Borden, J.H. and Gillespie, D.R. (1993) Identification and bioactivity of alarm pheromone in the western flower thrips, *Frankliniella occidentalis*. *Journal of Chemical Ecology*, 19 683-697.

Terry, L.I. and Gardner, D. (1990) Male mating swarms in *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Journal of Insect Behaviour*, 3 133-141.

Terry, I. and Schneider, M. (1993) Copulatory behaviour and mating frequency of the western flower thrips, *Frankliniella occidentalis* (Insecta: Thysanoptera). *Advances in Thysanopterology*, 4 339-354.

Terry, L.I. (1995) Competition of western flower thrips males: effects of density on behavior. In: *Thrips Biology and Management, NATO ASA Series A276*. Edited by:

B.L. Parker, M. Skinner and T. Lewis. Plenum Press, New York, USA. pp. 433-438.

Terry, L.I. and Dyreson, E. (1996) Behavior of *Frankliniella occidentalis* (Thysanoptera: Thripidae) within aggregations, and morphometric correlates of fighting. *Annals of The Entomological Society of America*, 89 589-602.

Terry, I., Moore, C.J., Walter, G.H., Forster, P.I., Roemer, R.B., Donaldson, J.D. and Machin, P.J. (2004) Association of cone thermogenesis and volatiles with pollinator specificity in *Macrozamia* cycads. *Plant Systematics and Evolution*, 243 233-247.

Teulon, D.A.J., Hollister, B., Butler, R.C. and Cameron, E.A. (1999) Colour and odour responses of flying western flower thrips: wind tunnel and greenhouse experiments. *Entomologia Experimentalis et Applicata*, 93 9-19.

Tommasini, M.G. and Maini, S. (2002) Thrips control on protected sweet pepper crops: enhancement by means of *Orius laevigatus* releases. *Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera*, 249-256.

Tschuch, G., Lindeman, P. and Moritz, G. (2008) An unexpected mixture of substances in defensive secretions of the tubuliferan thrips, *Callocccithrips fuscipennis* (Moulton). *Journal of Chemical Ecology*, 34 742-747.

Tsumuki, H., Ishida, H, Yoshida, H., Sonoda, S., Izumi, Y. and Murai, T. (2007) Cold hardiness of adult western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Applied Entomology and Zoology*, 42 223-229.

Ullman, D.E., Sherwood, J.L. and German, T.L. (1997) Thrips as vectors of plant pathogens. Chapter 14. In: *Thrips as crop pests*. Edited by: T. Lewis. CAB Interna-

tional, Wallingford, UK. pp. 539-565

Ullman, D.E. (2007) Thrips vectors and tospoviruses: on the edge of adaptation. *Journal of Insect Science*, 7 28.

van Lenteren, J.C. (2004) Foreword. In *The manual of biocontrol agents. Third edition of the biopesticide manual*. Edited by: L.G. Copping. British Crop Protection Council, Alton, UK. p. *v*.

van Rijn, P.C.J., Mollema, C. and Steenhuis-Broers, G.M. (1995) Comparative life history studies of *Frankliniella occidentalis* and *Thrips tabaci* (Thysanoptera: Thripidae) on cucumber. *Bulletin of Entomological Research*, 85 285-297.

Walbank, M.H. (1996) The sensory biology of the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) in relation to host location. Ph.D. thesis, University of Birmingham, Birmingham, UK.

Wilson, E.O. (1971) Communication: alarm and activity. Chapter 12. In: *The insect societies*. By: E.O. Wilson. Harvard University Press, London, UK. p. 233-246.

Whittaker, R.H. and Feeny, P. (1971) Allelochemicals: chemical interactions between species. *Science*, 171 757.

Zabaras, D., Wyllie, S.G., Spooner-Hart, R.N. and Tronson, D. (1999) Semiochemicals of rose aphid, black citrus aphid (Hemiptera: Aphididae) and greenhouse thrips (Thysanoptera: Thripidae). Australian Zoologist, 31 403-409.

Chapter 2

General materials and methods

2.1 Introduction

This section provides detailed information on the general methodology used for the experiments presented in this thesis. It is intended to be comprehensive although additional information may be found within specific experimental chapters.

2.2 Thrips rearing

F. occidentalis can be reared in an artificial environment in a variety of ways. Mixed sex, mixed age populations can be reared on caged host plants with relative ease. Populations of single sex thrips can also be obtained by rearing virgin females and removing unfertilised haploid male progeny. This is ordinarily achieved using plastic ring cages (Murai, 1990) with an appropriate substrate. For the purposes of this research project thrips were reared on potted chrysanthemum within several self-contained rearing cages. This method allows for the rearing of large numbers of mixed aged thrips with com-

parative ease. This method has been used at Keele previously and was adapted from Teulon (1992).

2.2.1 Rearing apparatus design

Chrysanthemums were introduced into 6 specially constructed perspex cages (600 × 430 × 430 mm)(Figure 2.1). The side walls of each cage were manufactured with transparent Perspex (Rubberfast Ltd., Fenton, UK). A UVA transmissible plastic sheet was used to cover the top of each cage in order to provide a more natural 'daylight'. The front wall of each cage (370 × 540 mm) was made into a removable panel to allow easy access. In an attempt to maintain constant humidity the front panel contained two incorporated vents (84 × 84 mm) and in an attempt to provide air throughflow, the back panel contained a 12VDC, 0.8W rotating fan (70 × 70 mm), (Papst-Motoren, St. Georgen, Germany). The base was left open and placed on double layer capillary matting (Vattex Black, Berrycroft Stores Ltd., UK). The capillary matting was cut to a size slightly larger than the area of the base of the cage (around 500 mm^2) and placed into a 525 × 525 mm black plastic tray. This allowed for plant watering without opening the cages and also served to provide a moist but not waterlogged cage base acting as a potential thrips pupation site.

Overhead 100Hz full spectrum lighting providing a visible light intensity of 10.8 Wm^{-2} and a UVA light intensity of 0.2 Wm^{-2} was placed directly above the cages (Activa 172 (58W), Sylvania Lighting International, West Yorkshire, UK (1.5 m length) in 4 strips, 240VAC) and the plants and thrips were subjected to a 16:8 light:dark photoperiod.

2.2.2 Chrysanthemum host plants

Mixed age, mixed sex *F. occidentalis* were reared within Keele University School of Life Sciences on a potted chrysanthemum culture. In an attempt to standardise host plants, every effort was made to use a specific cultivar variety of potted chrysanthemum, *Dendranthema* (*Chrysanthemum*) *grandiflora* Tzvelev, var. Yellow Princess Anne (Rydale Nurseries, Carlisle, UK), there were times when this was unachievable due to supply constraints and so commercially obtained chrysanthemums were used instead. New plants were added to the culture cage when flower heads had become severely damaged or the plants were desiccated. The approximate frequency of this was recorded to help ensure plants were in plentiful supply.

Chrysanthemums were placed in fours within each cage with the second oldest plant replacing the oldest plant at the back row of the four when new stock was added. The culture was maintained at 25 ± 2 °C providing an approximate egg to egg developmental time of 14 days (van Rijn *et al.*, 1995). Plants were ordinarily replaced once a week with remaining viable flowerheads being removed and placed in a central tray in the centre of the plant pots. This was done in an attempt to minimise larval and adult thrips loss from the colony with the removal of aged plant material. For the period 2004-2005 *Dendranthema* (*Chrysanthemum*) *grandiflora* Tzvelev, var. Swingtime (ADAS, Boxworth, UK) were also used for thrips culture.

2.2.3 Thrips culture

Several biotypes of *F. occidentalis* have been identified (Martin & Workman, 1994). The strain used for this work were obtained in 1997 from Horticulture Research International, Wellesbourne, UK (now Warwick University, HRI) and the population maintained as a colony. This strain was originally obtained from a population found in

a commercial UK crop. In an attempt to prevent the introduction into the culture of foreign *F. occidentalis* strains from external plant material, all new plants were visually inspected for thrips and thrips damage prior to their introduction into the culture. Thrips were sampled on a monthly basis and examined by dissecting microscope to ensure that the population consisted exclusively of *F. occidentalis*. Where appropriate a key was used to identify foreign thysanoptera species (Mound & Kibby, 1998). *Thrips spp.* were confirmed using Nakahara (1993). *Thrips nigropilosus* and *T. tabaci* were the only other thrips ever found to be present, with the former only being present in the glasshouse.

2.3 Thrips collection and handling

Thrips were collected when required from the chrysanthemum culture. In order to obtain either males or females, flower heads of infested chrysanthemums were gently tapped into a white plastic collection dish. Thrips were then manually aspirated using one of two types of aspirators.

A modified aspirator (E713 'Pocket Pooter', Watkins & Doncaster, Kent, UK) was used when collecting more than one individual thrips. This was a standard aspirator with a narrowed tip made from the addition of a 5 ml automatic pipette tip (Eppendorf, UK) with a widened 2 mm diameter hole in the tip. In order to prevent thrips inhalation by the operator a finer fabric gauze mesh (bridal veiling) than the one supplied was installed at the base of the suction tube (John Lewis, Cambridge, UK). The supplied aspirator plastic collection vessel was replaced with one of three types of compatible glassware. A corresponding sized glass cylindrical vessel (50 × 22 mm inside diameter (Watkins & Doncaster, Kent, UK)) was used for all thrips collection with the exception of the moving-air bioassay and the collection of thrips for filter disc entrainment. For

the moving-air bioassay (chapter 6) a reduced length glass vessel with a fluted edge was utilised instead (12 mm diameter at fluted edge rising to 20 mm diameter at base × 34 mm total height) enabling direct insertion into the thrips entry port. When exposing thrips to filter papers for prolonged semiochemical entrainment periods for use in the filter disc bioassay (chapter 4) reduced length glass vessels (25 × 22 mm, Scientific Glass Limited, Hanley, UK) were used in an attempt to minimise entrainment volumes.

When handling only one individual thrips, the thrips was gently transferred using a distilled water-moistened calligraphic paintbrush (Aquafine 2 AF62 Flat Shader, Daler-Rowney, UK) or aspirated using a custom made aspirator. This was constructed from the top of a 5 ml automatic pipette tip (Eppendorf, UK) which formed a 10 mm × 8 mm (inside diameter) cylinder. Into this cylinder a layer of gauze and a 15 cm tube (FEP grade, 4 mm inside diameter, Sigma-Aldrich, UK) was placed.

2.4 Treatment of glassware

In an attempt to ensure odour free operation of bioassays all glassware was immersed in 10% Teepol L (BDH laboratory supplies, Poole, UK) and rinsed through with large amounts of distilled water. Glassware was dried using acetone (AnalaR, 100035R, 99.5% purity, BDH laboratory supplies, Poole, UK) and where possible baked overnight at 180 °C in a laboratory oven. Care was taken to prevent glass fracture by avoiding placing glass directly on the metallic oven base.

All glass GC syringes were cleaned and rinsed-through thoroughly with hexane (n-hexane, pesticide residue analysis grade (1526764), BDH laboratory supplies, Poole, UK) prior to each use.

2.5 Storage of chemicals

All synthetic compounds, extracts and dilutions were stored as per MSDS guidelines where applicable. Dilutions were stored in flame-sealed glass ampules and kept in a freezer.

2.6 Bioassay conditions

All experiments were conducted in one of two designated constant temperature rooms. Temperature and humidity were monitored using multiple thermohygrometers (212-124, RS Components, Northamptonshire, UK) and these rooms were maintained at 25 ±2 °C.

2.7 Data processing and statistical analysis

Statistical tests were performed using Minitab for Windows Release 15.1 (Minitab Incorporated, USA) and SPSS Release 14 for Windows (SPSS Incorporated, USA). Parametric analyses were performed where appropriate and Anderson-Darling normality tests were used to confirm data normality. Where appropriate transformations were undertaken to enable parametric statistics to be used. Data were deemed to be statistically significant where $P<0.05$.

Dedicated chromatographic data acquisition software was used for obtaining all chromatograms (chapter 3). Chemical structures were illustrated where appropriate using ChemDraw Std 9.0.1 for Macintosh (CambridgeSoft EU, UK). All other graphics were created with Adobe Creative Suite (Adobe Systems Inc., San Jose, USA). All

illustrations and text were collated and typeset using TeXShop 2.15 for Macintosh (Free Software Foundation Inc., MA, USA) in conjunction with the LaTeX 2ε 2.09 document preparation system (Free Software Foundation Inc., MA, USA).

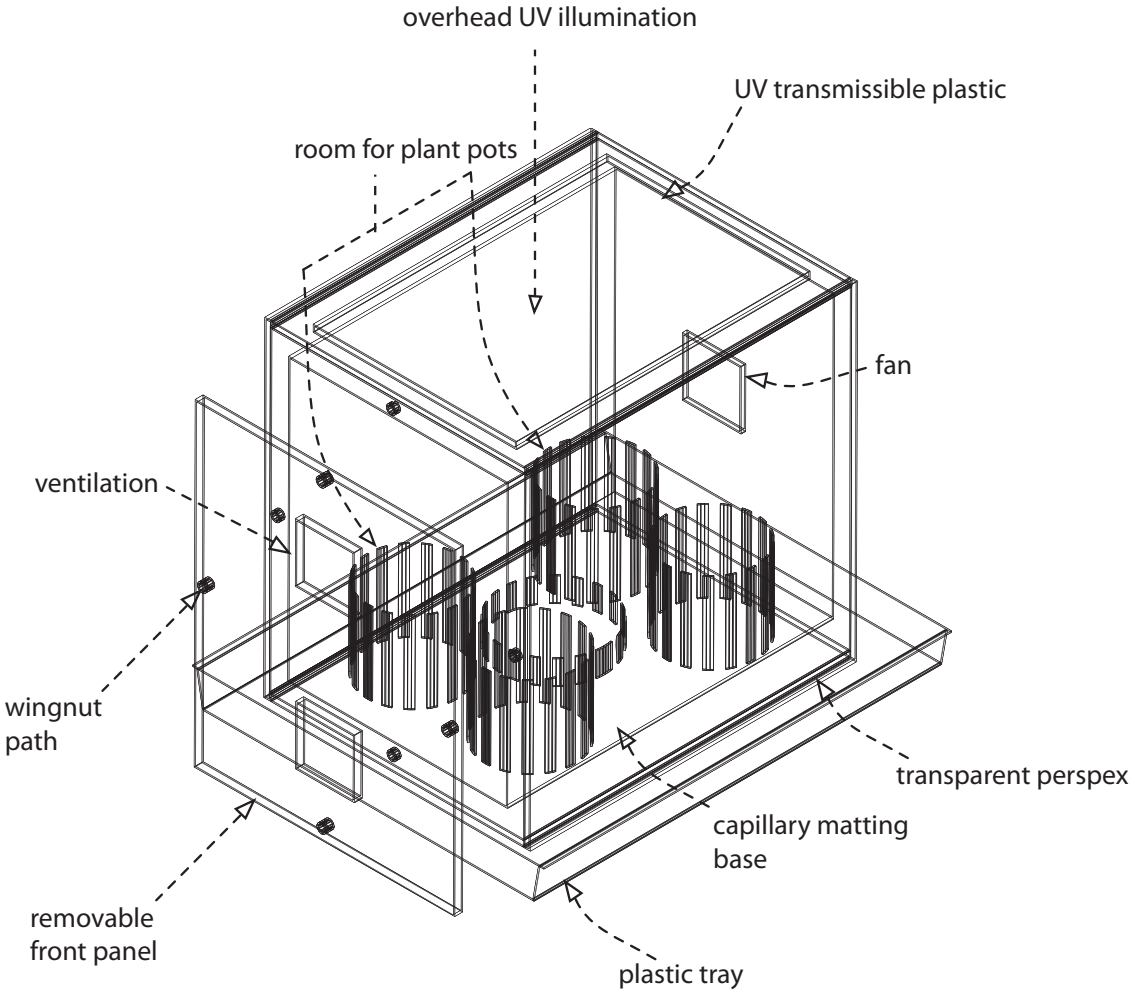

Figure 2.1: Individual rearing cage, isometric-left view

2.8 References

Martin, N.A., Workman, P.J. (1994) Confirmation of a pesticide resistant strain of western flower thrips in New Zealand. *Proceedings of the New Zealand Plant Protection Society, 1994.*

Teulon, D.A.J. (1992) Laboratory technique for rearing western flower thrips (Thysanoptera: Thripidae). *Journal of Economic Entomology*, 85 895-899.

Mound, L.A., Kibby, G. (1998) *Thysanoptera: An identification guide. 2nd edition.* CAB International, Wallingford, UK.

Murai, T. (1990) Rearing method and biology of thrips parasitoid, *Ceranisus menes*. *IOBC/WPRS Bulletin*, 13 142-146.

Nakahara, S. (1993) The genus *Thrips* Linnaeus (Thysanoptera Thripidae) of the New World. *U.S. Department of Agriculture, Technical Bulletin*, 1822 1-183.

van Rijn, P.C.J., Mollema, C. and Steenhuis-Broers, G.M. (1995) Comparative life history studies of *Frankliniella occidentalis* and *Thrips tabaci* (Thysanoptera: Thripidae) on cucumber. *Bulletin of Entomological Research*, 85 285-297.

Chapter 3

Measurements of production rates and elucidation of minor compounds

3.1 Introduction

As mentioned in chapter 1, pheromones can induce a variety of actions within individuals of the same species. Multiple pheromones can be produced with specific signalling roles.

Headspace solid phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (HS-SPME/GC-MS) has been used to identify two main male *F. occidentalis* headspace (HS) odour components, monoterpenoid esters, neryl (*S*)-2-methylbutanoate (Figure 3.3) $C_{15}H_{26}O_2$, MW= 238.37 and (*R*)-lavandulyl acetate

[0]This chapter forms part of the published manuscript Dublon, I.A.N., Hamilton, J.G.C. and Kirk, W.D.J. (2008) Quantification of the release rate of the aggregation pheromone of the western flower thrips, *Frankliniella occidentalis* (Pergande), using solid-phase microextraction (SPME). *Acta Phytopathologica Entomologica et Hungarica*, 43 249-256. DOI: 10.1556/APhyt.43.2008.2.6

(Figure 3.1) $C_{12}H_{20}O_2$, MW= 196.29 (Hamilton *et al.*, 2005). Both compounds were seemingly not detectable by whole body solvent extraction which suggests that the compounds are produced as required and not stored by males. In the above study, neryl (*S*)-2-methylbutanoate was shown to attract both sexes of *F. occidentalis* and it was suggested that the compound plays a role in male aggregation. Understanding the effect of density on *F. occidentalis* aggregation pheromone production rates is of key importance in enhancing the efficacy of field traps that utilise this compound.

SPME headspace extraction has been used to examine epicuticular hydrocarbon content of two species of social wasps *Vespa crabro* and *V. orientalis* (Moneti *et al.*, 1997). This technique has also been used with crushed thrips headspace volatiles in *Heliothrips haemorrhoidalis*. In this species, crushed headspace SPME was used to identify neryl and geranyl acetate. It was postulated that this was either part of an alarm response or due to the diet of the thrips (Zabaras *et al.*, 1999).

3.1.1 The effect of density on pheromone production

Dynamic pheromone production has been identified in several beetles and there is evidence of negative-feedback regulation. Certain nitidulid beetles, including *Carpophilus antiquus* have been shown to produce less aggregation pheromone in the presence of other individuals. Individually held male *C. antiquus* produce on average 25 times more (3*E*,5*E*,7*E*,9*E*)-6,8-diethyl-4-methyl-3,5,7,9-dodecatetraene per beetle than when held in groups containing 10 or more individuals (Bartelt *et al.*, 1993). The pine engraver beetle, *Ips pini* also appears to employ a negative feedback mechanism (Ginzel *et al.*, 2007). Antennectomised males that were therefore unable to detect ipsdienol pheromone produced significantly greater amounts of pheromone than intact control males and podectomised males. The antennectomised males produced on average approximately 4.5 times the amount of ipsdienol per male in comparison with the intact

control males (Ginzel *et al.*, 2007). Production of pheromones can often be costly in terms of selection pressures and so there is a selective advantage in regulating production (Raffa, 2001).

Male aggregation pheromone production may be affected by the presence of females, perhaps because there is no longer a need to attract them. In the grain borer beetle, *Prostephanus truncatus* aggregation pheromone production is suppressed in the presence of females (Smith *et al.*, 1996). Production rates decrease by over 9-fold, from 49.2 ng male^{-1} h^{-1} to 5.1 ng male^{-1} h^{-1} when the males are held in the presence of females (Smith *et al.*, 1996). This can be contrasted with the lesser grain borer beetle, *Rhyzopertha dominica* (F.) where the presence of conspecific female beetles did not inhibit production (Cork, *et al.*, 1991).

Neryl (*S*)-2-methylbutanoate is the only identified thysanopteran aggregation pheromone and little is known about production rates, although an estimate of 120 pg male^{-1} h^{-1} was suggested from an entrainment of over 50 males (Kirk & Hamilton, 2004). This rate may be affected by male density and it is possible that it is also affected by the presence or absence of a female, since this could influence their mating behaviour.

Violent escalations can be observed in male *F. occidentalis* when individuals are placed in close proximity and both (*R*)-lavandulyl acetate and neryl (*S*)-2-methylbutanoate are produced in varying amounts. The dynamics for the release of these two compounds has not been investigated and the relationship between the production of the two compounds and the number of males present has not been elucidated. Previous entrainment experiments suggested that there was little difference between the amount of (*R*)-lavandulyl acetate and neryl (*S*)-2-methylbutanoate production when entraining large numbers of males compared to small numbers (Hamilton, personal communication). If this is the case it is possible that (*R*)-lavandulyl acetate and neryl (*S*)-2-methylbutanoate production is attenuated at high densities.

3.1.2 Minor headspace components

Preliminary experiments showed that several additional compounds appear to be present in male headspace volatile entrainments that are absent in female entrainments. Identification of these minor compounds, which may act as synergists may prove worthwhile when developing commercial control products. Tentative identification by retention time and ionisation pattern suggests that these compounds may include nerol, (*E*)-ocimene, geraniol, and 3-carene (Hamilton, personal communication).

3.1.3 SPME fibre adsorption dynamics and potential affect on the live headspace entrainment process

When live male produced compounds are produced as headspace volatiles they are released into the sealed entrainment vessel and then adsorbed onto the SPME fibre. Over the time period of the entrainment process it is unclear if adsorption at the fibre site is linear and if efficiency varies with concentration. If the SPME fibre is very efficient at removing volatiles produced by males, this may mean that any aggregation pheromone produced by live males would be adsorbed too quickly for an accumulation of pheromone molecules in the air to occur. If this were the case the neryl (*S*)-2-methylbutanoate headspace level will remain relatively constant over time until saturation of the fibre ensues. If this were to occur it would be possible that males would not detect elevated levels of neryl (*S*)-2-methylbutanoate in their headspace and therefore there would be no production down-regulation. This would reduce the value of HS-SPME as a method for investigating the effects of density on *F. occidentalis* pheromone production.

3.1.4 Experimental aims

This chapter examines the effect of thrips density on neryl (*S*)-2-methylbutanoate production. Live males were entrained at two different densities in the presence or absence of a female and neryl (*S*)-2-methylbutanoate detection levels were calibrated against two neryl (*S*)-2-methylbutanoate standards entrained in the same way.

This chapter also attempts to identify some of the minor headspace components produced by live males. GC-MS is used for minor peak identification where the ionisation spectra of known synthetic standards are compared with those obtained from published spectral data and with those obtained from live SPME headspace entrainments.

The adsorption dynamics of SPME fibres were investigated with a view to determining if rapid adsorption at the fibre site is likely to reduce the headspace concentration of any male produced compounds.

3.2 Materials and methods

3.2.1 SPME fibre selection

SPME live insect headspace sampling was carried out using Supelco SPME fibre assemblies and their corresponding SPME holders. StableFlex fibres with a film-coated stationary phase consisting of divinylbenzene (DVB), Carboxen (CAR) and polymethylsiloxane (PDMS) were selected (57328-U, Supelco, Bellefonte, PA, USA) because the adsorbent bipolar DVB/CAR/PDMS stationary phase was suitable for molecular weights (MW) that fall within the range of neryl (*S*)-2-methylbutanoate (MW= 238) and (*R*)-lavandulyl acetate (MW= 196) (Supelco, 2005). See Figure 3.5 for a comparison of SPME stationary phases for various molecular weights. These fibres were exposed to

the thrips headspace sampling vessels using a dedicated holder set at maximum depth gauge (57330-U, Supelco, Bellefonte, PA, USA). This was also used during manual injection into the gas chromatograph. All fibres were initially conditioned by heating them in the GC injector block to 270°C for 1 h following the manufacturers guidelines (Supelco Data Sheet, 1999).

Temperature affects the analyte extraction recovery performance of the SPME fibre, and so all entrainments were carried out in a constant temperature room at 25 ± 2°C. A 6 h headspace entrainment time was used for all entrainments.

3.2.2 Gas Chromatography-Flame Ionisation Detection

Chromatographic separations were conducted using a Shimadzu GC-15A (Shimadzu Corp, Kyoto, Japan). The GC-15A injector system was fitted with a Jade SPME injector block (Alltech, UK) and a Shimadzu SPL-G15 split/split-less injector assembly (Shimadzu Corp, Kyoto, Japan). A dedicated low-volume SPME 0.75 mm internal diameter, 127 mm injector liner was used instead of the standard Shimadzu injector liner (26329,01 Supelco, Bellefonte, PA, USA) in order to increase the resolution and amplitude of the chromatograph retention peaks. Injector liner graphite ferrules were also renewed and conditioned prior to use in order to minimise the outgassing of graphite impurities. The GC-15A was fitted with a 30 m J&W DB-1 column (0.32 mm internal diameter, 0.25 μm film thickness, J&W, Agilent, UK). Column insertion distance was kept at 40 mm following the GC-15A operating instructions and Supelco guidelines. Detector blocks were unchanged and coupled to a flame ionisation detector. All SPME injections were conducted in splitless mode following Supelco guidelines. All other non-SPME, liquid samples were injected directly in 1 μl volumes using the Grob Split method. A split waiting time of 2 min was chosen throughout. SPME samples were desorbed for 5 min before removal of the fibre assembly.

Helium (British Oxygen Company, UK) was selected as a carrier gas and was set to enter the GC-15A at 3 kg cm^{-2} carrier head pressure in accordance with the operator guidelines. This was further regulated by adjustment of the instrument giving a constant 3 kg cm^{-2} primary flow pressure with 0.5 kg cm^{-2} capillary pressure. The FID used zero-grade air (British Oxygen Company, UK) and hydrogen (British Oxygen Company, UK) at 0.4 and 0.6 kg cm^{-2} respectively.

3.2.3 Temperature programme and run time

All chromatographic acquisitions were obtained using a set temperature programme. In a 26 min runtime, the oven temperature was first maintained at 45°C for 0-2 min. Following this it was set to rise at a rate of 15.77°C min^{-1} to 250°C. This was maintained isothermally for three min (15-18 min). Following this it was set to rise at a rate of 8.33°C min^{-1} for the period 18-21 min to a final temperature of 275°C. This temperature was then held isothermally for five min (21-26 min). The injection block was maintained at 180°C throughout with the detector block remaining at 250°C.

3.2.4 Electronic acquisition

The 0-1 V FID output from the Shimadzu GC-15A was coupled to a Waters SAT/IN bus interface (Waters Chromatography, Watford, Hertfordshire) and a Shimadzu CR5-A automatic integrator (Shimadzu Corp, Kyoto, Japan). A dedicated computer workstation running Millennium 2010 Chromatography Manager version 2.10 (Waters Chromatography, Watford, Hertfordshire) was used to acquire data from the GC.

3.2.5 Chemicals and synthesis of neryl (*S*)-2-methylbutanoate

Synthetic neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate standards were synthesised at the University of Greenwich using the method described by Hamilton *et al.*, (2005). Neryl (*S*)-2-methylbutanoate was selected as an external standard at 10 ng and 100 ng respectively. Both compounds were used to confirm retention times on SPME entrained peaks.

The use of an internal standard with similar properties and extraction characteristics to neryl (*S*)-2-methylbutanoate would have been more accurate but this was not done because of the potential effects of such a compound on the behaviour of the thrips.

3.2.6 Thrips aspiration and transfer

Mixed-age adult males were carefully aspirated in required quantities as described in 2.3 and immediately anaesthetised using a gentle 10 s stream of CO_2 (British Oxygen Company, UK). These were then carefully transferred using a modified thistle funnel (which had a reduced diameter tip) to a flame-cleaned entrainment vessel (0.2 ml, Gold grade glass, silanized, 02-MTVWG, Chromacol, Welwyn Garden City, UK) and sealed with two layers of high grade polytetrafluoroethylene (PTFE) tape (Teflon Z104388, Sigma-Aldrich, UK). Thrips numbers were verified in situ using a dissecting microscope (Olympus SZ40, Olympus, UK). In each entrainment the SPME fibre assembly was inserted through the PTFE tape and the fibre exposed. Each entrainment was conducted at 25°C in a constant temperature room with surrounding florescent low intensity overhead lighting. Males were observed to be engaging in patrolling and fighting behaviour as described by Terry & Gardener (1990).

3.2.7 External standard entrainment

As illustrated in Figure 3.6, live-insect SPME headspace entrainments were conducted using two identical aged conditioned SPME fibres with 5 and 15 males respectively. These trials were repeated with the addition of 1 female. For the external standard a droplet of 1 μl synthetic neryl (*S*)-2-methylbutanoate in hexane at 10 ng μl^{-1} was placed into the entrainment vessel in place of live individual thrips. All injections were made using a hexane-cleaned 10 μl GC syringe (Hamilton, Reno, Nevada, USA). This process was also carried out using 1 μl synthetic neryl (*S*)-2-methylbutanoate in hexane at 100 ng μl^{-1}.

3.2.8 SPME fibre performance

In a separate experiment 1 μl caryophyllene ((-)trans-caryophyllene, C-9653 Sigma-Aldrich, UK) at 10 ng μl^{-1} was produced as an internal standard. This compound was then headspace entrained using two identical aged fibres for a short time period (1 h), using the method described in 3.2.7. Over the course of a day, several 1 μl caryophyllene standard entrainments were carried out with each fibre in-turn and then with both fibres simultaneously. In order to allow SPME fibre headspace adsorption efficiencies to be investigated, GC-MS obtained peak areas from fibres entrained individually were compared with each other and moreover against peak areas obtained for each fibre when entrained simultaneously. When awaiting thermal desorbtion after the entrainment, SPME fibres were stored within a flamed pasteur pipette with its tip flame-sealed and its base covered with a layer of PTFE tape. Four repeats of the same experiment were carried out over four days.

3.3 Identification of minor headspace compounds using GC-MS

Standard solutions of synthetic compounds were sourced and injected on a Hewlett Packard 5890 series II plus (Agilent, UK) coupled to a quadrupole mass selective detector, HP 5972A (Agilent, UK). Identification of eluted compounds was done by comparing total ion chromatograms (TIC) and mass spectral fragmentation patterns with external standards and two databases (NIST spectral database version (Chemstation C.0200, NBS75K.L) and MassFinder 2.3.1.1: Mass Spectral Library "Terpenoids and Related Constituents of Essential Oils" 2001 (Dr Hochmuth, Scientific Consulting, Hamburg, Germany)).

3.3.1 Synthetic chemicals

10 ng μl^{-1}Standards were produced from 3-Carene (Sigma-Aldrich, UK), geraniol (Gold Grade 98%, Sigma-Aldrich, UK), (+)-limonene (97%, Sigma-Aldrich, UK), Nerol (Sigma-Aldrich, UK) and Ocimene (Isomeric mixture IPC151353 kindly provided by International Flavors & Fragrances Inc., Benicarló, Spain).

3.3.2 Gas Chromatography-Mass Spectrometry

Liquid standards and SPME headspace samples were introduced via a Merlin Microseal (Thames-Restek, UK) septumless injector maintained at 180°C. SPME samples were injected using a dedicated narrow bore injector sleeve (0.75 mm, Supelco, UK). A DB-5MS column was used for separations (30 m × 0.25 mm i.d., 0.25μm film coating). The column oven was programmed with a 2 minute isothermal at 40°C and a subsequent

10°C min^{-1} rise to 250°C. This was further maintained for 10 minutes.

The mass selective detector was operated in electron impact (EI) mode at 70 eV and 180°C.

3.4 Results

3.4.1 Neryl (*S*)-2-methylbutanoate

Male headspace SPME entrainment results are presented in Figure 3.7. Subsequent analysis and confirmation with a standard showed the presence of neryl (*S*)-2-methylbutanoate (retention time, t_R= 13.2 min). Neryl (*S*)-2-methylbutanoate was detectable in all of the entrainments whilst (*R*)-lavandulyl acetate was only present (t_R= 10.6 min) in some of the 15-male entrainments.

There was a significant difference in detectable peak area between a 5-male and a 15-male entrainment (ANOVA, $F_{1,15}$=22.45, P<0.0001) (Figure 3.7). There was no significant effect on detectable peak area when a female was present (ANOVA, $F_{1,15}$=0.01, P=0.93) and this was the case at both densities as there was no significant interaction between density and presence/absence of a female (ANOVA, $F_{1,15}$=0.61, P=0.45). Given that there was no significant effect of the female on detectable peak area it is possible to combine the data with and without a female for each of the two male entrainment densities. There was once again a statistically significant difference in detectable peak area between the male densities (2-sample t-test, $t_{(17)}$= 4.92, P<0.0001).

3.4.2 (*R*)-lavandulyl acetate

This compound was not detectable at the lower male density (Figure 3.7). In 15-male entrainments there was no significant difference in detectable peak area with and without the addition of a female (2-sample t-test, $t_{(7)}$= 0.25, P=0.81).

3.4.3 Synthetic neryl (*S*)-2-methylbutanoate standards

As expected, there was a significant difference in detectable peak area between 10 and 100 ng standards (ANOVA, $F_{1,6}$=36.26, P=0.001). A 10-fold increase in dose produced a peak area that was increased 7-fold. It is possible that at 100 ng the fibre is saturated but this is unlikely given the technical properties of the SPME fibre.

The observed 7-fold increase is not significantly different from a linear 10 fold increase (ANOVA, $F_{1,6}$=2.51, P=0.164). This suggests that we are observing a linear peak area response with dose.

3.4.4 Production rates and quantification

Detectable neryl (*S*)-2-methylbutanoate produced per male at the two densities was significantly different and higher at the 15-male density (ANOVA, $F_{1,17}$=6.61, P=0.020). Although there was a slight deviation from normality in these data when using the Anderson Darling normality test (P= 0.036, A^2=0.78), subsequent square root transformation normalised the residuals and confirmed the significance (ANOVA, $F_{1,17}$=9.84, P=0.006).

3.4.5 External standards compared with live entrainments

Using the entrained 10 and 100 ng neryl (*S*)-2-methylbutanoate average detected peak areas and comparing these with the average detected peak areas for 5 and 15 males it is possible to produce an estimate of pheromone production rate at the two densities. At a 5-male density an average of 0.1±0.055 (mean±SE) ng male h^{-1} is produced. This increased to 0.3±0.056 ng $male^{-1}$ h^{-1} at a 15-male density. These data confirm the estimate made by Kirk and Hamilton (2004), of 120 pg $male^{-1}$ h^{-1}.

3.5 SPME fibre adsorption dynamics

As is visible from Figure 3.8, when two fibres are entrained simultaneously for a short 1h period they adsorb around half the amount of standard as one fibre. Performance between each fibre is not statistically different when entrained individually (ANOVA, $F_{1,3}$=4.85, P=0.12), nor when entrained simultaneously (ANOVA, $F_{1,3}$=0.20, P=0.69). If observed peak areas of two fibres entrained simultaneously are multiplied by two, the resulting peak areas are not significantly different from peak areas for each fibre entrained singularly (2-sample t-test, $t_{(7)}$= 0.74, P=0.47).

Over a shorter time period (1 h), two fibres effectively adsorb half of what one fibre would adsorb. It is likely therefore, that any standard or male produced headspace compound is likely to be adsorbed by the fibre over a longer duration (6 h). As adsorption to the fibre will rapidly reduce concentration of headspace volatiles, this suggests that male produced neryl (*S*)-2-methylbutanoate does not reach elevated headspace concentrations during the entrainment process.

3.6 Minor compounds

Limonene (Figure 3.12) was apparently present at t_R= 9.112 min in one live male headspace entrainment. Obtained chromatograms for the limonene standard and the live male headspace entrainment are shown below (Figure 3.9). Mass spectra for published and entrained standards are shown in Figure 3.10. Entrained spectra are then compared with published spectra in Figure 3.11. Based on comparison with the published limonene standard there is a 93% probability that the observed compound is limonene. Based on comparison with the Keele 10 ng limonene standard this probability rises to 99%. In all incidences the molecular ion is present at m/z 136.

3.7 Discussion

There was a significant difference in neryl (*S*)-2-methylbutanoate detectable peak area between entrained males at the two different densities. This increase appears to be non-linear and unaffected by the addition of an individual female. It is important to note that neryl (*S*)-2-methylbutanoate production at the 5-male density is likely to be towards the lower detection limits of our system and that this may impact on the linearity of detectable peak area. Failure to detect neryl (*S*)-2-methylbutanoate in some 5-male entrainment instances will have reduced entrainment averages. In these instances it is possible that no compound, or too little, was produced and hence it was undetectable. Pilot experiments with less than 5 males sometimes produced detectable neryl (*S*)-2-methylbutanoate. Experiments at additional densities could be conducted to elucidate this.

No (*R*)-lavandulyl acetate was detected at the 5-male density. It is likely that we were below the lower detection limits of our system at this point. Kirk & Hamilton

(2004) also detected less headspace (*R*)-lavandulyl acetate than neryl (*S*)-2-methylbutanoate.

The use of known external neryl (*S*)-2-methylbutanoate standards entrained by the same method provided us with an indication of rate of production when these results were compared with the live entrainments. The estimated rate of 0.1-0.3 ng male^{-1} h^{-1} is similar to that of Kirk & Hamilton (2004).

Fibre performance and GC performance are also likely to affect entrainment results. Whilst every care was taken to use comparable age and condition fibres it is accepted that performance decreases over time when using StableFlex fibres. It is unlikely that the fibre was saturated when entraining 100 ng neryl (*S*)-2-methylbutanoate because much higher peak areas have been detected on our system with the fibres used.

It is likely that during the entrainment adsorption to the SPME fibre of neryl (*S*)-2-methylbutanoate reduced the detectable levels in the entrainment vessels thus negating any negative feedback mechanisms. In this case, production rate would have been unaffected by density. If the entrainment vessel was undisturbed and headspace sampling carried out towards the end of the volatile collection period then this may reduce this potential effect.

Mixed age males were used in this study and whilst there is no known evidence for age affecting *F. occidentalis* aggregation pheromone production, it is likely and thus a potential source of variability. Coleopterans such as the pine engraver beetle, *I. pini* display variations in pheromone production with age (Miller et al., 1989).

The majority of pheromone quantification studies are of the amounts in the bodies of individual insects, not the rate of production. The observed rate of aggregation pheromone production in male western flower thrips is notably lower than in some larger insects, such as larger borer beetles *P. truncatus*, which is around 3 mm in

length and produces 5.1-49.2 ng male^{-1} h^{-1} (Smith et al., 1996).

Limonene was identified as a minor compound present in *F. occidentalis* headspace. This is a new finding and it would be interesting to explore the role of this compound when added to neryl (*S*)-2-methylbutanoate. Unfortunately production levels of the minor compounds appear to vary quite substantially and as a result it is unclear if these compounds would necessarily increase trap catch when added as a synergist.

Identifying potential sources of limonene in chrysanthemum leaves and petals could easily be achieved using solvent plant extraction and GC-MS. This would help in deciding if this compound was present as a contaminant. Limonene occurs in *Dendranthema boreale* leaves (Kim, 1997).

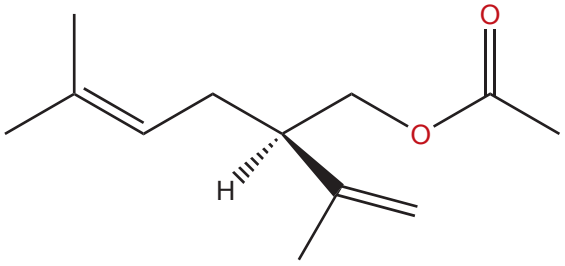

Figure 3.1: Chemical structure of (*R*)-lavandulyl acetate, (*R*)-5-methyl-2-(prop-1-en-2-yl)-hex-4-enyl acetate

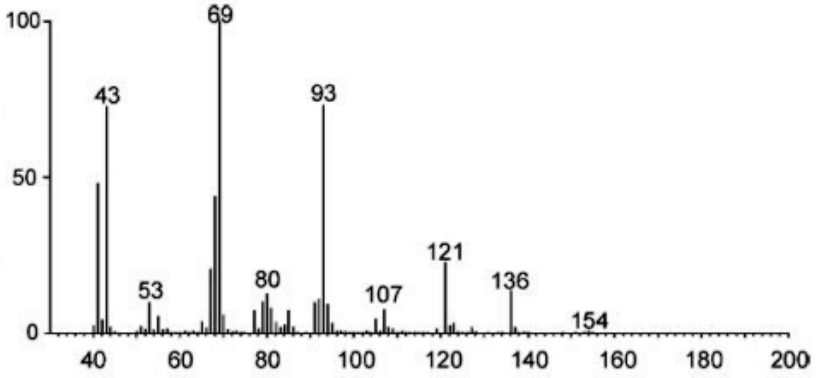

Figure 3.2: Mass spectra for (*R*)-lavandulyl acetate, (*R*)-5-methyl-2-(prop-1-en-2-yl)-hex-4-enyl acetate [From: Hamilton *et al.*, 2005]

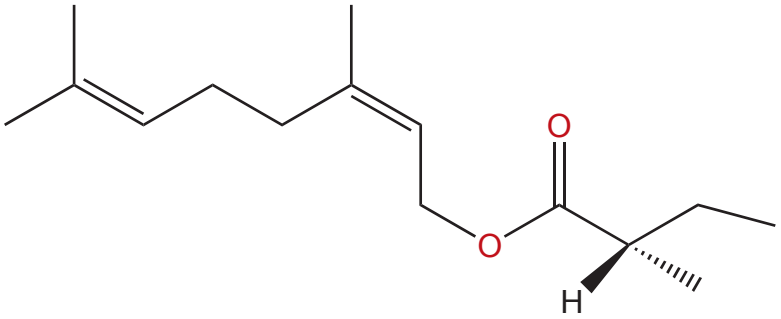

Figure 3.3: Chemical structure of neryl (*S*)-2-methylbutanoate, (*S*)-(*Z*)-3,7-dimethyl-2,6-octadienyl 2-methylbutanoate

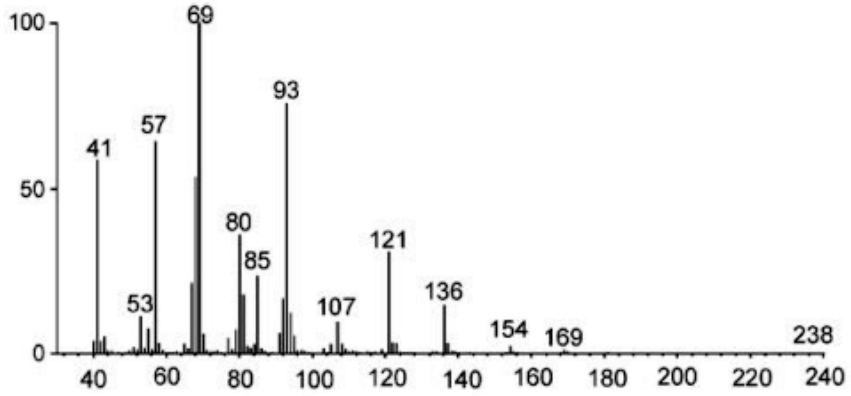

Figure 3.4: Mass spectra for neryl (*S*)-2-methylbutanoate, (*S*)-(*Z*)-3,7-dimethyl-2,6-octadienyl 2-methylbutanoate [From: Hamilton *et al.*, 2005]

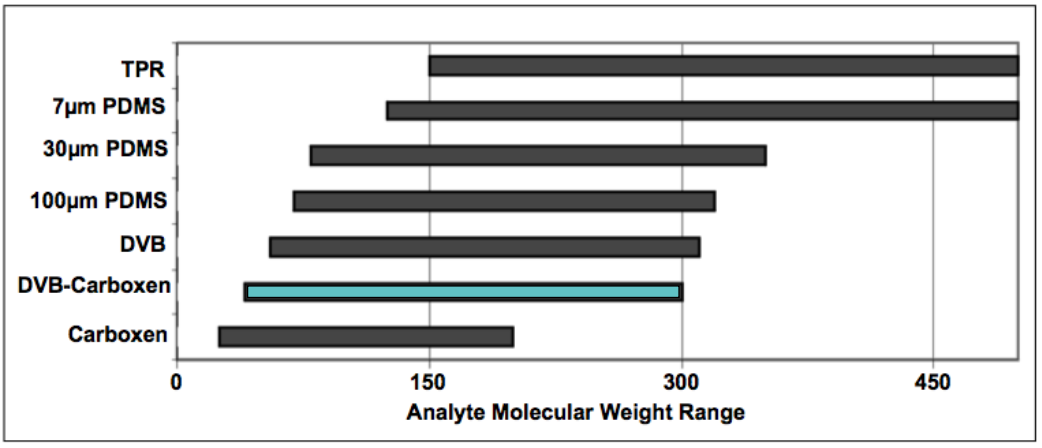

Figure 3.5: Molecular weight range for SPME fibres [Supelco, 2005]

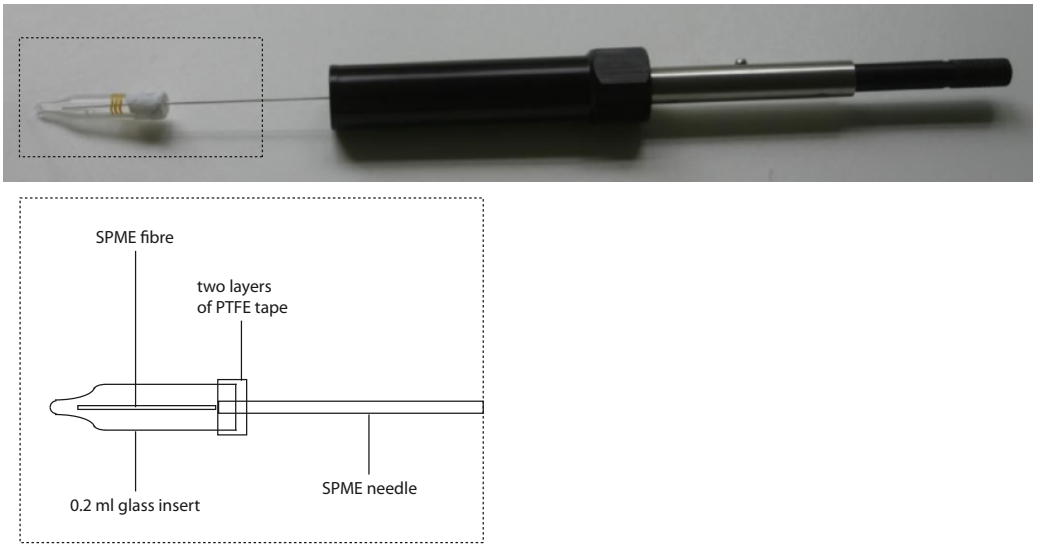

Figure 3.6: HS-SPME fibre assembly sampling setup [Photo: Dublon, 2007]

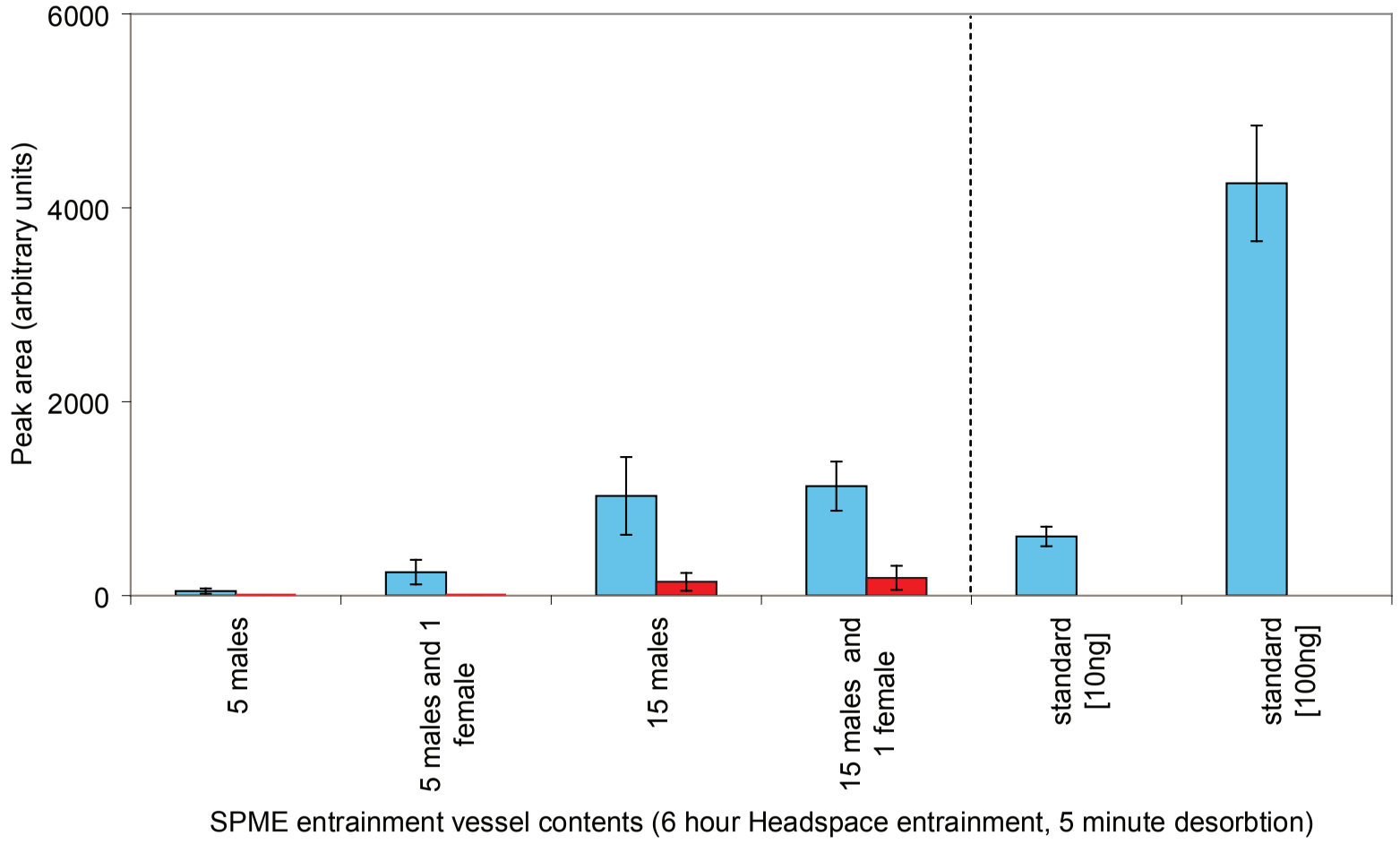

Figure 3.7: Mean peak areas from live insect headspace entrainments and synthetic neryl (*S*)-2-methylbutanoate standards. Bars denote mean ±SE. Key: blue bars are for neryl (*S*)-2-methylbutanoate and red bars are for (*R*)-lavandulyl acetate.

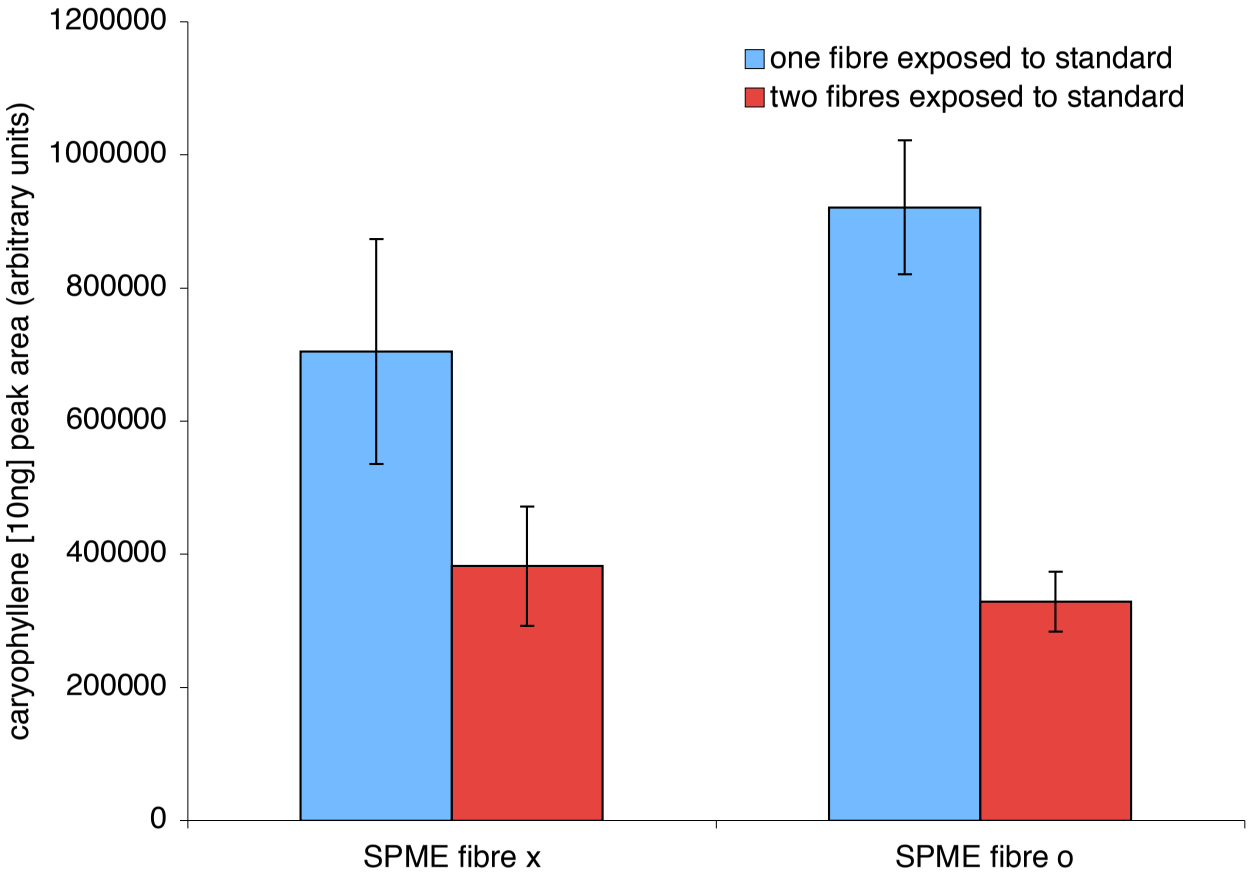

Figure 3.8: Mean peak areas from 1 μl 10 ng μl^{-1} SPME caryophyllene standard SPME entrainments. Two fibres (x and o) were exposed for 1 h to the standard. This was done individually and then repeated with the two fibres entraining the standard simultaneously. Bars denote mean $\pm$SE. Detected peak areas between fibres x and o are not significantly different.

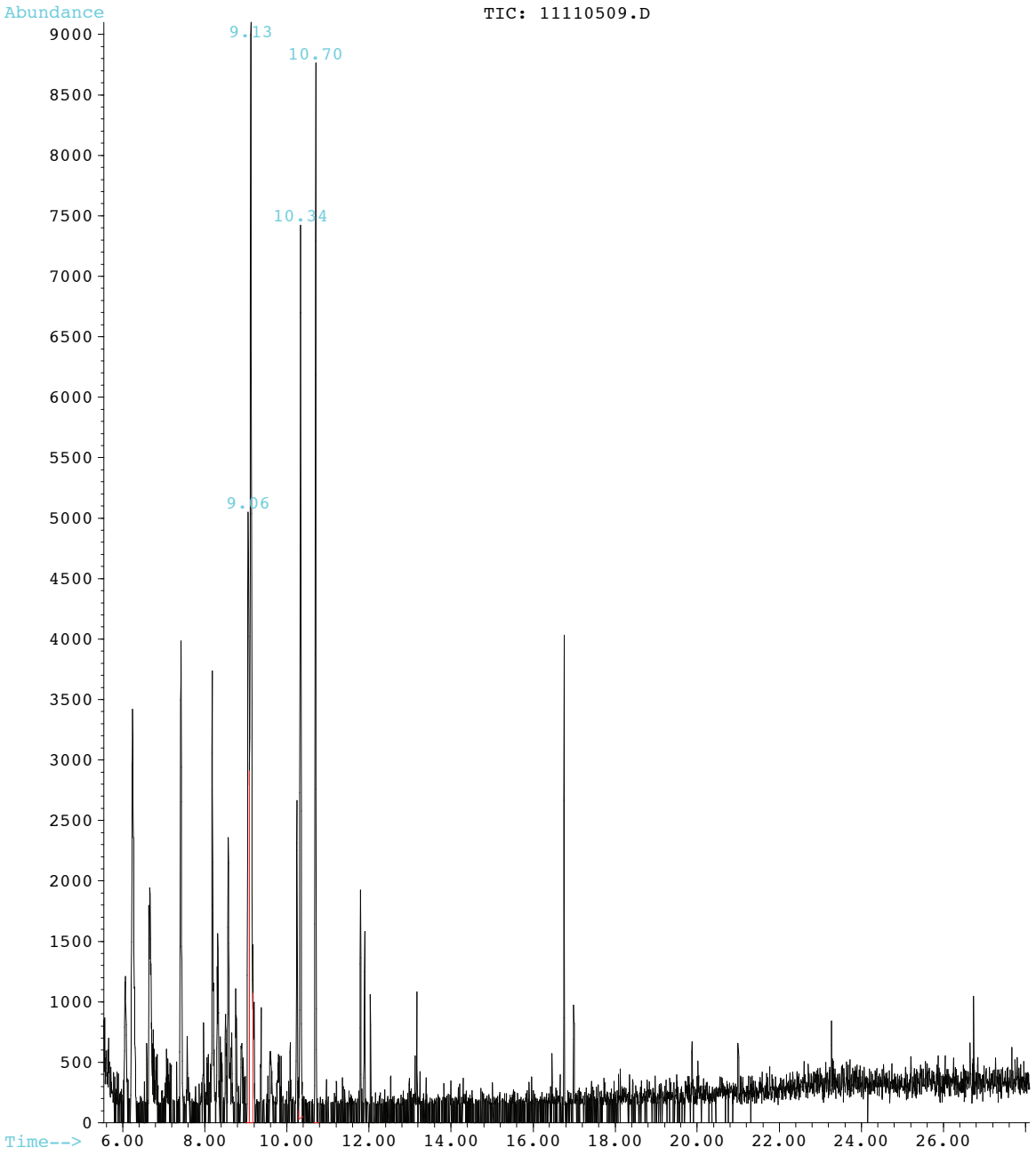

Figure 3.9: Identification of minor male-produced compounds: Total ion chromatogram from a seven hour headspace entrainment of 9 male and 1 female *F. occidentalis*. Of interest is the peak at t_R= 9.125 min which has a similar retention time to limonene (1-methyl-4-(1-methylethenyl)-cyclohexene) t_R= 9.112 min.

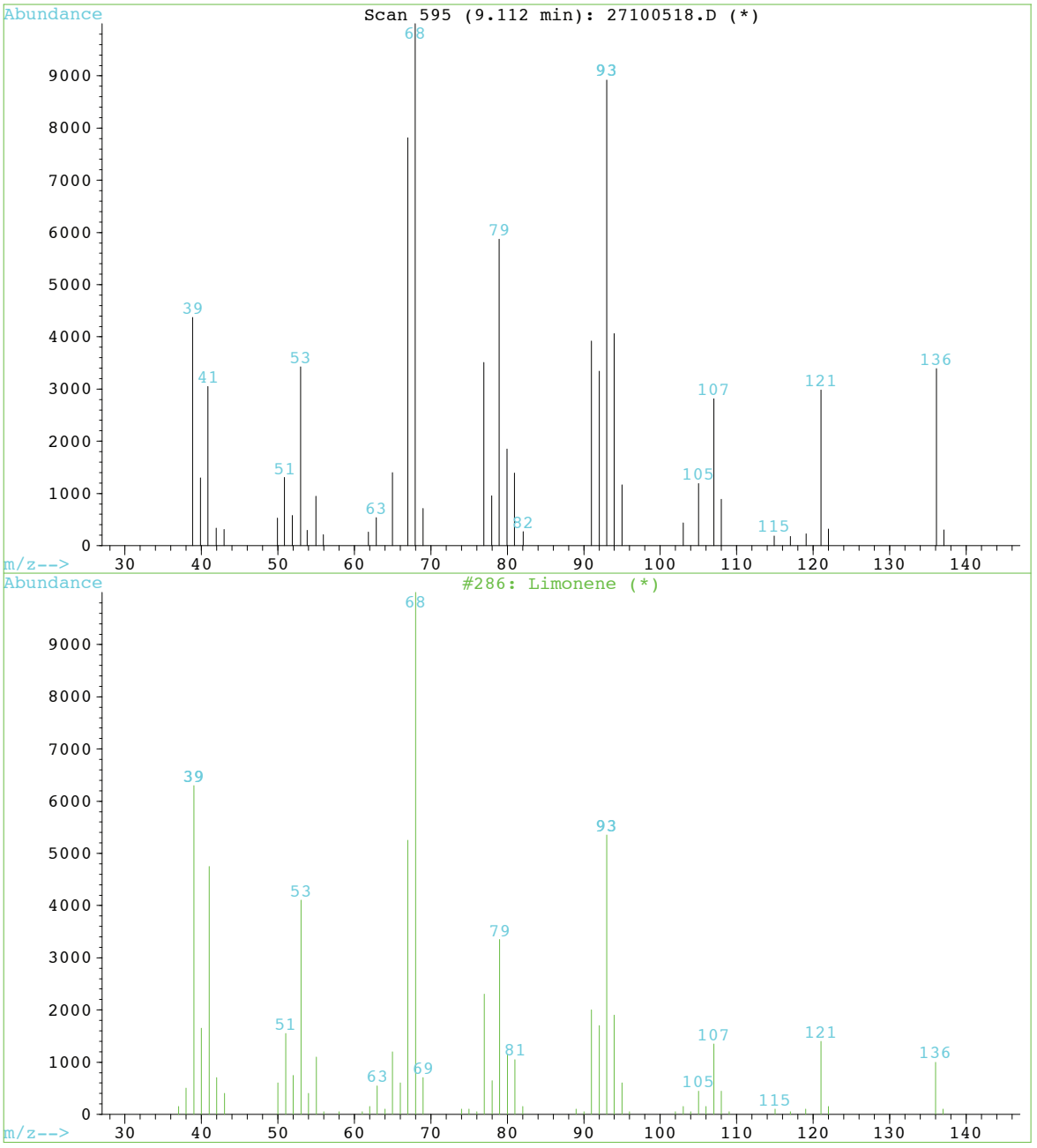

Figure 3.10: Identification of minor male-produced compounds: mass spectra for a prepared 10 ng limonene (1-methyl-4-(1-methylethenyl)-cyclohexene) standard. The topmost spectra (black) shows limonene from a standard injection, t_R= 9.112 min. The lower spectra (green) is the published entry for limonene (MS Library "Terpenoids and Related Constituents of Essential Oils", 2001).

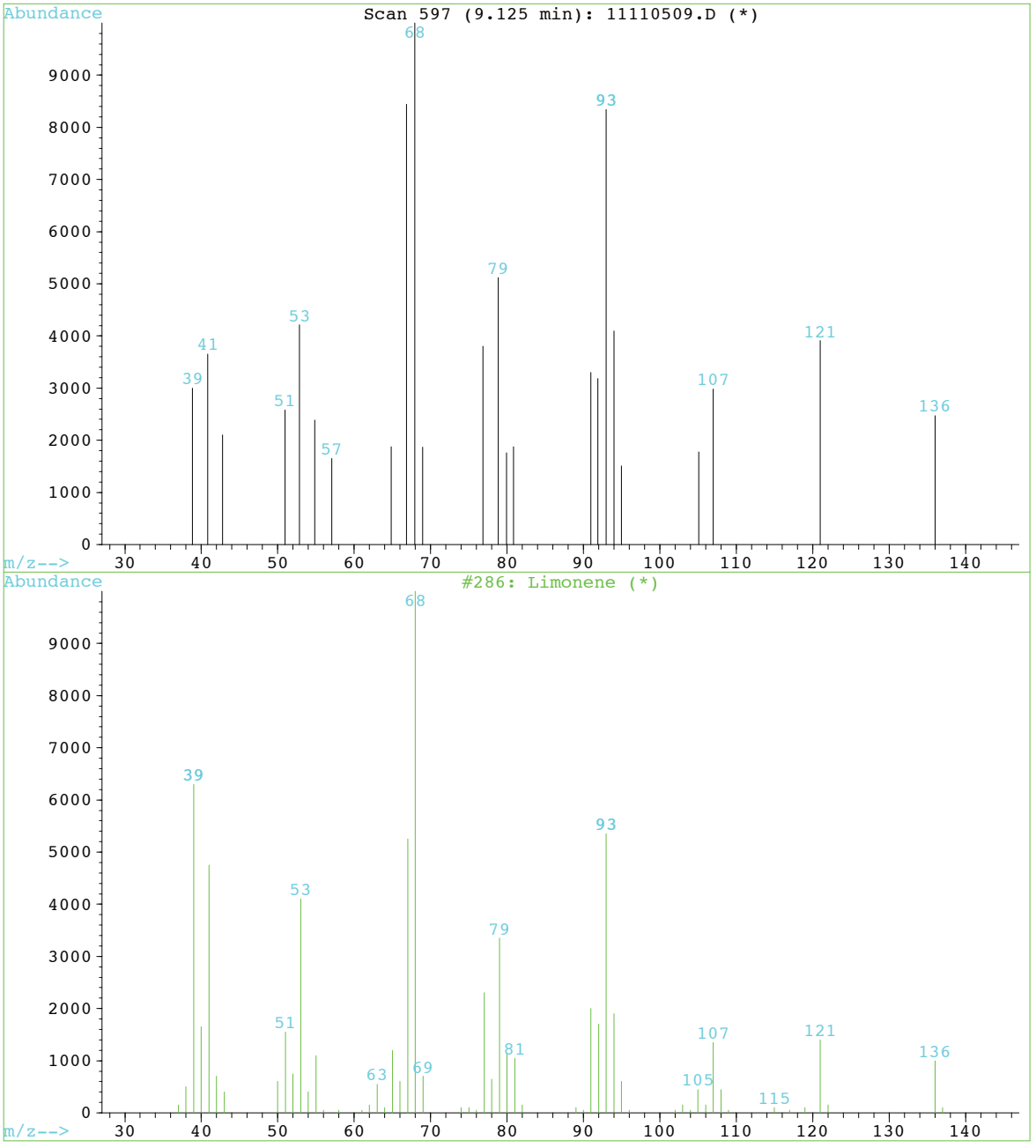

Figure 3.11: Identification of minor male-produced compounds: mass spectra for limonene. The top spectra (black) shows detected limonene at t_R= 9.125 min, from a seven hour headspace entrainment of 9 male and 1 female *F. occidentalis*. The lower spectra (green) is the entry for limonene (MS Library "Terpenoids and Related Constituents of Essential Oils", 2001). Based on comparison with the library standard there is a 93% probability that the observed spectra is limonene and this rises to 99% when the observed entrained spectra is compared with the Keele 10 ng limonene standard.

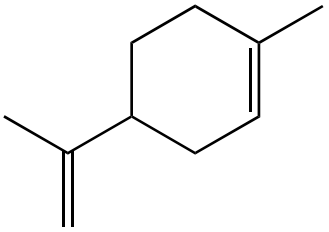

Figure 3.12: Chemical structure of limonene (1-methyl-4-(1-methylethenyl)-cyclohexene)

3.8 References

Bartelt, R. J., Seaton, K. L. and Dowd, P. F. (1993) Aggregation pheromone of *Carpophilus antiquus* (Coleoptera: Nitidulidae) and kairomonal use of *C. lugubris* pheromone by *C. antiquus*. *Journal of Chemical Ecology*, 19 2203-2216.

Cork A., Hall D. R., Hodges R. J. and Pickett J. A. (1991) Identification of major component of male-produced aggregation pheromone of larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae). *Journal of Chemical Ecology*, 17 789-803.

Ginzel, M. D., Bearfield, J. C., Keeling, C. I., McCormack, C. C., Blomquist, G. J. and Tittiger, C. (2007) Antennally mediated negative feedback regulation of pheromone production in the pine engraver beetle, *Ips pini*. *Naturwissenschaften*, 94 61-64.

Hamilton, J.G.C., Hall, D.R., Kirk, W.D.J. (2005) Identification of a male-produced aggregation pheromone in the western flower thrips *Frankliniella occidentalis*. *Journal of Chemical Ecology*, 31 1369-1379.

Kim, J.-H. (1997) Variation of concentration of terpenes in *Chrysanthemum boreale*. *Korean Journal of Ecology*, 20 397-403.

Kirk, W.D.J. and Hamilton, J.G.C. (2004) Evidence for a male-produced sex pheromone in the western flower thrips *Frankliniella occidentalis*. *Journal of Chemical Ecology*, 30 167-174.

Miller, D. R., Borden J. H., Slessor, K. N. (1989) Inter- and intra-population variation of the pheromone, ipsdienol produced by male pine engravers *Ips pini* (Say) (Coleoptera: Scolytidae). *Journal of Chemical Ecology*, 15 233-247.

Moneti, G., Dani, F.R., Pieraccini, G. and Turillazzi, S. (1997) Solid-phase microextraction of insect epicuticular hydrocarbons for gas chromatographic/mass spectrometric analysis. *Rapid Communications In Mass Spectrometry*, 11 857-862.

Raffa, K. F. (2001) Mixed messages across multiple trophic levels: the ecology of bark beetle chemical communication systems. *Chemoecology*, 11 49-65.

Smith, J. L., Cork, A., Hall, D. R. and Hodges, R. J. (1996) Investigation of the effect of female larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), and their residues on the production of aggregation pheromone by males. *Journal of Stored Products Research*, 32 171-181.

Supelco Data Sheet, (1999) *T794123M Solid Phase Microextraction Fiber Assemblies*. Supelco, Bellefonte, PA, USA.

Supelco SPME Training Seminar Presentation (2005) *SPME Principles and Recent Developments 2005*. Presented by Roberto Ferrari at Culham Science Centre, Culham, Oxford, 11th October 2005. Supelco, Bellefonte, PA. USA.

Terry, L. I. and Gardner, D. (1990) Male mating swarms in *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *Journal of Insect Behavior*, 3 133-141.

Zabaras, D., Wyllie, S.G., Spooner-Hart, R.N. and Tronson, D. (1999) Semiochemicals of rose aphid, black citrus aphid (Hemiptera: Aphididae) and greenhouse thrips (Thysanoptera: Thripidae). Australian Zoologist, 31 403-409.

Chapter 4

Walking responses of adult females to male-derived compounds

4.1 Introduction

The effects of inter- and intra-specific semiochemicals on insect behaviour can be studied in the laboratory and the field using various bioassays and traps. Studying behaviour in this way can contribute to the understanding of chemically mediated insect interactions. Such interactions can then be exploited with a view to developing new or enhancing existing methods of insect control. Carefully regulated laboratory experiments can provide indications of natural behaviours exhibited in the field though they have some limitations in that they are ordinarily unable to mirror natural environments.

Male *F. occidentalis* produce an aggregation pheromone, neryl (*S*)-2-methylbutanoate (Hamilton *et al.*, 2005; chapter 3). This compound has been shown to attract both males and females in the field (Hamilton *et al.*, 2005; Gómez *et al.*, 2006) and it would be advantageous to replicate and further examine this response in the laboratory. Close

inspection of thrips behaviours could also provide valuable information with a view to enhancing trap efficacy.

4.1.1 Types of responses

Three behavioural processes assist insects in odour orientation (Shorey, 1973). Chemotaxis occurs where the insect aligns its body in the direction of the odour source. This response can be further divided into chemotropotaxis where the insect uses multiple organs to ascertain if the odour molecules reside to one side of the body or another and turns accordingly or chemoklinotaxis where the insect sways from side to side gradually reducing oscillations when it faces the chosen source (Finch, 1980).

Alternatively, responses can occur where the insect does not use the direction of the odour gradient but instead is stimulated to move at different rates (orthokinesis) or turn at different frequencies (klinokinesis).

As a third alternative, odours can act as sensitisers and cause the insect to orientate to another stimulus such as turning and flying upwind into an air current (anemotaxis). This was thought to be the primary method of orientation to a distant odour source (Finch, 1980). It has been argued that for phytophagous insects that behave anemotactically and fly upwind, assays should only be tested on insects in flight (Wright, 1958) though as is evident from the large number of papers describing laboratory walking assays there is convincing evidence that the walking response can provide a valid indication of the flight response.

Arrestment occurs when an arrestant chemical causes an aggregation with a mechanism involving either ortho- or klinokinesis (Dethier, 1960). Attraction is defined as an orientated movement towards an attractant source.

4.1.2 Behavioural responses in bioassays

Small-scale olfactometers are normally used to assay rates of movement and directional choice during walking with larger flight tunnels being used to assess the above whilst the insect is in-flight (Hare, 1998). Assays without air-flow are ordinarily used to examine behaviours over a short distance from the test source and are ordinarily restricted to demonstrating attraction as an orientated movement towards, or conversely, away from a source (Dethier, 1960).

Measurements in such assays involve analysis of movement and this can be achieved by scoring counts, for example movements to or away from a source, contacts, oviposition, number of grooming individuals or number of flight attempts. In addition quantification of response can be possible by recording time spent engaged in respective activities such as quiescence on source and time spent grooming. Where more than one individual is being observed, observations can be made of the group or of a specific individual.

In all bioassays consideration needs to be given to endogenous and exogenous factors that may affect outcome and so rigourous control methods are necessary (Hare 1998).

4.1.3 Existing walking bioassays

Several small scale walking bioassays have been developed for screening chemicals in insects. Most walking bioassays have one common limitation in that the insect is only able to walk in a still-air environment or against an airflow and not take-off and land. Further strengths and limitations of principal designs are briefly expanded upon below.

4.1.4 The Y-tube bioassay

The Y-tube olfactometer based on the type designed by Barrows (1907), as reviewed by Varley & Edwards (1953) is an accepted design of laboratory bioassay. The insect is able to enter a Y or T-shaped glass tube which has air flowing through it towards the base. Entering at the base of the Y the insect is able to choose to either remain still, move backwards, move forwards and at the junction of the arms and stem choose either arm of the Y. One of the two arms can be loaded at known concentrations with either intraspecific attractants such as pheromones, plant kairomone attractants, plant botanical repellants or live individuals. The insect is able to select its preferred odour and orientate itself towards it. When properly constructed and free of instrument bias the Y-tube olfactometer is therefore an excellent indication of a walking orientated response towards a substance. This technique has been used for numerous insects and for several thrips species including *F. occidentalis* as detailed in Koschier (2006). Whilst Y-tubes are widely reported in the literature it is commonplace for instruments to be made where the insect does not express a preference even if the tube is unbiased (W.J. de Kogel, personal communication, Wageningen 2004).

The original Y-tube bioassay used for *F. occidentalis* at Keele was successful in getting the thrips to discriminate between odours however various instruments made subsequently since 2002 although unbiased have failed to replicate this. After attempting and failing to get the thrips to discern between odours using existing Keele Y-tubes and subsequently a Y-tube kindly provided by CFR Christchurch, New Zealand, it was decided that a new type of bioassay with the capacity for a wider range of responses would be beneficial.

Y-tube bioassays are custom-manufactured according to insect size and can be manufactured in one of three ways and this difference is likely to affect the airflow at the junction of the Y and thus may affect the ability of the instrument to distinguish

between odours. Variation in manufacture may account for bias between seemingly identical instruments where there may be too much mixing of substances at the junction of the Y and hence a loss of response:

1) the whole instrument is made from three interconnecting glass pieces joined at the centre to form the Y. Connections were made so that the Y-tube provided a 90° junction angle between each arm. This method was used to make several bespoke Y-tubes for the phlebotomine sand fly *Lutzomyia longipalpis* at Keele. In addition a set of around 40, comparatively smaller bespoke *F. occidentalis* Y-tubes were also constructed in this way. Whilst many were unbiased there was only one 'working' tube that allowed the thrips to distinguish between odours. This tube ceased to function in this way and the reasons for this remain unclear. It appears that in such a Y-tube, two separate streams of airflow exit the base of the Y with little mixing at the junction.

2) the whole instrument is made from two interconnecting glass pieces with one piece being straight and the other curving around to from a U shape. The two are then joined. This process has created a successful *F. occidentalis* Y-tube at Wageningen Universiteit en Researchcentrum (WUR), Wageningen, Netherlands (W.J. de Kogel, personal communication) and a similar technique has provided a successful *F. occidentalis* Y-tube at CFR, Christchurch, New Zealand (M.M. Davidson, personal communication).

3) the whole instrument is made from two interconnecting glass pieces with one piece forming one entire length of one side of the Y and the other piece being straight and forming one of the arms. This piece is then joined subsequently.

Airflow systems vary between designs with some Y-tubes being designed to run from regulated air-flow entering at both arms of the Y whilst others employ pulsed air-flow with the air being drawn out from the base of the Y via a flowmeter. If this protocol difference is combined with varied exogenous factors (semiochemical release systems, varied air-flow sources, varied gas purifiers, varied temperature and varied

lighting conditions) then it is conceivable that there may be differences in individual responses when exposed to the same test compound.

Only two choices are possible in the Y-tube and should the insect fail to choose either arm within a specific timeframe it is ordinarily treated as a non-responder and thus often not included in subsequent analyses. This reduces the statistical resolving power of the device where an apparent 'attraction' could result from a random movement in one direction. Increasing the number of arms (see 4.1.6) and the number of replicates can reduce this possibility. In an ordinary Y-tube, two test components cannot be assayed at the same time without a blank control being present in one arm; without this it is impossible to discriminate between attraction to one arm and repellence to the other. Additionally the combination of odours at the junction may inhibit a response where attractiveness is lost (Hare, 1998).

As with all bioassays, the semiochemical concentrations and volumes used over the very small scale of the Y-tube also affect the attractiveness of the compound being assayed. This can lead to different conclusions as to the attractiveness of the compound if the dose being assayed is incorrect. Additionally if there is a slight difference in flow rates between the two arms, air from one direction can exceed that from another. The relationship between concentration resulting in either attraction or repellence is something that needs to be considered and it necessitates that test semiochemicals are tested over a range of different doses. Variations in concentration combined with time-of-day variations in response have been displayed in *Cycadothrips chadwicki* Y-tube responses (Terry *et al.*, 2007).

When separating an insect from its cohort and adding it to the assay, there is likely to be some interruption in natural behaviour and this may affect subsequent behaviour. Whilst this may not be as much of a concern for larger insects that are easier to handle this is a key consideration when using the Y-tube with *F. occidentalis*; as a result of the

insects small size, it is very difficult even with the utmost care and manual dexterity to state with certainty that the insect being introduced into the tube remains entirely unaffected by the transfer process.

4.1.5 Linear track olfactometers

Where insects are inclined to walk along the path of a straight line their responses can be assayed in a twin track olfactometer (Sakuma & Fukami, 1985). In this system insects walk upwards on a thin metal wire and make a choice at the junction when presented with two odour choices. This system has been used to measure chemotaxis and odour modulated anemotaxis for the Dictyopteran German cockroach, *Blattella germanica* (Sakuma & Fukami, 1985) and the Hemipteran aphid *Megoura viciae* (Hardie *et al.*, 1990). Whilst effective, these assays rely on the insect remaining on the wire and are difficult to construct.

4.1.6 Additional multiple choice assays

Multiple choice olfactometers have been designed with a view to increasing statistical power. A fan-shaped, five choice, 'multichoice olfactometer with air flow' is described by Burkholder (1970) which was used to assay black carpet beetle, *Attagenus megatoma* males when presented with female extract. Constructed from brass and glass and featuring flowmeter regulated air-flow this system allows for multiple zones with partly separated odour streams (Burkholder, 1970).

A four-choice Pettersson 'star' olfactometer system (Pettersson, 1970) has also been devised and is more commonly utilised. This device features several arms with flowmeter regulated air-flow and a central exhaust. Again there is relatively little mixing of

air-flows and there is less chance of a random choice being made as the insect is able to walk around and sample each air stream. A 'star' olfactometer has been used to screen *F. occidentalis* responses to volatiles obtained from fresh flowers (Shamshev *et al.*, 2003). In this assay modified from Pow *et al.*, (1999) a weak air stream is directed into the centre with one odour source placed around three controls.

Whilst these systems are statistically more powerful (in the 'star' the insect has only a 25% chance of randomly choosing the treatment odour where three controls are used, instead of the Y-tubes 50%) they are both exceedingly difficult to construct and ensure bias free operation. They can however be used to separate attraction from arrestment where the time spent by test individuals in each respective quarter is recorded.

4.1.7 Still-air bioassays

Still air bioassays can be used to examine behaviour over a short range. These assays are suitable for demonstrating attraction or repellence though care must be taken to ensure odour is not saturating the closed environment making orientation to the source less likely.

A still air bioassay made from closed petri dishes and glass tubes was used to assay responses to mustard oils (allyl isothiocyanate and phenyl isothiocyanate) of the vegetable weevil *Listoderes obliquus* larvae (Matsumoto, 1970). Test compounds were applied to a cotton cloth in the base of a small tube. This was left to evaporate for a prolonged period (and at an elevated temperature to aid evaporation) and four such tubes were placed facing each other, crossways per dish. Two tubes contained treatment odour and two control solvent alone. The arena was left for 1 h and the number of larvae present in the respective tubes recorded at this timepoint. In this design, arrival is recorded but departure recording was not possible and therefore not

taken into account.

To address this issue this assay was also modified to assay newly emerged *Delia antiqua* onion maggot larvae with odorous sulphurous compounds from plants (n-propyl disulphide and methyl disulphide) (Matsumoto, 1970). Two tubes were prepared as above and placed in a petri dish at right angles to one another. The number of larvae reaching the mouth of the tubes was recorded over a 10 min period.

Detailed information on behavioural changes such as arrestment and corresponding behaviours such as slowing or stopping (orthokinesis) or altered rate of turning (klinokinesis) (Kennedy, 1978) can be obtained in such a system by video observation or by coating the surface of the dish with a medium that allows for recording of trails. Agar has been used to record *Psila rosae* carrot rust fly larval tracks (Jones & Coaker, 1977). In larger insects such as ants, smoked glass (a soot coating is obtained from a candle or similar source) is often used to record movement (Gobin *et al.*, 2001; J. Billen, personal communication Keele, 2004).

Webster *et al.*, (2006) explored the attractive properties of filter discs exposed to male Kelly's citrus thrips, *Pezothrips kellyanus*. Filter discs were split into segments and exposed over a 60 min period to males, females or no individuals and subsequently frozen (for storage). Female thrips were exposed the following day to unfrozen discs and were found to be attracted only to male-exposed discs.

As more *P. kellyanus* were found to be in contact with male-exposed disc segments in comparison to non-exposed disc segments a similar experiment was devised for *F. occidentalis*. It was possible that male *F. occidentalis* exposed filter discs would retain more mixed age female thrips at specific timepoints than blank control discs. It was also possible to substantially modify this assay to enable the screening of potential attractant semiochemicals including neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate in a contact two-choice binomial system (as described in 4.3.2). The small size

of the petri dish allows for a walking response and limited flight is also possible.

4.2 Experimental aims

This chapter attempts to examine the behaviour of female *F. occidentalis* in a laboratory environment, using a simple assay that allows for a walking response. Male-derived compounds are assayed in this chapter and synthetic compounds assayed in chapter 5.

4.2.1 Behavioural assays

Assuming a contact response could be established, i.e. freshly exposed females make more or less contacts with a treatment disc in comparison with a control, it was hoped that this response could be further categorised in a no-choice system without a point odour source (see Chapter 5). It was hoped to differentiate arrestment from attraction and detect ortho and klinokinesis if present. In the event of such a behavioural response it was hoped to artificially recreate this response with application of synthetic semiochemicals (Chapter 5).

If females responded to male-exposed discs, it was hoped to solvent extract the male-derived compounds from the discs, re-apply the extract to new discs and show a response to the extract. Basic chromatographic techniques could then be used to attempt to detect any bioactive compounds.

4.3 Materials and methods

4.3.1 Obtaining filter discs exposed to live individuals

Individual cellulose filter discs (Whatman International Limited (1001020) grade 1, 2 cm diam.) were carefully placed into the base of a reduced length soda glass aspirator vessel (25 × 22 mm (length × diam.), Scientific Glass Limited, Hanley, UK). A specified number of thrips (ordinarily 15 mixed-age males) were then aspirated directly into the treatment vessel, or not in the case of the control. Each aspirator vessel was carefully detached from the aspirator and quickly covered with a stretched piece of 30 mm × 50.8 (2") mm length of Parafilm membrane (Parafilm M, Pechiney Plastic Packaging, WI, USA). As mentioned in 1.2 thrips possess 'punch and suck' mouthparts (Moritz, 1997). These allow for individuals to penetrate stretched Parafilm. It was therefore possible to provide a water source for the thrips during the disc exposure process. 100μl of distilled water was placed on the top of an initial Parafilm membrane (Finnpipette, Digital 40-200μl, Finland) and the entire surface was covered in an additional layer of stretched Parafilm. A water source was thus provided for both test and control exposure vessels.

Numbers of live individuals were checked *in situ* using a dissecting microscope (Olympus SZ40, Olympus, UK) prior to commencing the exposure process. The vessels were placed in a constant temperature room maintained at 25 ±2 °C room for a known time-period, 24 ±2 h and illuminated by a light-rig containing 4 × fluorescent tubes (F65W/35 General Electric, Hungary) providing approximately 11 Wm^{-2} of visible light.

The above process was performed in an identical way for the control vessels with the only difference being that no live individuals were present.

4.3.2 Filter disc binomial-choice assay

A set of six 100 mm diameter glass petri dishes and their corresponding lids (Duroplan Duran, Schott AG, Stafford, UK) were prepared as described in 2.4. Two equidistant positions were marked on the base of the petri dish so as to provide a guide for positioning two filter discs. Two cellulose filter discs (Whatman International Limited (1001020) grade 1, 2 cm diam.) were prepared as described in 4.3.1 and placed centrally on these two marks using hexane cleaned forceps. See Figure 4.1 for an exploded view of the assay.

In order to observe the test thrips throughout the experiment it was necessary to provide strong illumination. Light from below the dish was necessary to allow for observation of the thrips when on the underside of the filter disc. To allow for this the petri dish was carefully placed on a piece of transparent 6 mm thickness Perspex plate (Rubberfast Ltd., Fenton, UK) which was placed on a lightbox (556-272 'Artwork Lightbox', RS Components, Northamptonshire, UK). Overhead lighting was also provided as above (4.3.1).

Test thrips were obtained by aspirator in required quantities (20 mixed-age females were used per bioassay) as described in 2.3 and immediately anaesthetised by exposing them to a gentle 10 s stream of carbon dioxide (British Oxygen Company, UK). Whilst still under anaesthetic the thrips were transferred into the middle of the petri dish containing the discs. The lid was added and a 90 $\times$ 20 mm strip of Parafilm membrane was stretched around the edges of the lid and base to create a thrips-proof seal. During the bioassay process, treatment and control filter discs were randomly alternated between sides of the dish with each replicate to reduce possible directional room bias. Treatment and control positions were assigned using random number tables.

4.3.3 Behavioural observations

Pilot observations of live female individuals exposed to male-exposed filter discs showed that individuals often did not remain stationary. Indeed where *P. kellyanus* were observed to remain in contact with respective filter discs at successive time-points (Webster *et al.*, 2006) *F. occidentalis* were not ordinarily quiescent upon the filter discs and comparatively active. As a result, for subsequent pilot experiments the arena was divided into two halves and the number of thrips in each half counted at successive time-points.

Given the difficulty of accurately mapping the positions of the 20 test thrips this was further refined and the number of individuals making contact with the two respective filter discs during the initial 30 min of the experiment was recorded and compared statistically. A contact was defined as a contact when a minimum of one leg or entire head area of the contacting thrips were placed either on the top or the glass underneath of the filter disc. Once the contacting thrips had departed any repeated contacts were treated as new contact incidents. In final experiments the 30 min was split into three consecutive 10 min sections so that initial and subsequent responses could be separated.

Individual thrips behaviour varied during trials and between trials. Flitting, grooming, quiescence, dish circling and female-female antennation interactions were observed and noted when most pronounced. Where thrips remained in contact with the disc this was often on the underside. These variations appeared to be erratic and it was not possible to adequately quantify given the difficulty of simultaneous observation of 20 individuals and the small size and rapid movement of female *F. occidentalis*. An attempt was made at mapping movements in discs exposed to live male movements (see Figure 4.2).

During the exposure of discs to live males there were instances where the inside

of the exposure vessel appeared to be more moist. This was assumed to be a direct result of the live individual thrips piercing the Parafilm membrane when feeding and this moisture being transferred via condensation to the inside of the exposure vessel. Additionally it was thought possible that live thrips could be producing excretory droplets.

The increased moisture could have unintentionally made the treatment discs more moist and attractive and thus potentially affected the number of contacts. To establish if this were the case a set of filter discs were weighed (NA114, Oertling, London, UK) before and after thrips exposure to test whether discs gained weight. Filter discs were thus exposed to 15 live female *F. occidentalis* and the disc weight compared with control discs not exposed to live individuals.

Mean weight gain was recorded for discs exposed to live females and a corresponding weight of distilled water added to dry discs using a hexane rinsed chromatography syringe. The biological responses of newly added females to both dry (control) and moistened filter discs were thus compared.

It is often possible to observe thrips excreta on lightly coloured substrates if their secretions contain residues of plant pigments such as anthocyanin. In initial trials adults were observed to produce bright purple anal excretions when fed on purple chrysanthemums. In an attempt to examine if there was strong evidence of excretion on the filter discs during the exposure process, live females and males were exposed as described in 4.3.1 with the addition to the distilled water source of a minute amount of Page-Blue 83 crystals (75970 Fluka, Sigma-Aldrich, UK). Filter discs and reduced size TLC plates (see 4.4.3) were visually inspected for blue dots after 48 hours. If thrips fed on the blue-stained water, it was hoped to detect this stain in any deposited excretory droplets on the filter discs.

4.3.4 Statistical analysis

Contact data were analysed within each trial using a two-way ANOVA, with odour and trial as factors. This enabled detection of significant trial response variation.

When testing live exposed female discs a male exposed disc was included as a positive control to provide further comparisons. Differences between the tested disc treatment and the positive control were examined by comparing treatment *minus* control levels for each and these were in-turn compared using a General Linear Model ANOVA (GLM). This was necessary as computation of a standard two-way ANOVA is impossible for unbalanced designs where there is a difference in replicate number between each level.

All data were tested for normality as described in 2.7. Where parametric data were expected and where the majority of analyses within the same experiment were parametric, occasional non-parametric data was still analysed in the same way.

4.4 Extraction and chromatographic analysis of male-derived compounds

4.4.1 Solvent extraction for re-application

Discs exposed to male thrips (prepared as described in 4.3.1) and their respective control discs were solvent extracted by adding 250 μl of *n*-hexane to the disc using a 250 μl hexane cleaned syringe and concentrating this down to 5 μl by gentle evaporation. This was then re-applied by hexane cleaned syringe to new discs in a five-point arrangement as illustrated in Figure 4.3. New females were added to the test arena and their

contact response assayed. In addition female exposed filter paper extracts were also processed in the same way. It was hoped that should these discs be attractive to newly exposed females, any compounds remaining on the disc surface could be extracted and transferred to new discs, with these being assayed in-turn.

4.4.2 Solvent extraction for chromatographic analysis

Glass filter discs (GF/A 24 mm, 1820024, Whatman International Limited, Maidstone, UK) were prepared in the same way as the cellulose discs (4.4.1). Cellulose discs were used for the binomial choice assay as they were thin enough to allow partial illumination through them and hence allow observation of any thrips on the underside of the disc. Glass filter discs were used here as they were easy to clean with solvent. Prior to this process the discs were cleaned thoroughly by rinsing them with *n*-hexane and baking them overnight as described in 2.4. Several discs were exposed to high numbers of males and females and these along with their respective controls were extracted by adding 250 μl of *n*-hexane to the disc using a 250 μl hexane cleaned syringe and concentrating this down to 1 μl by gentle evaporation. Extracted solutions were stored in a freezer until required.

In an attempt to observe any male-derived compounds present on the filter disc, filter disc extracts were analysed using thin layer chromatography (TLC) plates or analysed using GC-MS.

4.4.3 Chromatography

For basic detection of compounds TLC plates (HP-K (High Performance Silica Gel) 4807-700, 200 μm, Whatman International, Maidstone, UK) were cut to around 20 $\times$

50 mm strips and the disc extracts spotted accordingly using a 10 μl calibrated pipette (Sigma-Aldrich, UK). Plates were carefully transferred into a small solvent chamber and placed in petroleum ether until the solvent front reached the last few mm of the plate. Plates were stained by transferring to iodine crystals and subsequently visualised using UV at short 254 nm and at long 366 nm wavelengths.

HP-K plates have high detection sensitivity and allow samples in the nanogram and picogram range to be analysed with short distance and development times (Whatman Data Specification, 2007).

In addition unmodified standard silica polyester backed plates were also used after being cut to size (805013 SIL G, 200 μm, Macherey-Nagel GmbH & Co. KG, Düren, Germany). These featured similar properties to the above HP-K plates but were easier to cut.

GC-MS (configuration as described in 3.3.2) was used to measure and tentatively identify any compounds present in the hexane extract. Multiple male, female and control disc extract solutions were obtained over several days. These were then merged and each filter paper solution further evaporated down under air with the last 1μl being injected.

4.5 Results

4.5.1 Filter disc binomial choice assay bias tests

Bias tests were conducted to ensure no room bias. The arena was kept in the same position. The number of contacts made by 20 newly added females in 30 min was measured. As is visible from Figure 4.4, there was no significant difference in mean

contacts between left and right discs and therefore no positional room bias was detected. (ANOVA, $F_{1,6}$=0.75, P=0.420).

4.5.2 Effect of time on response

It was important to establish if the biological response varied over time as perhaps the majority of contacts would be immediate and then decrease in number as the thrips acclimatised. Trials were split into three 10 min periods; it was hoped to separate initial responses from subsequent responses in this way. Cumulative average treatment *minus* control contacts made in the initial 10 min were compared with those made in the subsequent 20 and 30 min. As is evident from Figure 4.5, (an example male-exposed disc dataset) cumulative treatment *minus* control contacts appear to be linear over time. When the coefficient of variation ($c.v.$) (Sokal & Rohlf, 1995) is calculated for these three values:

$$c.v. = \frac{s \times 100}{\bar{Y}} \tag{4.1}$$

Where s is the standard deviation and $\bar{Y}$ is the mean.

it became apparent that the coefficient of variation in this dataset is similar for the three timepoints. For the three successive timepoints $c.v.$=90%, 89% and 102.7% respectively. This suggests contact results stayed approximately the same, showing no trend with time. Whilst reducing the duration of the experiments by 10 min would have been possible ($c.v.$ is lowest at 20 min) it was decided that the slowest part of the experiment was the disc exposure process and due to prior collection of valid 30 minute data it was decided to continue with 30 minute duration.

4.5.3 Effect of increased moisture on bioactive response

As mentioned in 4.3.3 discs exposed to thrips appeared more moist. It was thus important to compare the effect of moisture on contacts. Discs exposed to 15 live individual thrips gained on average 4 mg in weight when compared to non-exposed discs prepared in the same way. See Table 4.1.

To determine if this moisture gain affected disc preference and thus increased treatment contacts 4μl distilled water was added to test blank discs and compared with dry ordinary discs. There was no significant difference in the number of contacts at 30 min (Figure 4.6) (ANOVA, $F_{1,14}$=0.01, P=0.921). There was a significant difference in response between trials (ANOVA, $F_{1,14}$=2.74, P=0.034).

4.5.4 Presence or absence of thrips excretory droplets on the disc surface

It was important to establish if excretory droplets were being deposited on the surface of the filter disc. The presence of plant compounds within the droplets could have contributed to their attractiveness. Discs and TLC plates exposed to 15 female thrips that had access to water containing Page-Blue 83 dye were thus visually examined. After several 48 h exposure replicates no droplets were detectable on cellulose discs nor on appropriately post-processed silica polyester-backed TLC plates. In some cases droplets were observed on the underside of the Parafilm surface though these were not easily quantifiable due to the stretched brittle nature of the Parafilm substrate.

4.5.5 Male-exposed discs: cellulose discs

Discs previously exposed to 15 male *F. occidentalis* were tested in the binomial-choice assay against a blank control. As is evident from Figure 4.7 there was a significant difference in the number of contacts made by newly added females after 30 min between treatment (15 male exposed) and control (no thrips exposed) filter discs (ANOVA, $F_{1,9}$=32.32, P<0.0001). There was also a significant difference in response between trials (ANOVA, $F_{1,9}$=2.60, P=0.049). The behaviour of one randomly selected female thrips presented with a male exposed cellulose disc is shown in the trace in Figure 4.2. This data provides an indication of the behaviours exhibited in this assay.

4.5.6 Male exposed discs: hexane-cleaned glass fibre discs

It was important to establish that glass discs, like cellulose discs, exposed to male thrips also increased the number of contacts made by newly added females. A two-way ANOVA was carried out to investigate difference in contacts within each trial and comparisons were made between the two types of discs using an ANOVA general linear model (Figure 4.8). There were increased numbers of contacts observed on both discs after 30 mins with the number of contacts on the glass treatment discs being significantly increased in comparison to glass control discs. A significant difference in contacts was observed between glass discs exposed to males and their respective control discs over 12 replicates (ANOVA, $F_{1,11}$=10.77, P=0.007).

No significant difference in contacts was detected between treatment and control cellulose discs. This is likely because only four replicates in this instance were carried out (ANOVA, $F_{1,3}$=6.78, P=0.080 (odour), $F_{1,3}$=2.68).

There was no significant difference in contacts between the two types of treatment

disc types. Treatment *minus* control contacts were compared between the two disc types (ANOVA, $F_{1,3}$=0.00, P=0.989). This suggests that both discs, when exposed to male *F. occidentalis* are attractive to newly exposed females.

4.5.7 Female-exposed discs: cellulose discs

Adult female *F. occidentalis* are not known to produce any known pheromones. It was therefore considered a good negative control to test female exposed discs for a contact response with newly added females. Female exposed discs were produced as described in 4.3.1. These were then tested against their respective control discs. An additional positive control replicate containing a male exposed disc was also tested each day (Figure 4.9). As is visible, after 40 female-exposed disc replicates, there was a significant difference between number of contacts made with both female and male discs in comparison to their respective controls (ANOVA, $F_{1,39}$=7.16, P=0.011). There was also a significant difference in contact response between trials (ANOVA, $F_{1,39}$=4.12, P=0.001). A significant difference in contact response for males after 10 replicates (ANOVA, $F_{1,9}$=6.54, P=0.031).

There was a significant difference between the two disc types with the male exposed discs being significantly more attractive than the female exposed discs. Treatment *minus* control contacts for both male and female exposed discs were compared (ANOVA, $F_{1,9}$=12.92, P=0.001).

4.5.8 Male-derived compounds: hexane-extracted cellulose discs

Increased female contacts where observed when exposing new females to a new cellulose disc containing manually applied male filter paper extract (ANOVA, $F_{1,11}$=7.41,

P=0.020). See Figure 4.10.

4.5.9 Female-derived compounds: hexane-extracted cellulose discs

When assaying a cellulose disc containing manually applied female filter paper extract, no increase in female contacts was observed (ANOVA, $F_{1,16}$=0.12, P=0.737). There was also a significant difference in response between trials however suggesting this response is highly variable (ANOVA P<0.0001). See Figure 4.11.

4.5.10 Chromatographic analysis

Neither neryl (*S*)-2-methylbutanoate nor (*R*)-lavandulyl acetate were found in GC-MS tested extracts. Several male-exposed disc extractions and their controls (nine sets of discs exposed to 40 males, females or no insects) were combined and analysed. Combined chromatograms are shown in Figure 4.12. Unknown peaks with t_R 14.77, 18.64, 21.44 and 21.96 were present in the female extract and unknown peaks with t_R 14.76, 18.64, 21.44, 21.97 and 25.15 were present in the male extract. 1 μl 20 ng μl^{-1} neryl (*S*)-2-methylbutanoate was injected and eluted at 15.907 min. All other peaks were present in the control chromatograms and were therefore contaminants. Identification of eluted compounds was attempted by comparing mass spectral fragmentation patterns with those in the NIST spectral database (Chemstation C.0200, NBS75K.L) and MassFinder 2.3.1.1: Mass Spectral Library "Terpenoids and Related Constituents of Essential Oils" 2001 (Dr Hochmuth, Scientific Consulting, Hamburg, Germany). No compounds were identified using this method.

No male-derived compounds were detectable using TLC. Neryl (*S*)-2-methylbutanoate

standard [100 ng μl] (NRI, University of Greenwich) spotted too close to the start of the solvent front so a small amount of ethyl acetate was added to the solvent to increase its polarity and thus increase the retention factor (Rf) values for the standard and for all other compounds that may have been present.

An Rf of around 0.488 was detected for neryl (S)-2-methylbutanoate standard on HP-K 200 μm plates where:

$$Rf = \frac{b}{a} \tag{4.2}$$

Where b= distance travelled by the spot centre of sample analyte
and a= distance travelled by solvent front.

Recommended Rf values lie between 0.3 and 0.7 with 0.5 being optimal (Fried & Sherma, 1999).

4.6 Discussion

Females exposed to discs previously exposed to live males make more contacts with the disc surface. This appears to be attraction as an orientated movement towards a source (Dethier, 1960) though it is also possible that arrestment is occurring. Whilst the compound(s) present on the disc cause a response it was not possible to resolve their identity using the chromatography systems employed. It was however possible to transfer the effect to a new disc, though there was some loss of response.

Male exposed discs appeared to be on average around 2.5-3 times more attractive to females than their respective controls. There was considerable variation in baseline response within controls or where discs are untreated. As all experiments were carried out within the same daily time period this variation appears to arise from day-to-day variation in behaviours within individuals. Variation with time has been recorded in

flight responses in *F. occidentalis* (O'Leary, 2005).

It is likely that the active compound(s) involved in attraction to male-exposed and female-exposed discs are not the aggregation pheromone neryl (*S*)-2-methylbutanoate, or at least are not this compound in isolation.

4.6.1 Female exposed discs and attractiveness

It was not expected that female exposed discs would cause increased contacts with newly introduced females. This effect is weak but significant with contacts being on average 1.25 times more than respective control contacts. It is unlikely that this effect arises from anomalous experimental design or statistical anomalies though it is worthy of notice that in this instance, the statistical power of the two-way ANOVA is high due to the large number of replicates so the test is more likely to reveal a real difference.

Moistened filter discs were tested against ordinary dry discs in order to establish that added moisture on the disc surface does not make the disc more attractive (see 4.5.3). Whilst the results above show no significant difference between the two discs when additional water is added to one using a chromatography syringe it is worth considering the possibility that this application method fails to uniformly coat the entire disc surface.

Cellulose is insoluble in normal aqueous solutions though it is likely to retain water by capillarity. 4-point application certainly differs from gradual osmosis in a moist humid environment. This difference may have contributed to the mild contact effect of the female exposed discs, especially if the edges of the disc were more moist. Whilst moisture itself may not be attractive it may increase arrestment where the surface of a moist cellulose disc more closely resembles that of a plant substrate.

Whilst it is possible that both male and female exposed discs were made more attractive due to the presence of residual *F. occidentalis* excretion upon the disc surface this is unlikely to be the sole reason given that female exposed discs, also possibly containing residual female excreta, were not equally attractive. As females are larger than males it could be surmised that more excretion will ordinarily occur in females and that this will in-turn result in more excretory droplets being produced during the exposure period. Studies on insect excreta are uncommon and the composition of thrips excretory droplets is unknown. Whilst likely to be mostly water with waste metabolites it is clear from visual observation that plant colourants visible in petals are often present.

As mentioned above in 4.5.4, thrips appear to excrete droplets on the surface of the Parafilm membrane whilst feeding and not on the filter discs themselves. Whilst excretion on the disc is still possible it can be considered less likely.

There is little evidence in the literature of females being attracted by female-produced pheromones however a "female-released" pheromone (methoxybenzene) with this effect has been described in the melolonthine scarab beetle, *Holotrichia consanguinea* (Coleoptera: Scarabaeidae) (Leal *et al.*, 1996). The adaptive function of this pheromone is unclear. It has been suggested from results obtained using GC-EAG, that the majority of melolonthines produce "primitive" compounds that are used both as sex pheromones and may also be involved in other primary functions (Yarden *et al.*, 1996; Leal *et al.*, 1996).

There is no evidence of adult female *F. occidentalis* producing pheromones of any kind and given the comparatively complex chiral structure of the *F. occidentalis* male-produced aggregation pheromone neryl (*S*)-2-methylbutanoate, it is unlikely though not impossible that a new female produced pheromone is being detected. As mentioned in 1.2 and 1.3, several Phlaeothripidae (Idolothripinae and Phlaeothripinae) produce

anal secretions of varying composition that may have roles in intra- and inter-specific communication. Only one adult Thripidae, *Heliothrips haemorrhoidalis* is known to produce anal secretions and whilst larval *F. occidentalis* release anal secretions containing alarm pheromone, no such compounds have been identified from adult females. In addition females lack the defined sternal glands present in males (Moritz, 1997) and this further reduces the likelihood of a female produced, non-anal droplet compound being detected.

Where thrips walk over plant surface tissue and then subsequently, the discs it is possible that plant contaminants such as pollen grains could be transferred and deposited as part of feet residue. Substrate attachment in most insect orders is facilitated by the presence of specialised tibial, tarsal or pretarsal attachment systems. Two adhesion mechanisms exist, hairy and smooth, and both require the release of an adhesive secretion in order for attachment to smooth surfaces (for a review see Beutel & Gorb, 2001).

Thysanoptera possess balloon-shaped pretarsal adhesive structures at the distal end of a a one or two segmented tarsus. An arolium is present that lacks adhesive pulvilli. The pad appears to be flat though there are specialised adhesive tarsal thorns (plantar lobes) present and it is thus comparable with Coleoptera and Hymenoptera (Beutel & Gorb, 2001). It is unlikely that any adhesive secretions produced would be attractive to new females; production levels are very low (around 0.5 μg being obtained from 1200 black bean aphid, *Aphis fabae* footprints (Dixon *et al.*, 1990)).

		mean weight (g)	weight change
no exposure (control)	pre-exposure	0.02698	+0.00144
	post exposure	0.02842	
15-male exposure	pre-exposure	0.0274	+0.00562
	post exposure	0.03302	

Table 4.1: Mean filter disc weights before and after exposure to thrips compared with non-exposed controls, n=5.

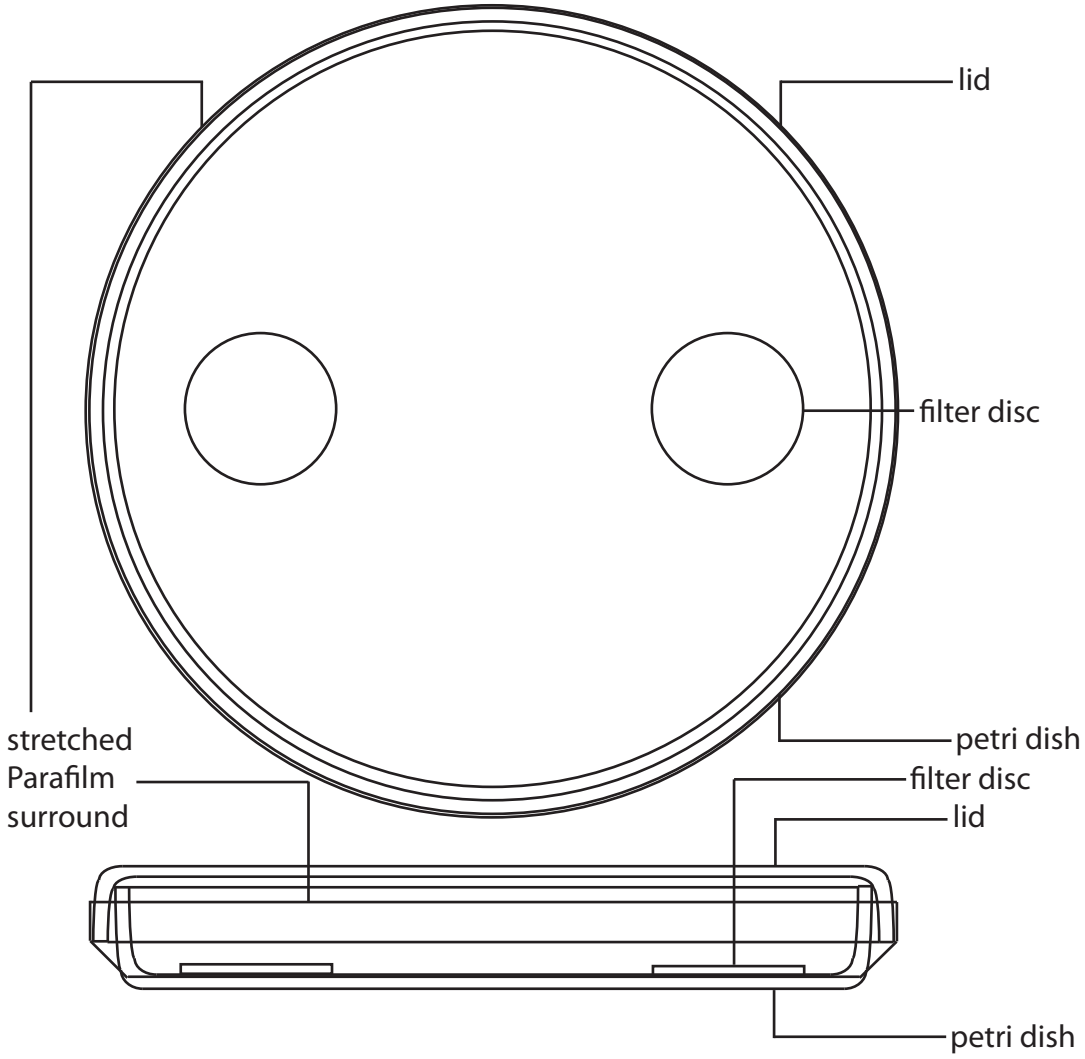

Figure 4.1: Filter disc binomial choice assay, top view and side view. Diagram scale is approximate.

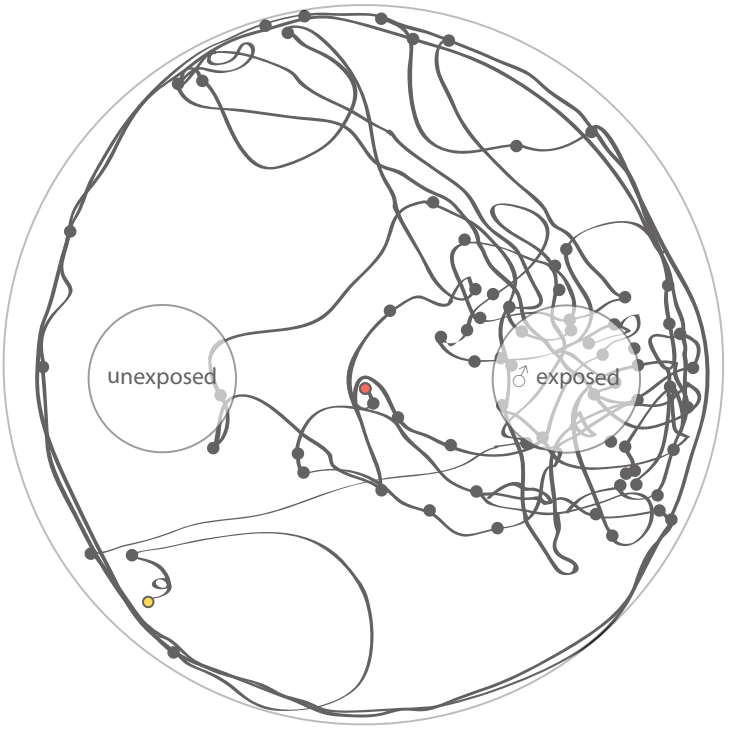

Figure 4.2: Movement in the filter disc binomial choice assay of one individual female thrips over 30 min when presented with a male-exposed filter disc and a no-male exposed control disc. The trace is transcribed from video footage and provides an indication of the possible behaviours exhibited. The red dot denotes the starting position and the orange dot denotes end position. Black dots indicate sedentary periods. Diagram drawn to scale.

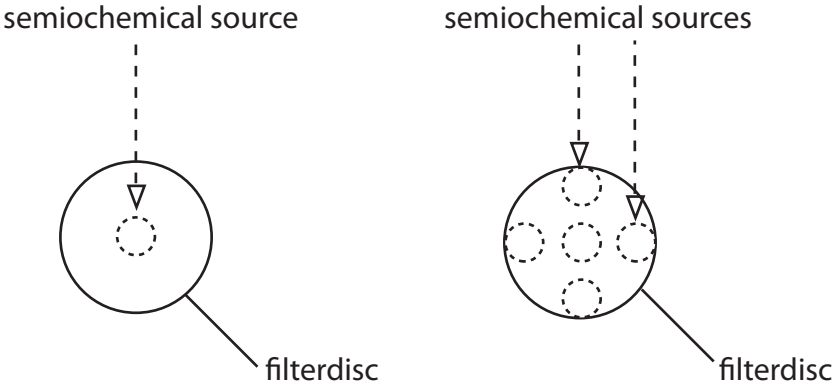

Figure 4.3: Dispersed semiochemical application sites, top view. Left: 1μl central application, right: 5μl application. Diagram scale is approximate.

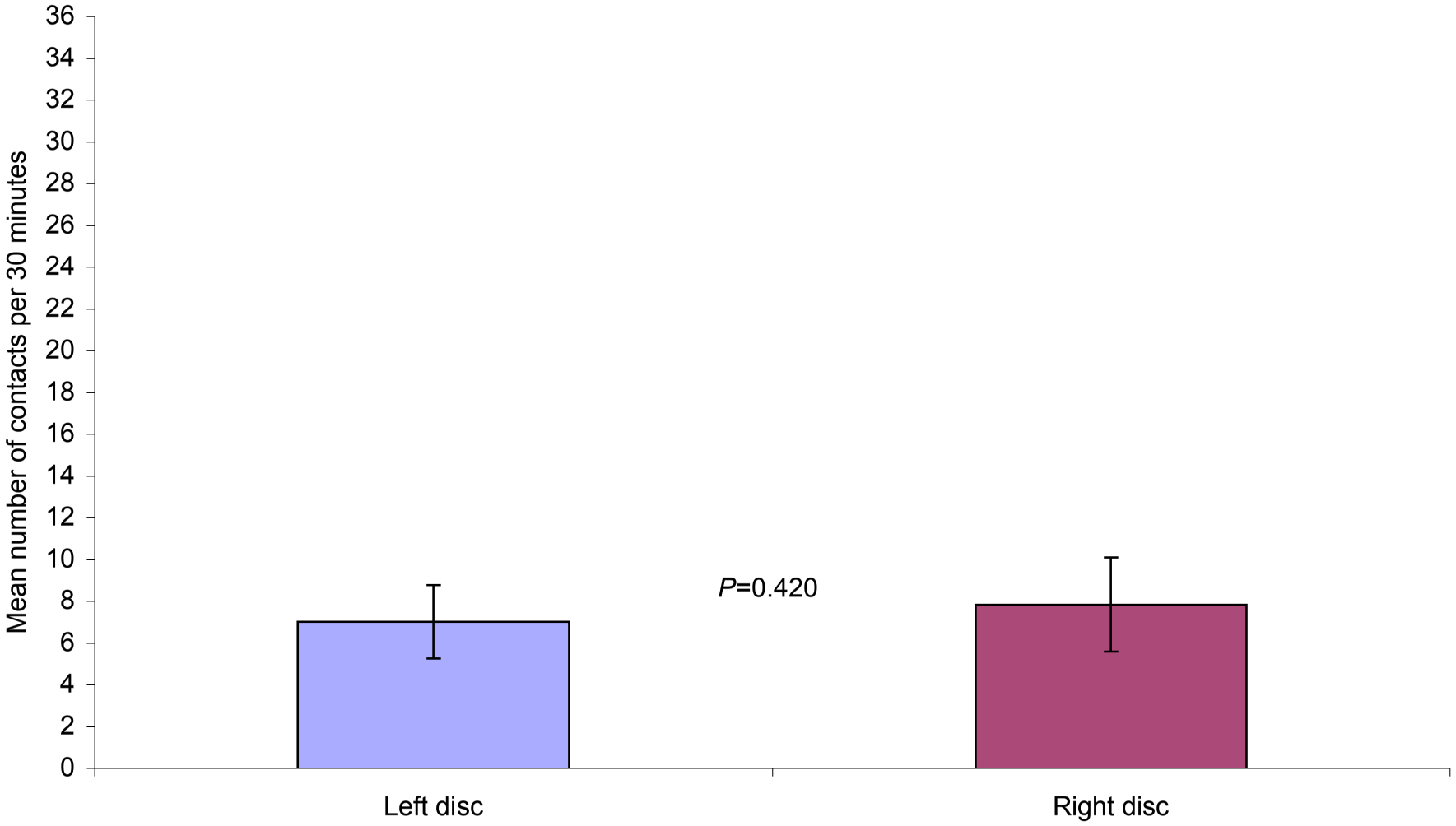

Figure 4.4: Filter disc binomial choice assay, female response in room bias test. Mean number (±SEM) filter disc contacts with blank filter discs placed on the left and right of the dish, n=7.

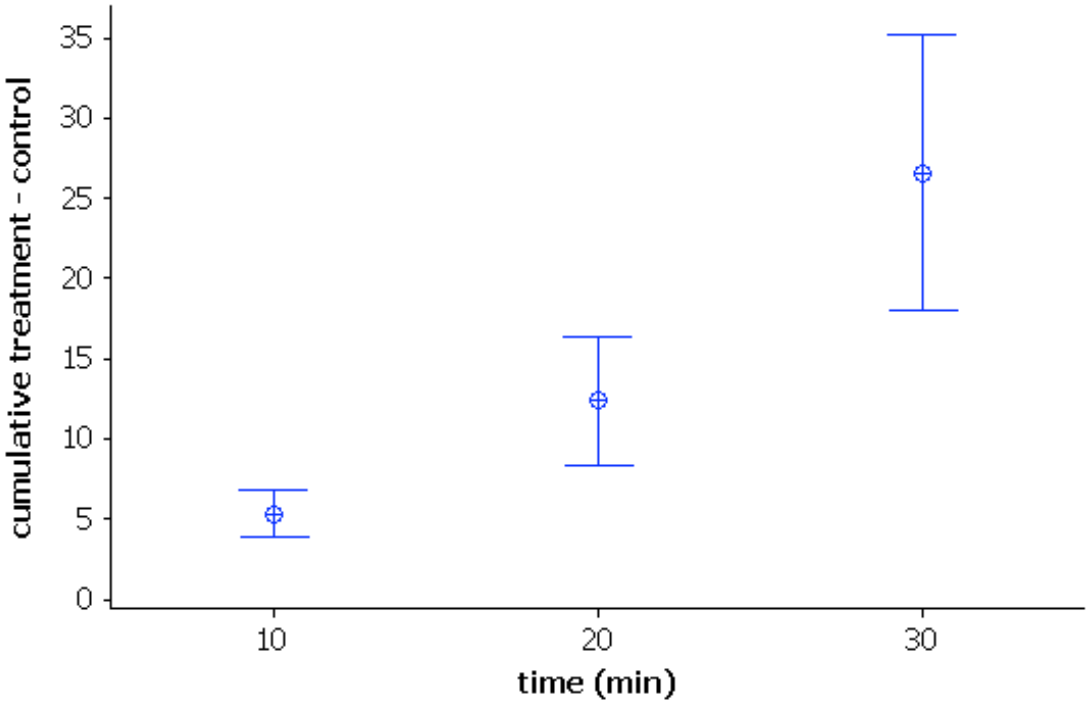

Figure 4.5: Filter disc binomial choice assay, female response. Mean number (±SEM) treatment *minus* control filter disc contacts at three successive timepoints with live 15 male exposed disc (treatment) compared with non-exposed filter disc (control), n=10. Coefficient of variation values.

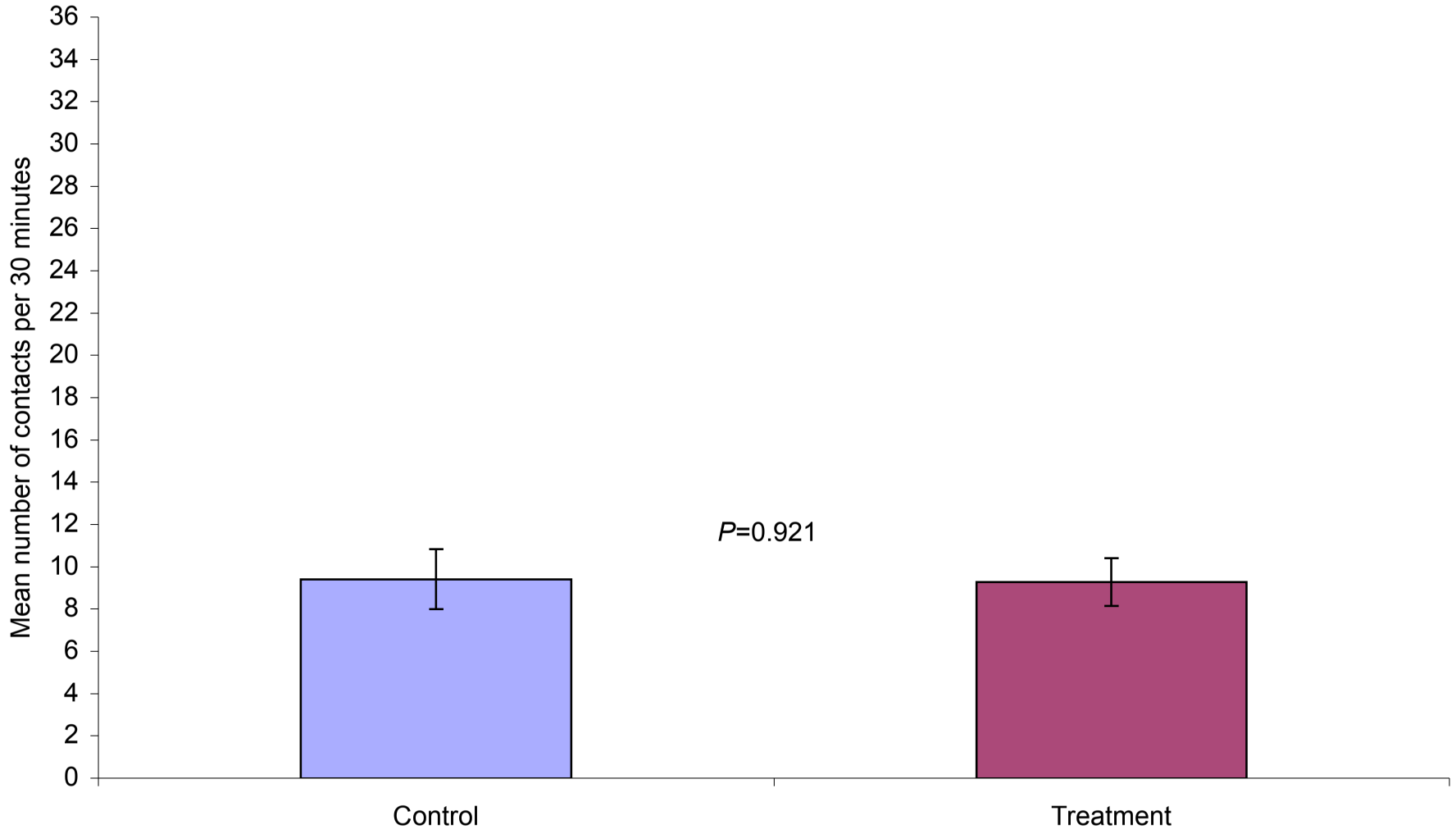

Figure 4.6: Filter disc binomial choice assay, female response. Mean number (±SEM) filter disc contacts with filter disc with an additional 4μl distilled water (treatment) compared with ordinary filter disc (control), n= 15.

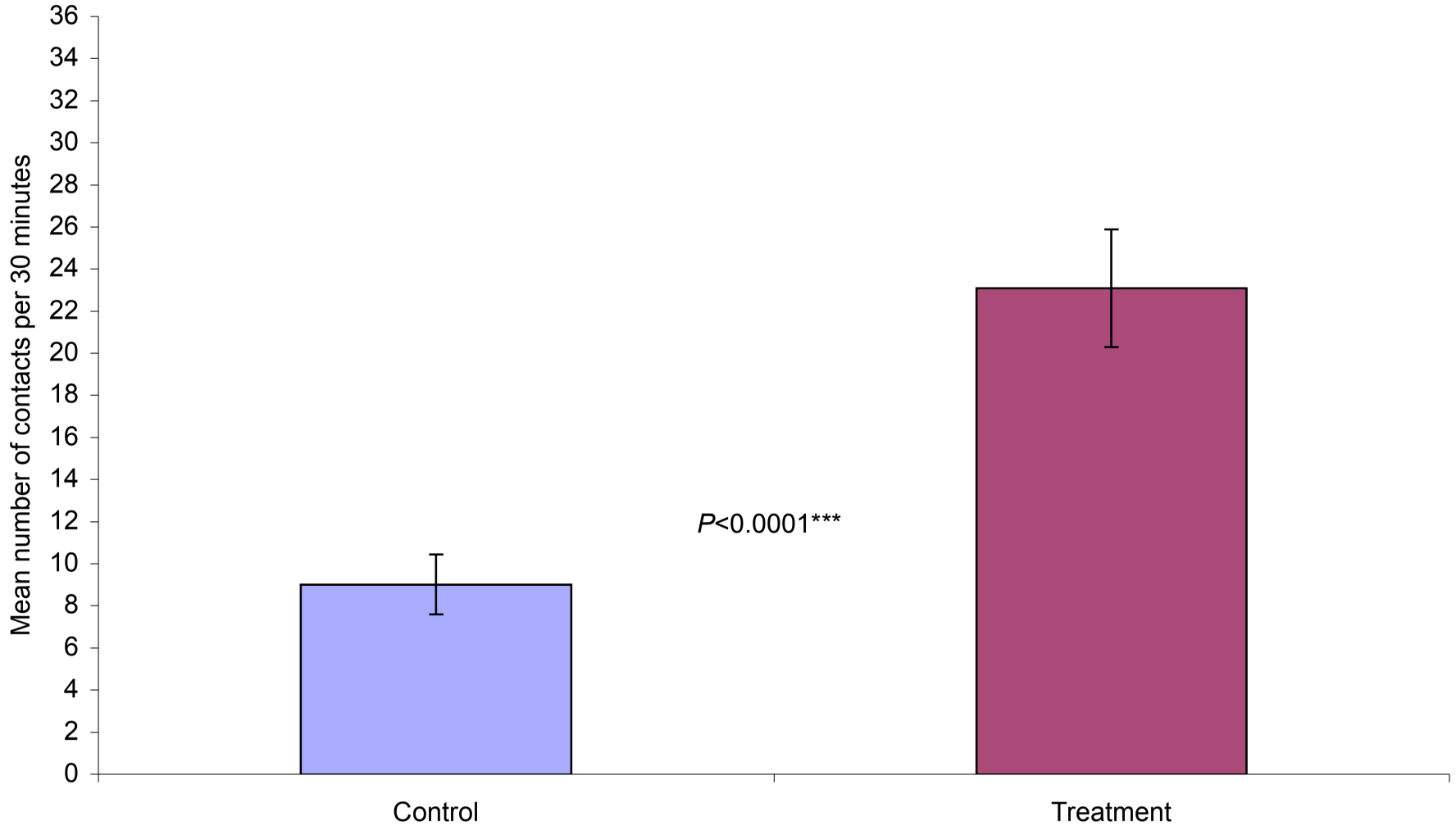

Figure 4.7: Filter disc binomial choice assay, female response. Mean number (±SEM) filter disc contacts with filter disc exposed to 15 male *F. occidentalis* (treatment) compared with non-exposed filter disc (control), n= 13.

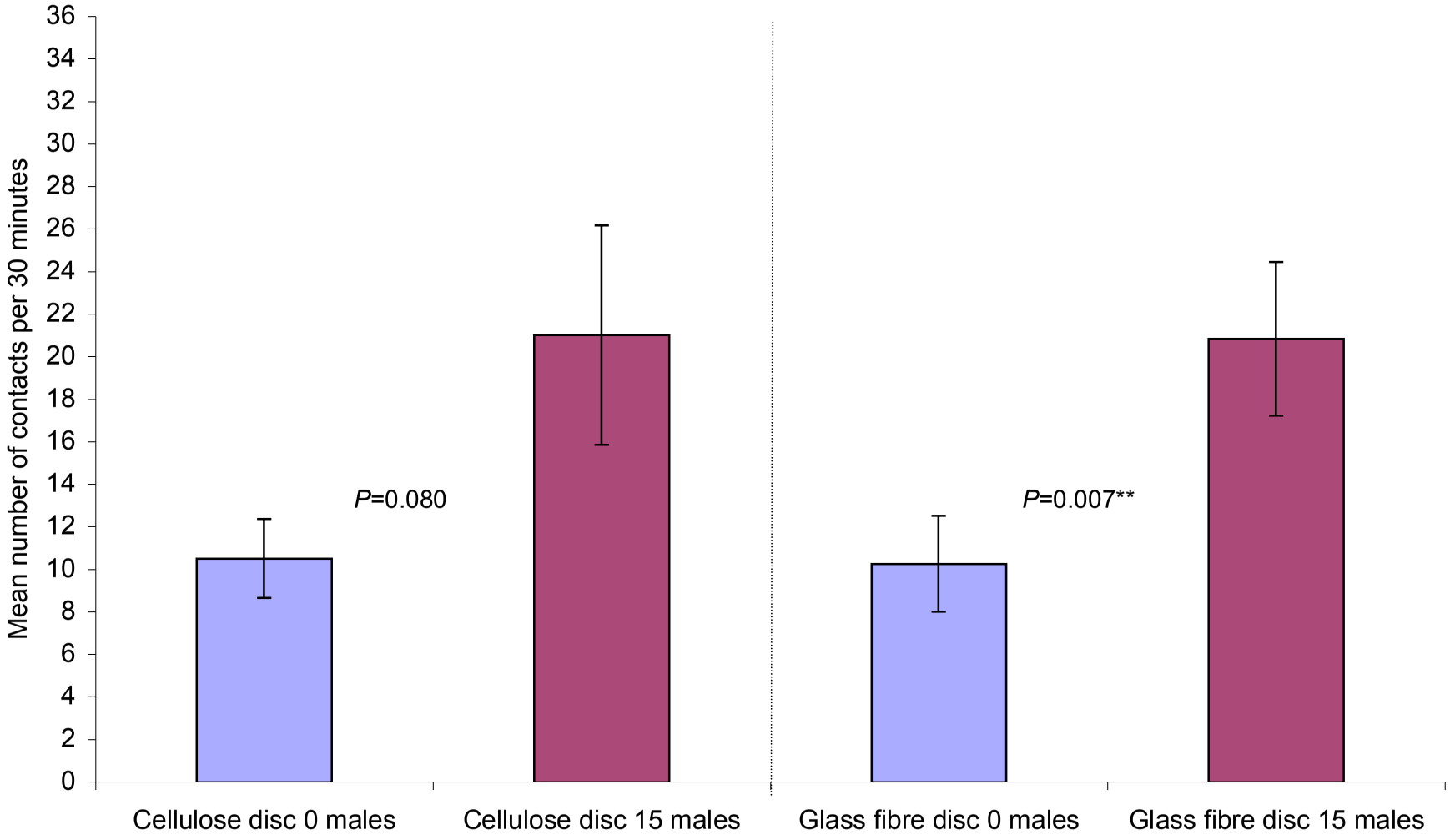

Figure 4.8: Filter disc binomial choice assay, female response. Comparison of cellulose disc with glass fibre disc. Mean number (±SEM) filter disc contacts with filter disc exposed to 15 male *F. occidentalis* (treatment) compared with blank filter disc (control), 4 blocks. glass fibre discs n= 12, Cellulose discs n=4.

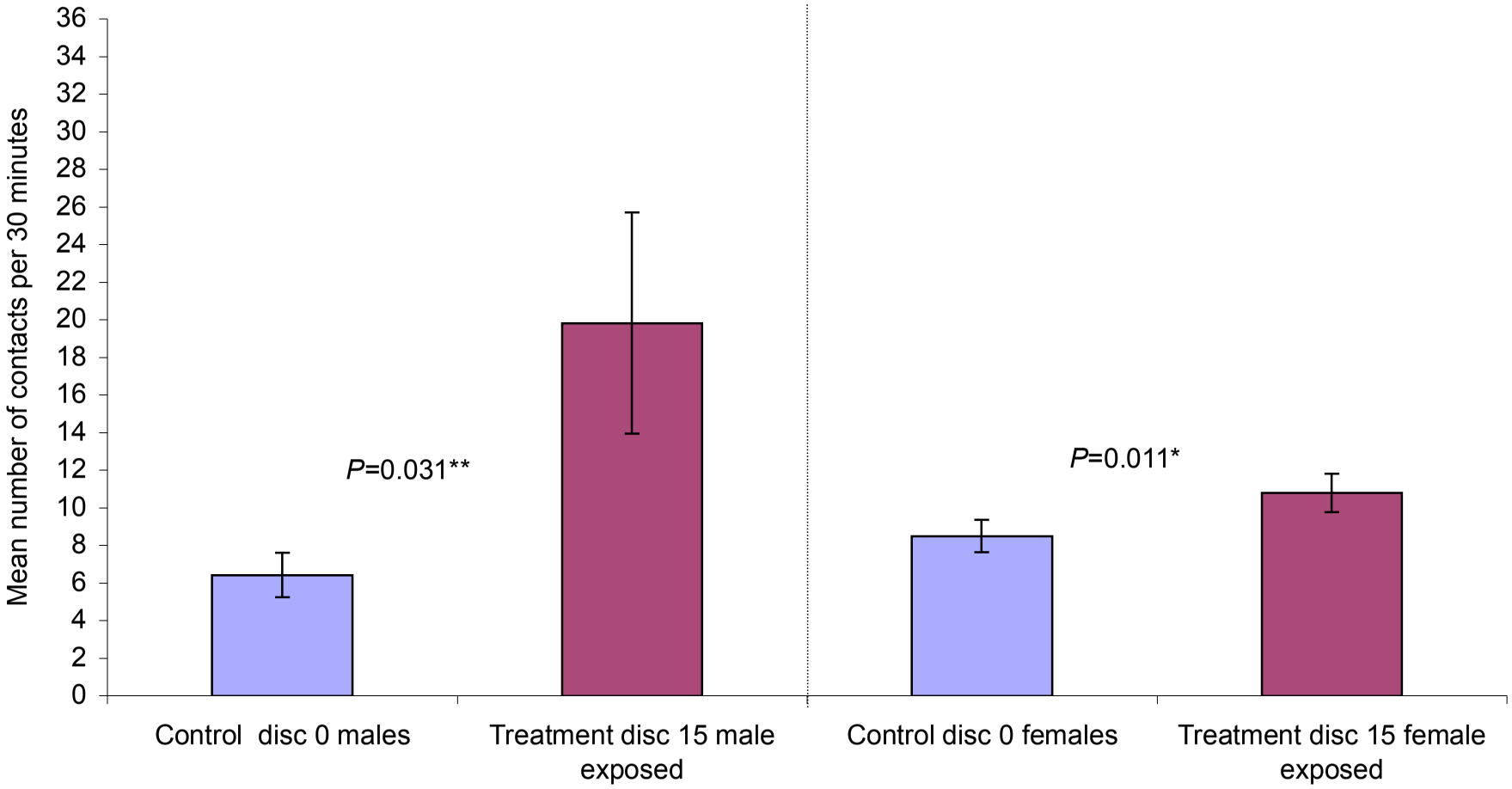

Figure 4.9: Filter disc binomial choice assay, female response. Comparison of female and male exposed discs. Mean number (±SEM) filter disc contacts with filter disc exposed to 15 male or 15 female *F. occidentalis* (treatment) compared with non-exposed filter disc (control). 10 blocks, male trials n=10, female trails n=40

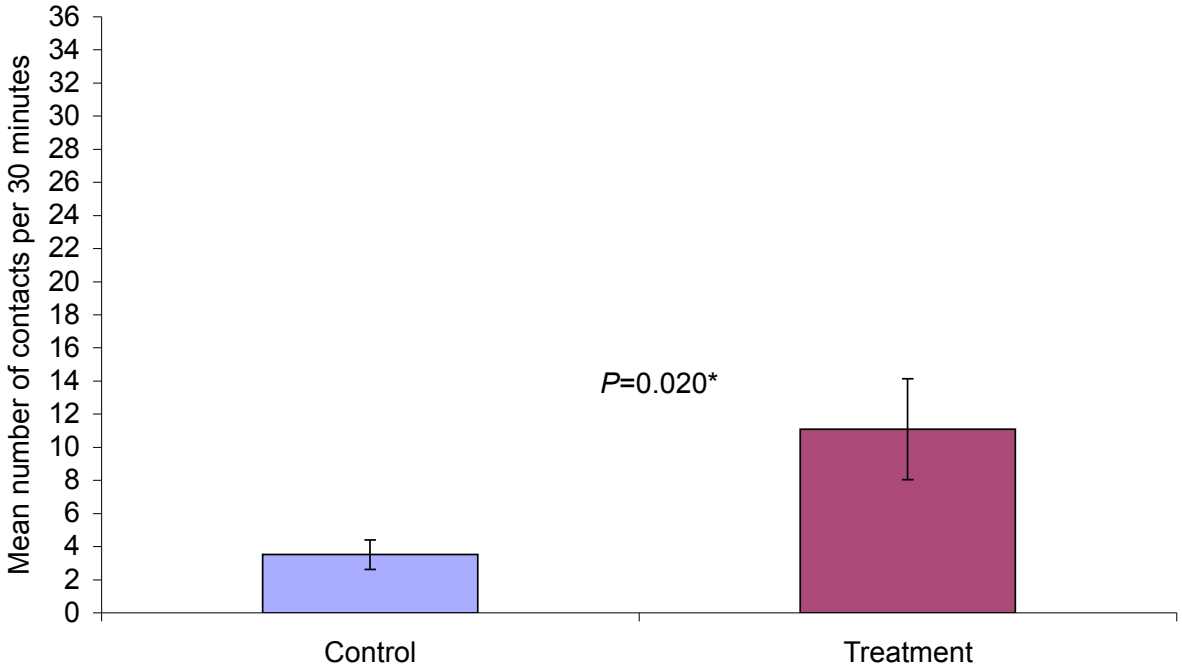

Figure 4.10: Filter disc binomial choice assay, female response. Mean number (±SEM) filter disc contacts with male filter paper extract present on the disc (treatment) compared with hexane injected filter disc (control), n= 12.

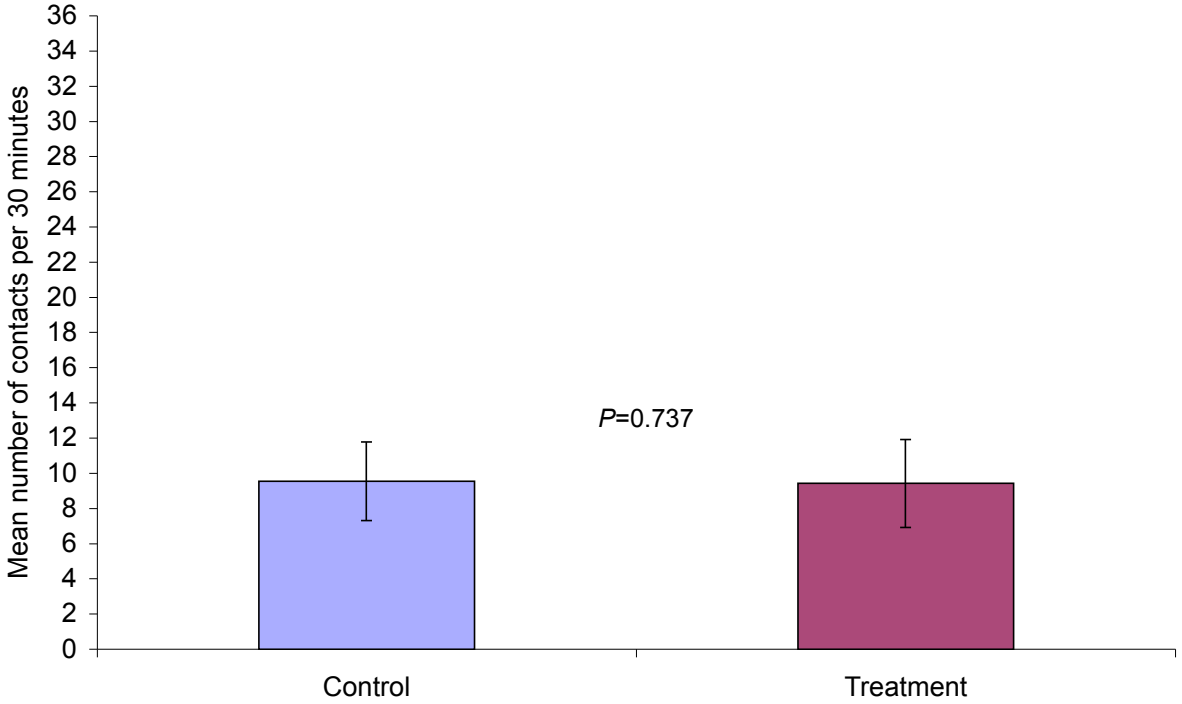

Figure 4.11: Filter disc binomial choice assay, female response. Mean number (±SEM) filter disc contacts with female filter paper extract present on the disc (treatment) compared with hexane injected filter disc (control), n= 17.

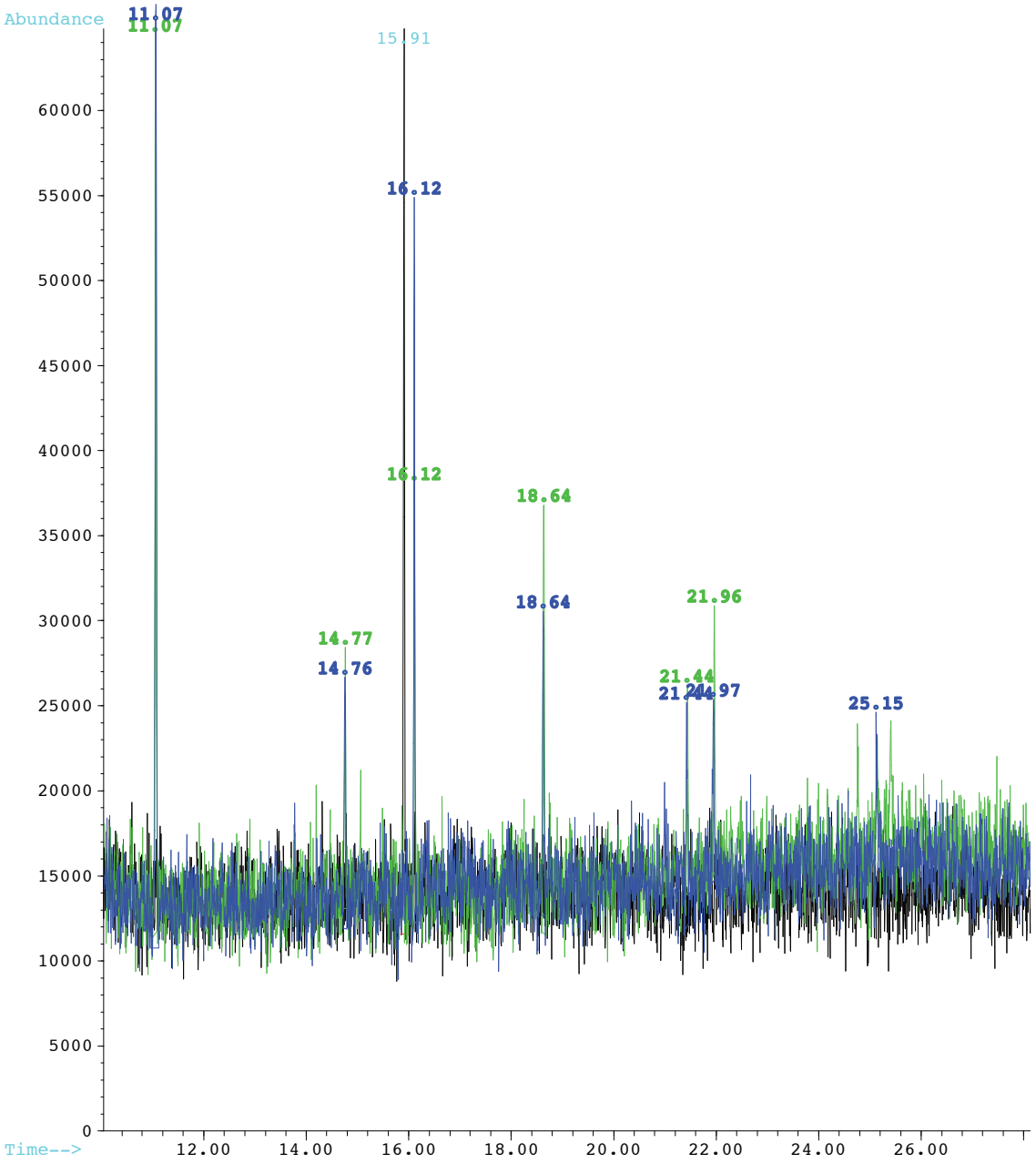

Figure 4.12: GC chromatogram of filter paper extracts. Retention times are printed on integrated peaks. The male GC trace (9 sets of discs each exposed to 40 males) is coloured purple, the female trace (9 sets of discs each exposed to 40 males) coloured green and neryl (*S*)-2-methylbutanoate [20 ng] standard is coloured turquoise. For ease of viewing two control GC traces (9 sets of discs each exposed to no thrips) are omitted. Unidentified peaks are present in the female exposure at t_R 14.77, 18.64, 21.44 and 21.96. Unidentified peaks present in the male exposure occur at t_R 14.76, 18.64, 21.44, 21.96 and 25.15. All other integrated peaks shown were present in control chromatograms and are therefore contaminants.

4.7 References

Barrows, W. M. (1907) The reaction of the pomace fly, *Drosophila ampelophila* Loew, to odorous substances. *Journal of Experimental Zoology*, 4 515-537.

Beutel, R.G. and Gorb, S.N. (2001) Ultrastructure of attachment specializations of hexapods (Arthropoda): evolutionary patterns inferred from a revised ordinal phylogeny. *Journal of Zoological Systematics and Evolutionary Research*, 39 177-207.

Burkholder, W.E. (1970) Pheromone research with stored-product coleoptera. In: *Control of insect behavior by natural products.* Edited by: D.L. Wood, R.M. Silverstein and M. Nakajima. Academic Press, London, UK. pp. 1-20.

Dixon, A.F.G., Croghan, P.C. and Gowing, R.P. (1990) The mechanism by which aphids adhere to smooth surfaces. *Journal of Experimental Biology*, 152 243-253.

Dethier, V.G. (1960) The designation of chemicals in terms of the responses they elicit from insects. *Journal of Economic Entomology*, 53 134-136.

Finch, S. (1980) Chemical attraction of plant-feeding insects to plants. *Applied Biology*, 5 67-143.

Fried, B. and Sherma, J. (1999) *Thin-layer chromatography.* 4th edition. CRC Press UK, London, UK.

Gobin, B., Rüppell, O., Hartmann, A., Jungnickel, H., Morgan, E.D. and Billen, J. (2001) A new type of exocrine gland and its function in mass recruitment in the ant *Cylindromyrmex whymperi* (Formicidae, Cerapachyinae). *Naturwissenschaften*, 88 395-

399.

Gómez, M., García, F., GreatRex, R., Lorca, M. and Serna, A. (2006) Preliminary field trials with the synthetic sexual aggregation pheromone of *Frankliniella occidentalis* on protected pepper and tomato crops in south-east Spain. *IOBC/WPRS Bulletin*, 29 153-158.

Hamilton, J.G.C., Hall, D.R., and Kirk,W.D.J. (2005) Identification of a male-produced aggregation pheromone in the western flower thrips *Frankliniella occidentalis*. *Journal of Chemical Ecology*, 31 1369-1379.

Hardie, J., Holyoak, M., Nicholas, J., Nottingham, S.F., Pickett, J.A., Wadhams, L.J. and Woodcock, C.M. (1990) Aphid sex pheromone components: age-dependent release by females and species-specific male response. *Chemoecology*, 1 63-68.

Hare, J.D. (1998) Bioassay methods for terrestrial invertebrates. In: *Methods in chemical ecology, volume II: bioassay methods*. Edited by: K.F. Haynes and J.G. Millar. Kluwer Academic Publications, Massachusetts, USA.

Jones, O.T. and Coaker, T.H. (1977) Orientated responses of carrot fly larvae, *Psila rosae*, to plant odours, carbon dioxide and carrot root volatiles. *Physiological Entomology*, 2 189-197.

Kennedy, J.S. (1978) The concepts of olfactory 'arrestment' and 'attraction'. *Physiological Entomology*, 3 91-98.

Koschier, E.H. (2006) Plant allelochemicals in thrips control strategies. In: *Naturally occurring bioactive compounds*. Edited by: M. Rai and M.C. Carpinella. Elsevier

Science, Oxford, UK. pp. 221-249.

Leal, W.S., Yadava, C.P.S. and Vijayvergia, J.N. (1996) Aggregation of the scarab beetle *Holotrichia consanguinea* in response to female-released pheromone suggests secondary function hypothesis for semiochemical. *Journal of Chemical Ecology*, 22 1557-1566.

Matsumoto, Y. (1970) Volatile organic sulfur compounds as insect attractants with special reference to host selection. In: *Control of insect behavior by natural products*. Edited by: D.L. Wood, R.M. Silverstein and M. Nakajima. Academic Press, London, UK. pp. 133-158.

Moritz, G. (1997) Structure, growth and development. In: *Thrips as crop pests*. Edited by: T. Lewis. CAB International, Wallingford, UK. pp. 15-63.

O'Leary, A.T. (2005) Flight and oviposition in the Western flower thrips. Ph.D. thesis, Keele University, Keele, Staffordshire, UK.

Pettersson, J. (1970) An aphid sex attractant. *Entomologica Scandinavica*, 1 63-73.

Pow, E.M., Bennison, J.A., Birkett, M.A., Luszniak, M.J., Manjunatha, M., Pickett, J.A., Segers, I.S., Wadhams, L.J., Wardlow, L.R. and Woodcock, C.M. (1999) Behavioural responses of western flower thrips (*Frankliniella occidentalis*) to host plant volatiles. *Proceedings of the Sixth International Symposium on Thysanoptera*, 121-128.

Sakuma, M. and Fukami, H. (1985) The linear track olfactometer: an assay device for the taxes of the german cockroach *Blattella germanica* (L.) (Dictyoptera: Blattellidae) toward their aggregation pheromone. *Applied Entomology and Zoology*, 20

387-402.

Shamshev, I.V., Selytskaya, O.G., Chermenskaya, T.D., Burov, V.N. and Roditakis, N. (2003) Behavioural responses of western flower thrips (*Frankliniella occidentalis* (Pergande)) to extract from meadow-sweet (*Filipendula ulmaria* Maxim.): laboratory and field bioassays. *Archives of Phytopathology and Plant Protection*, 36 111-118.

Shorey, H.H. (1973) Behavioral responses to insect pheromones. *Annual Review of Entomology*, 18 349-380.

Sokal, R.R. and Rohlf, F.J. (1995) *Biometry: the principles and practice of statistics in biological research.* 3rd edition. W.H. Freeman and Company, New York, USA.

Terry, I. Moore, C., Walter, G., Hull, C. and Roemer, R. (2007) *Macrozamia* cycad cone traits mediate thrips and weevil pollinator behavior. *Journal of Insect Science*, 7(28).

Varley, G.C. and Edwards, R.L. (1953) An olfactometer for observing the behaviour of small animals. *Nature*, 4357 789-790.

Webster, K.W., Cooper P. and Mound, L.A. (2006) Studies on kelly's citrus thrips, *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae): sex attractants, host associations and country of origin. *Australian Journal of Entomology*, 45 67-74.

Whatman Data Sheet, (2007) *Partisil High Performance TLC Plates.* Whatman plc., Maidstone, UK.

Wright, R.H. (1958) The olfactory guidance of flying insects. *The Canadian Ento-*

mologist, 90 81-89.

Yarden, G., Shani, A. and Leal, W.S. (1996) (*Z*,*E*-α-farnesene-an electroantennogram-active component of *Maladera matrida* volatiles). *Bioorganic and Medicinal Chemistry*, 4 283-287.

Chapter 5

Walking responses of adult females to synthetic compounds

5.1 Introduction

As demonstrated in Chapter 4, female western flower thrips make significantly more contacts with male-exposed (and to a lesser extent female-exposed) discs in comparison with non-exposed control discs. As no thrips-derived filter disc compounds were resolvable in the GC-MS and TLC systems employed (Chapter 4), the cause of the contact response remains unclear. In addition it is unclear if the observed response is one of arrestment or attraction (Dethier, 1960).

The male-produced *F. occidentalis* aggregation pheromone, neryl (*S*)-2-methylbutanoate, increases trap catch in both sexes in the the glasshouse (Hamilton *et al.*, 2005; Gómez *et al.*, 2006; Chapter 7). Little is known about the mode of action of this aggregation pheromone and so laboratory assays are needed to expand upon this. In addition, little is known about the other main male headspace

compound, (*R*)-lavandulyl acetate, which does not increase glasshouse trap catch.

As mentioned in chapter 4, several types of walking bioassays have been developed with a view to examining behaviours. Designs based on two of these are utilised below.

It was unknown if synthetic neryl (*S*)-2-methylbutanoate would affect the walking behaviours of *F. occidentalis* in a laboratory environment and if any observed responses would be strongly influenced by semiochemical concentration.

Whilst lavandulyl acetate naturally occurs in male *F. occidentalis* headspace in the *R* enantiomeric configuration it was not known if a racemic lavandulyl acetate mixture would elicit a biological response in the laboratory. As mentioned in 1.6.1, where the insect is able to discern between chiral enantiomers, racemic mixtures can be ineffective as synthetic pheromones.

Geraniol has been shown to attract western flower thrips in Y-tube olfactometers (de Kogel *et al.*, 1999). It was hoped to replicate this result in a walking bioassay. In addition, the plant allomone *p*-anisaldehyde has also been used for this purpose. This compound also attracts *F. occidentalis* in the Y-tube (Tuelon *et al.*, 1993; Koschier *et al.*, 2000).

5.2 Experimental aims

This chapter attempts to examine the behaviour of female *F. occidentalis* in a laboratory environment, when presented with male produced compounds and synthetic attractants. Simple assays are used that allow for a walking response. As females respond to male-exposed filter discs, it was aimed to replicate this with synthetic neryl (*S*)-2-methylbutanoate. In addition it was hoped to further examine the behaviours involved in such a response. Additional plant attractant semiochemicals were also

assayed with the aim of gaining a biological response.

5.3 Materials and methods

Synthetic versions of male-produced headspace compounds were assayed in a similar method to male-derived compounds as described in chapter 4. In the first instance, the filter disc binomial choice assay was used to examine the effects on females of artificially applied neryl (*S*)-2-methylbutanoate and racemic lavandulyl acetate. A wide range of concentrations were selected when assaying neryl (*S*)-2-methylbutanoate and lavandulyl acetate in this way, in an attempt to ensure any biological contact response if present, could be detected.

In an attempt to further examine contact responses and detect possible arrestment or attraction behaviours in females, test semiochemicals were further examined in two additional bioassays. In the no-choice observational arena, possible female arrestment in response to neryl (*S*)-2-methylbutanoate and lavandulyl acetate was tested for by measuring activity in the absence of a point semiochemical odour source. In addition, in an attempt to rapidly gain an attraction response and perhaps indications of activation, a simple straight-tube bioassay was also developed.

5.3.1 Assaying specific semiochemicals using the filter disc binomial-choice assay

The filter disc binomial-choice system (see 4.3.2) was used to assay synthetic male *F. occidentalis* headspace compounds. Two cellulose filter discs (Whatman International Limited (1001020) grade 1, 2 cm diameter) were placed in two equidistant positions in a 100 mm diameter glass petri dish (Duroplan Duran, Schott AG, Stafford, UK) as de-

scribed in 4.3.2. A randomly assigned treatment disc was injected with a known volume of semiochemical and a corresponding control disc was likewise injected, with an equivalent volume of *n*-hexane. Test thrips were obtained by aspirator in required quantities (20 mixed-age females were used per bioassay) and anaesthetised using a gentle 10 s stream of carbon dioxide (British Oxygen Company, UK). Whilst still anaesthetised the thrips were transferred into the middle of the petri dish. Semiochemical and control injections were made prior to adding the lid and sealing the arena, but immediately after the thrips introduction. Treatment and control discs were alternated as described in 4.3.1. Lighting, additional apparatus and aspiration details were as described in 4.3.2 and 2.3. As described in 4.3.2, light was provided from below (556-272 'Artwork Lightbox', RS Components, Northamptonshire, UK) in order to provide illumination of the undersides of the disc and from above for clear observation (4 × fluorescent tubes (F65W/35 General Electric, Hungary) providing approximately 11 Wm^{-2} of visible light). Temperature was maintained by performing the experiment in a constant temperature room maintained at 25 ±2 °C.

In initial experiments 1μl (at a concentration of 10 pg μl^{-1}, 100 pg μl^{-1}, 1 ng μl^{-1}, or 10 ng μl^{-1}) of test substance was added in the centre of the treatment disc and an equal volume of hexane added to the control disc. In subsequent experiments an attempt was made to ensure that the disc surface was more evenly covered in test semiochemical and so an increased 5μl volume was used and presented in five 1μl points. The range of concentrations tested was also further expanded to ensure that any responses, if present, were detected; 0.1 pg μl^{-1}, 10 pg μl^{-1}, 1 ng μl^{-1}, or 100 ng μl^{-1} were assayed. As illustrated in Figure 4.3 these points were added as four points around the edge and one in the centre with four of the droplets reaching the edges of the disc. In this later presentation method the concentration remained unaltered with the volume being increased five-fold.

Several experimental repeats were performed per day (block) and a positive control

(15 male exposed cellulose disc) was included in each block. This exposed disc was prepared using the method described in 4.3.2.

5.3.2 Filter disc binomial-choice assay: observations and statistical analyses

The number of filter disc contacts made by 20 mixed-age female *F. occidentalis*, per dish over 30 min was measured as described in 4.3.3.

ANOVA was carried out to investigate differences in the number of contacts within each trial and was also used to make comparisons between the male-exposed discs and the discs containing synthetic test semiochemical.

5.4 No-choice petri dish observation arena

In an attempt to detect arrestment behaviours and view *F. occidentalis* in the presence of semiochemical but in the absence of a point-odour source a set of six 40 mm petri dishes and corresponding lids (Anumbra, Fisher Scientific, UK) were prepared as described in 2.4. The lid of each dish was coated in either 250μl of hexane control or test semiochemical. In an attempt to prevent the thrips from being in constant contact with the semiochemical application area a thin wire mesh was inserted between the lid and the base. A central acetate strip was used to create a thin transparent line and this was placed underneath the centre of the petri dish base, thus demarcating two halves of the arena. A mono CCD video camera (Sanyo VCB-3385P, Sanyo, UK) with an analogue 12 mm, f:1.4 lens (H1214 FICS-3, Computar, Japan) was placed above the dish at a height (around 30 cm) that enabled focus on the base of the dish. This camera was connected to a video recorder (CTR-3024, Computar, UK) and the events

recorded for the 15 min trial duration.

Lighting and temperature was kept as described in 5.3.1. Thrips were obtained in the required quantities by aspirator as described in 2.3; three mixed-age females were used per bioassay as it would have been difficult to simultaneously observe the behaviours described in 5.4.2 for 20 individuals.

The top edge of the petri dish base was carefully coated with a thin strip of Parafilm M (Pechiney Plastic Packaging, WI, USA). When stretched this was as tall as the base of the petri dish and covered the entire circumference. The Parafilm enhanced the lid seal by increasing the width of the glass base and also provided a malleable surface for the wire mesh to imprint into. Given the small number of individuals it was not necessary to anaesthetise the thrips after aspiration; instead prior to commencing the experiment they were left for a 2 min period in the base of the open petri dish. A fine gauze mesh (20 μm) was then applied to the top of the base and compressed into the Parafilm. The lid (section 5.4.1) was added and an additional 10 $\times$ 50 mm strip of Parafilm M membrane was placed around the edges to create an external thrips-proof seal. See Figure 5.1.

5.4.1 Applying test and control semiochemicals

Prior to being added to its base, the lid of the petri dish was coated in either 250 μl of hexane control or the test semiochemical applied in the same volume. The compounds were rapidly applied in a separate room, and the lid loosely applied in order to reduce possible cross-contamination of odour. Neryl (*S*)-2-methylbutanoate (97.8% enantiomeric excess, NRI, University of Greenwich) and racemic lavandulyl acetate (99% purity, TCI America, Portland, Oregon, USA) were assayed over a broad range of concentrations in this way. Unfortunately (*R*)-lavandulyl acetate was not available

so the racemate was tested instead. The effects of the racemate in the field are discussed in Chapter 7 and it was unclear if similar effects would be observed in this assay. As with the filter disc binomial choice assay, 0.1 pg μl^{-1}, 10 pg μl^{-1}, 1 ng μl^{-1} and 100 ng μl^{-1} concentrations were assayed.

5.4.2 No-choice petri dish observation arena: observations

The number of line crosses over the central acetate line and rapid movements involving departure from, and further contact with a surface, termed here as 'flits', were recorded over a 10 min period. During a 'flit', the individual was required to leave either the base or the lid of the dish by attempted flight and land. Each instance of departure from a glass surface or filter disc and the corresponding landing were recorded as a flit event. Whilst wing flexing prior to the flit was not observable on the video in all incidences, during visual observation thrips were observed to be engaging in this activity prior to flitting and there were no observed incidences of uncoordinated plummeting from the lid or sides of the dish. Individuals leaving the lid in this way were thus counted as flitting individuals.

The 10 min time period composed of two consecutive 5 min time periods following one 5 min period of unrecorded habituation. One block consisting of six trials were performed per day, with four concentrations of the test semiochemical being assayed in four of the trials and two of the trials being hexane controls. Trial order within blocks was allocated randomly.

5.4.3 No-choice petri dish observation arena statistical analysis

In order to normalise the data a $\log_{10}(x+1)$ transformation was applied to both line-crossing and flit data as they were found to be non-parametric. The transformation type was selected due to the presence of some unusually high and low values. ANOVA was used to compare observed counts in treatment levels with observed counts in hexane control levels and a Dunnett's *post-hoc* test was used to compare treatment levels against control.

5.5 Straight-tube bioassay

A no-choice straight-tube bioassay was developed for individual thrips with the aim of rapidly examining the attractiveness of a point odour source to individual thrips. It was envisaged that simple attraction and activation could be easily observed and recorded and that due to the simplicity of the assay, numerous replicates could be carried out per day.

A set of six 75 mm × 4 mm (internal diameter) soda glass tubes (BOC Edwards, Sussex, UK) were placed parallel to each other underneath the camera. See Figure 5.2. Camera setup was as described in 5.4. Each tube was cleaned as described in 2.4. Each tube contained a refuge consisting of half a rolled filter paper disc, (Whatman International Limited (1001020) grade 1, 2 cm diam.) which was carefully inserted 5 mm from the end using forceps. A Parafilm M membrane was added to this end in order to create a thrips-proof seal. For ease of construction, no air-through flow was incorporated into this design.

An individual thrips was then carefully added to the non-sealed side of each of the

six tubes using a distilled water moistened calligraphic paint brush as described in 2.3, and an additional Parafilm M seal added subsequently. In order to reduce potential tube position disruption from the paintbrush, individual thrips were introduced within the first 20 mm of the tube.

Lighting was kept as described in 4.3.2. Thrips were obtained in the required quantities by aspirator as described in 2.3; one non-anaesthetised mixed-age female was used per trial.

5.5.1 Straight-tube bioassay: semiochemical application and observations

Sets of six straight-tubes, containing individual *F. occidentalis* were left in view but untouched for a period of 5 min to allow the thrips to acclimatise. Two acetate strips were placed underneath, and at right angles to the tubes to identically demarcate them each into four, with two lines being positioned 20 mm from each end. A mono CCD video camera was used to record the number of line crossings observed and recorded for 10 min. This duration was split into two and at the end of the initial recorded 5 min phase an injection was made through the parafilm membrane on the non-refuge side in each of the tubes. 1μl of either test semiochemical or hexane control was added using three separate 10μl hexane cleaned syringes (Hamilton, Reno, Nevada). Behaviour continued to be observed and recorded following this for the remaining 5 min providing 10 min total recorded observation.

Six tubes were used per trial with each trial containing two hexane control applications, two treatment neryl (*S*)-2-methylbutanoate applications and two treatment racemic lavandulyl acetate applications. Six 15 min trials (with 10 min observation) were carried out per day, making a block. The experiment was carried out for five days

providing 60 trial replicates for each compound.

It was decided to use the filter disc binomial-choice assay and no-choice observational arena results to select the concentration most likely to elicit a response and assay this with numerous replicates. A concentration of 10 pg μl^{-1} neryl (*S*)-2-methylbutanoate and racemic lavandulyl acetate was thus selected (see 5.6).

5.5.2 Straight-tube bioassay statistical analysis

Line crossing data were compared between treatments. As baseline activity was recorded for five min after the unrecorded acclimatisation period it was possible to compare line crossings in this period with five min period line crossings post injection stimulus. Multiple Mann-Whitney U tests were performed on post-stimulus 5 min line crossing data where non-parametric.

5.6 Results

5.7 Filter disc binomial-choice assay

5.7.1 Male headspace semiochemicals: neryl (*S*)-2-methylbutanoate

As is visible from Figure 5.3, at 30 min females made significantly more contacts with the male exposed disc in comparison to the control disc (ANOVA, $F_{1,7}$=14.06, P=0.007).

When comparing treatment with control contacts within each trial for applied neryl

(*S*)-2-methylbutanoate treatments at several concentrations there was a significant difference only at 10 pg μl^{-1} (ANOVA, $F_{1,7}$=9.66, P=0.017) (Figure 5.3). Whilst no other concentrations gave a significant increase in contacts, average contacts made with treatment discs was higher in all cases suggesting a weak effect may have been present across the assayed concentration spectrum.

As mentioned previously, in an attempt to ensure even coating of the disc and to ensure that some of the compound reached the edge of the disc (this was visually observed), the previous experiment 5.7.1, was repeated using 5 μl neryl (*S*)-2-methylbutanoate, presented in five points (see 4.3) with an expanded concentration range. As is visible from Figure 5.4, and as observed previously, females made significantly more contacts with the male exposed disc in comparison to the control disc (ANOVA, $F_{1,9}$=41.05, P<0.0001).

When comparing treatment versus control contacts within each trial for applied neryl (*S*)-2-methylbutanoate treatments spread over a 5-point application process there was, as with the 1 μl presentation, a significant difference only at 10 pg μl^{-1} (ANOVA, $F_{1,9}$=7.01, P=0.027). As was the case with the previous neryl (*S*)-2-methylbutanoate presentation, no other concentrations resulted in a significant increase in contacts though the average number of contacts made with treatment discs were again higher in all cases indicating the possible presence of a weak effect between 10 pg and 100 ng.

In both 1 μl and 5 μl applications there was a significant difference between the disc containing neryl (*S*)-2-methylbutanoate and the 15-male exposed disc with the male exposed discs eliciting around 3.5 times more contacts than the synthetic neryl (*S*)-2-methylbutanoate exposed discs. Treatment *minus* control contacts for both sets of discs were compared with this value being significantly different (ANOVA, $F_{1,7}$=10.40, P<0.0001 (1-point application); ANOVA, $F_{1,9}$=23.03, P<0.0001 (5-point application)).

5.7.2 Male headspace semiochemicals: racemic lavandulyl acetate

Racemic lavandulyl acetate at 5 μl volumes was tested at a wide range of concentrations (Figure 5.5). As previously a 15-male exposed disc trial was included in each block. There was no significant difference in treatment and control contacts at any of the concentrations tested.

There was a significant difference in the number of contacts between discs containing applied racemic lavandulyl acetate and 15-male exposed discs. As previously, the male-exposed discs in this instance were significantly more attractive than discs with applied synthetic neryl (*S*)-2-methylbutanoate. Treatment *minus* control contacts for racemic lavandulyl acetate discs were compared with treatment *minus* control contacts in 15-male exposed discs in order to confirm this (ANOVA, $F_{1,11}$=12.02, P<0.0001). The data were non-parametric in this instance as a result of several unusual observations (Anderson-Darling normality test P=0.005). It was reasonable to assume an underlying parametric distribution as was the case with neryl (*S*)-2-methylbutanoate synthetic application so this test was still applied.

5.8 Additional known attractants

5.8.1 Semiochemicals: geraniol

Geraniol was tested at 10 ng μl^{-1} (Figure 5.6). As mentioned previously, this compound has shown to be attractive in Y-tube olfactometers (Koschier *et al.*, 2000). No increased contacts with the treatment disc in comparison to the control disc were observed after 30 min (ANOVA, $F_{1,9}$=1.39, P=0.315).

5.8.2 Semiochemicals: *p*-anisaldehyde

The plant fragrance allomone *p*-anisaldehyde was also tested at 10 ng μl^{-1} (Figure 5.7). As with geraniol, no increased contacts with the treatment disc in comparison to the control disc were observed after 30 min (ANOVA, $F_{1,9}$=1.66, P=0.230).

5.9 No-choice petri dish observation arena

5.9.1 Neryl (*S*)-2-methylbutanoate

No significant difference in activity (line-crossing or flit activity) was observed when neryl (*S*)-2-methylbutanoate was tested at a range of concentrations (Figure 5.8).

5.9.2 Racemic lavandulyl acetate

As with neryl (*S*)-2-methylbutanoate, no significant difference in activity (line-crossing or flit activity) was observed when racemic lavandulyl acetate was tested at a range of concentrations (Figure 5.9).

5.10 Straight-tube bioassay

There was no significant difference in post-injection line crossings between hexane control and neryl (*S*)-2-methylbutanoate (Mann-Whitney U, P=0.454). In addition there was no significant difference in post-stimulation line crossings between hexane control and racemic lavandulyl acetate (Mann-Whitney U, P=0.474) nor between neryl (*S*)-

2-methylbutanoate and racemic lavandulyl acetate (Mann-Whitney U, P= 0.108). See Figure 5.10.

5.11 Discussion

5.11.1 Synthetic neryl (*S*)-2-methylbutanoate compared with male-exposed discs

Synthetic neryl (*S*)-2-methylbutanoate causes a contact response when applied at 10 pg μl^{-1} in the filter disc binomial choice assay. This effect is present when the compound is presented in both a central point application and in a higher volume application that enables the compound to reach the edges of the disc.

As can be seen in Figures 5.3 and 5.4, whilst it is possible that neryl (*S*)-2-methylbutanoate is active over a very narrow concentration range, it is likely that the compound is actually active over the full range of tested concentrations (10-100 ng). Though this response is consistently strongest and significant at the 10 pg concentration (30.6% mean trap catch increase with 5 μl application), if the treatment to control ratio is examined for the assayed concentrations it becomes apparent that t:c can be high with no significant difference being present (for example 1 ng resulted in a non-significant 36% mean trap catch increase with 5 μl application). This illustrates a potential discrepancy between statistical significance and observation that is most likely caused by day-to-day variation.

Mean control levels are relatively constant across concentrations suggesting that activation of individuals is not occurring; if this were the case it is possible that thrips would make more random contacts with control discs. Whilst no orthokinesis was

detectable it does not rule out the possibility that klinokinesis is occurring. When neryl (*S*)-2-methylbutanoate contact control levels are contrasted with corresponding live-male exposed controls it is clear that contacts in the latter are higher suggesting activation and/or orthokinesis. As displayed in Figure 4.2, the walking response of a female thrips when presented with discs containing male-derived compounds is quite complicated and it is not easy to elucidate such behaviours in this assay.

The presence of the contact response at 30 min for 10 pg neryl (*S*)-2-methylbutanoate discs suggests that some neryl (*S*)-2-methylbutanoate remains on the disc. An estimate of the rate of production of neryl (*S*)-2-methylbutanoate by live male *F. occidentalis* is around 0.1-0.3 ng male^{-1} h^{-1} (obtained in chapter 3; Dublon *et al.*, 2008). 15 males exposed to the filter disc over a 24 h period, as occurred above would thus result in between 36 - 108 ng neryl (*S*)-2-methylbutanoate being produced. As neryl (*S*)-2-methylbutanoate is produced as a headspace volatile and not detectable by GC in hexane extracted male-exposed filter discs, it is not clear how much of this compound may be retained on the disc surface. This amount may be comparable to the bioactive assayed 10 pg amount.

Estimated male neryl (*S*)-2-methylbutanoate production rates during the disc exposure process (likely to exceed 10 pg) ordinarily result in a strong contact response with many contacts being made, whilst very low concentrations of the compound presented on the disc surface result in a comparatively weaker response. This would suggest that the aggregation pheromone in isolation is not the sole cause of the response as the two responses are not equal in magnitude. This idea is further strengthened where, as established in 4.5.7, female exposed discs are weakly attractive to female *F. occidentalis* though females produce no known aggregation pheromone. Furthermore, the contract response obtained with live male discs is transferrable to new discs upon solvent extraction and reapplication (4.5.8) even though the contents of the extracts are below the GC detection limit and thus unknown.

It is possible that a separate additional male produced compound is responsible for the contact response obtained with live male exposed discs, or alternatively the aggregation pheromone may require a synergist in the form of an additional compound (see 5.11.2). Multiple roles can be associated with multiple male produced pheromones as is the case with the Dictyopteran male speckled cockroach *Nauphoeta cinerea*. Males in this species release two compounds at low concentration that are highly volatile attracting females from a distance (2-methylthiazolidine and 4-ethylguaiacol) and two compounds (3-hydroxy-2-butanone and 2-methyl-2-thiazoline) that cause females to remain close, acting as either an attractant, a sexual stimulant or an arrestant (Sirugue *et al.*, 1992). It is possible that the mode of activity of neryl (*S*)-2-methylbutanoate as a male-produced aggregation pheromone is further aided by additional headspace volatiles such as (*R*)-lavandulyl acetate, or as yet undetected, higher molecular weight hydrocarbons acting over a shorter range.

There was no significant difference in flit or line crossing activity when neryl (*S*)-2-methylbutanoate was compared with corresponding hexane controls or presented without a point-odour souce in the no-choice petri dish observation arena. In addition no increased line crossings were detectable in the straight-tube bioassay. It is clear that the response is highly variable in the no-choice assay. Whilst there appeared to be little difference in line crossings between treatments the mean number of flits was much higher than in the control at all concentrations tested, although this difference was not significant. The variability in flit response necessitates additional replication before any possible difference can be confirmed. This reduces the value of the no-choice assay in this instance.

5.11.2 Synthetic racemic lavandulyl acetate compared with male-exposed discs

Racemic lavandulyl acetate when presented in the filter disc binomial choice assay (Figure 5.5), the no-choice petri dish observational arena (Figure 5.9) and the straight-tube bioassay (Figure 5.10) failed to cause a bioactive response. The role of this compound remains unclear. It is possible that the racemate itself is not bioactive, or that the *R* enantiomer is present at an insufficient concentration and therefore fails to elicit a response. As is the case in nettle caterpillars *Darna trima* (see 1.6.1), it is not uncommon for insects to be able to discern between chiral enantiomers where only one is bioactive. It is also possible in few instances that the presence of the alternate enantiomer may inactivate the bioactive enantiomer. This scenario occurs in the Japanese beetle; *Popillia japonica* where racemic (5*Z*)-tetradecen-4-olide completely lacks biological activity (Howse *et al.*, 1998). When racemic lavandulyl acetate was tested in the filter disc binomial choice assay, treatment contacts remain similar to control contacts at the majority of concentrations. Observed t:c ratio is high when compared to neryl (*S*)-2-methylbutanoate filter disc binomial-choice t:c ratio; this further validates the statistical analysis as they reflect the observations.

In the no-choice assay, in all instances, flits are below corresponding control levels whereas line crossings remain similar. This difference is not statistically significant so whilst it would imply reduced activity where the thrips are arrested or inactivated by this compound it is not possible to state this without additional replication.

In field trials, traps baited with (*R*)-lavandulyl acetate, catch a comparable amount when compared with control traps suggesting the compound whilst not repulsive, does not cause attraction (Hamilton *et al.*, 2005). Further field trial analysis suggests that this compound can be repulsive where control trap catches are significantly more than for treatment (see Chapter 7).

Whilst no effect is detectable in the laboratory assays used above, it was possible that this effect may have been visible in the binomial choice assay where increased control contacts would have been expected if the thrips became agitated and thus activated to move from the source. Such a response was not observed.

5.11.3 Additional compounds and potential artefacts

As established in chapter 4, water when artificially applied to the disc does not appear to increase contacts when compared with dry control. However, as discussed in Chapter 4, point application may not mirror gradual water absorption into the disc and the latter may make the disc more uniformly moist and attractive. It is possible that if the aggregation pheromone is presented on a pre-moistened disc it may become more attractive. This would need to be examined further.

Neither geraniol, nor *p*-anisaldehyde at 10 ng μl^{-1} caused increased contacts when assayed in the filter disc binomial choice assay. Due to time constraints only one concentration was assayed and so additional replications over a wider concentration range are essential. *p*-anisaldehyde (4-methoxybenzaldehyde) has been shown to be effective over a range of concentrations in a Y-tube olfactometer (0.001 - 10% tested in $\times 10$ increments) though other similar volatiles such as salicylaldehyde (2-hydroxybenzaldehyde) were only effective (in this instance they repelled) at 1% and undiluted concentrations (Koschier *et al.*, 2000). Both geraniol (de Kogel *et al.*, 1999) and *p*-anisaldehyde (Tuelon *et al.*, 1993; Koschier *et al.*, 2000) have been shown to be attractive in the Y-tube olfactometer and the former has also been shown to affect trap catch in two moving air bioassays (Frey *et al.*, 1994; Chapter 6).

When investigating the attractive properties of the male and female exposed filter-discs (Chapter 4), no additional compounds were resolvable in GC-MS solvent extracted

filterdiscs exposed to males and females. Whilst several additional compounds were resolvable in male headspace volatiles it is possible that a higher molecular weight hydrocarbon with a signalling role, may be present and hitherto undetected. Cuticular hydrocarbon profiles have been elucidated for several insects including in *F. occidentalis* (Gołębiowskia *et al.*, 2007). DVB/Carboxen/PDMS coated SPME fibres are able to detect compounds with a molecular weight of 40-275 Da. It would be advantageous to use a PDMS 7 μm bonded phase fibre to resolve any higher weight compounds that may be present. Alternative chromatographic columns may aid detection. *Formica pratensis* a species of European red wood ant produces C_{33}-C_{43} methyl alkanes that are implicated in pheromonal communication. These compounds were only detectable using a higher temperature column (S.J. Martin, personal communication, Keele 2008).

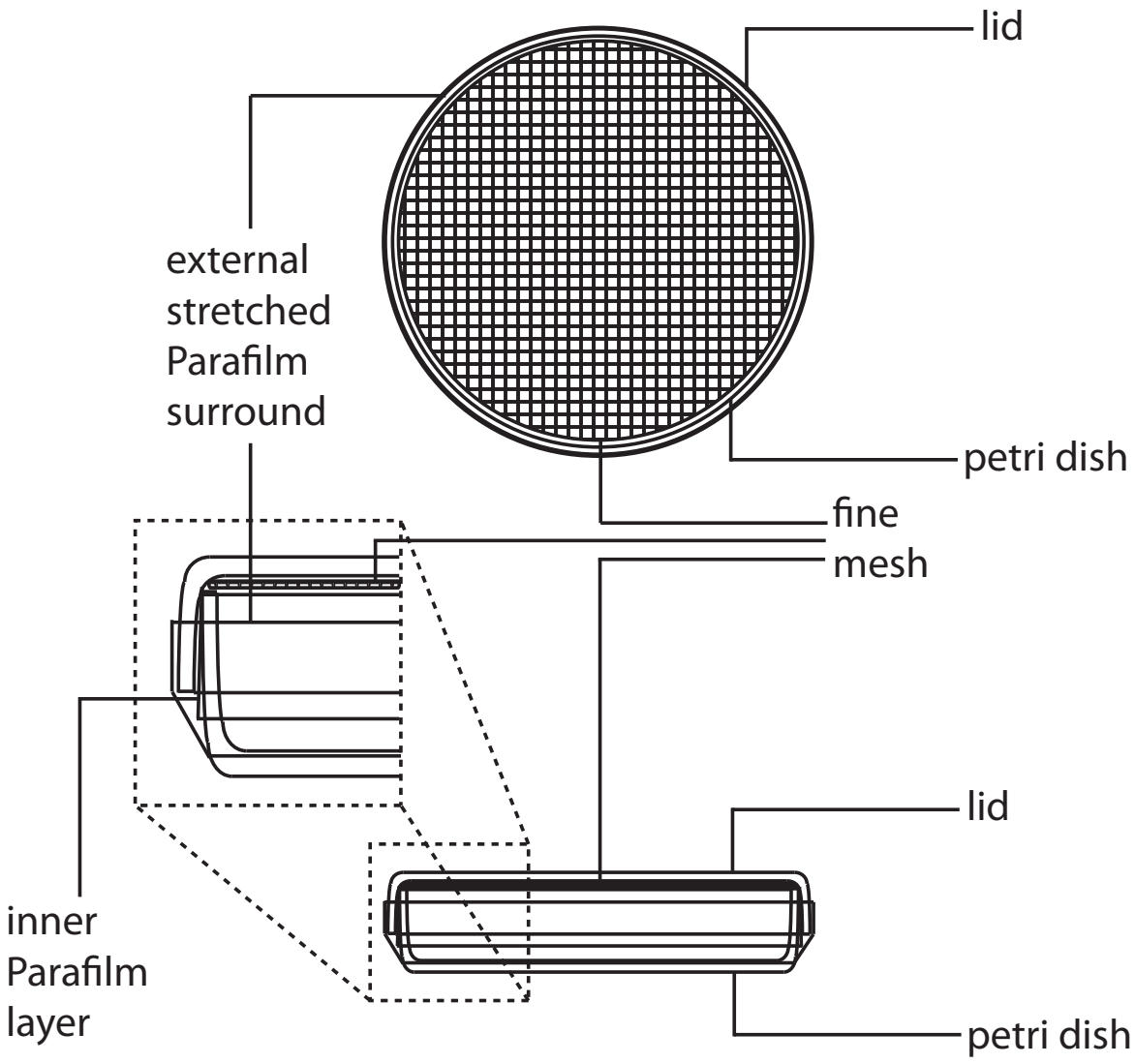

Figure 5.1: No-choice petri dish observation arena, top and side view

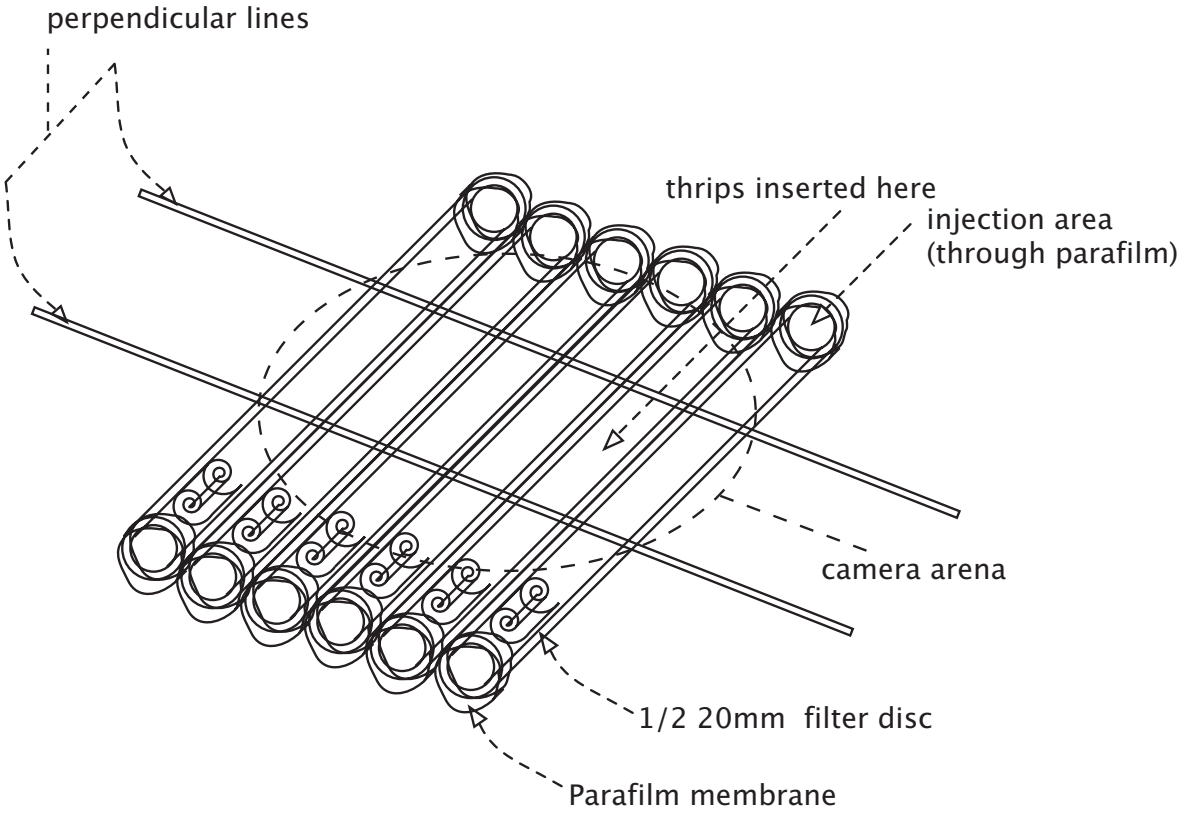

Figure 5.2: Straight-tube no-choice bioassay, exploded view

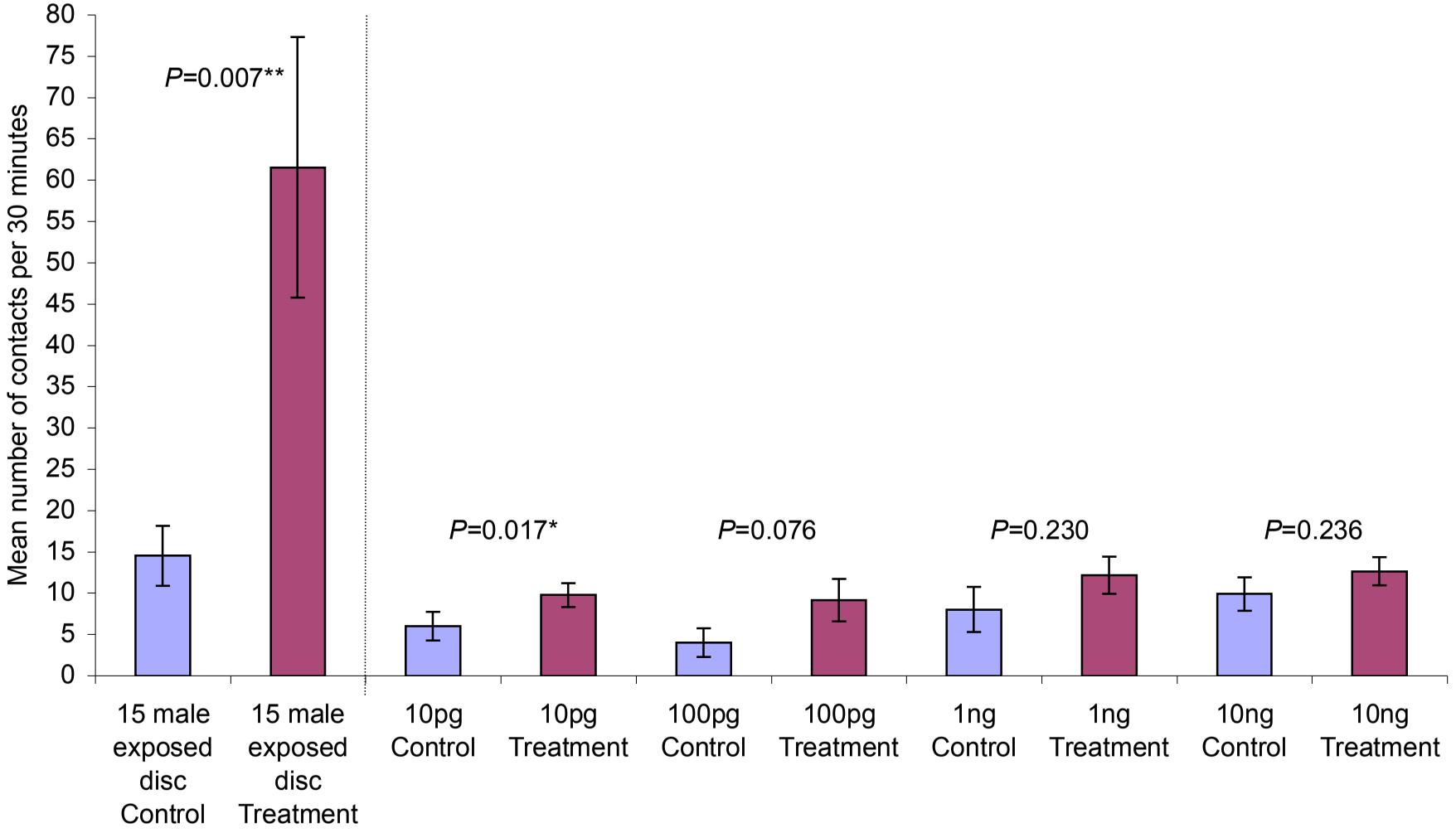

Figure 5.3: Filter disc binomial choice assay. Application of synthetic compounds to treatment filter discs. Mean number (±SEM) filter disc contacts made by 20 females in 30 min on a disc with 1 μl applied neryl (*S*)-2-methylbutanoate compared with hexane control discs (control), n=8.

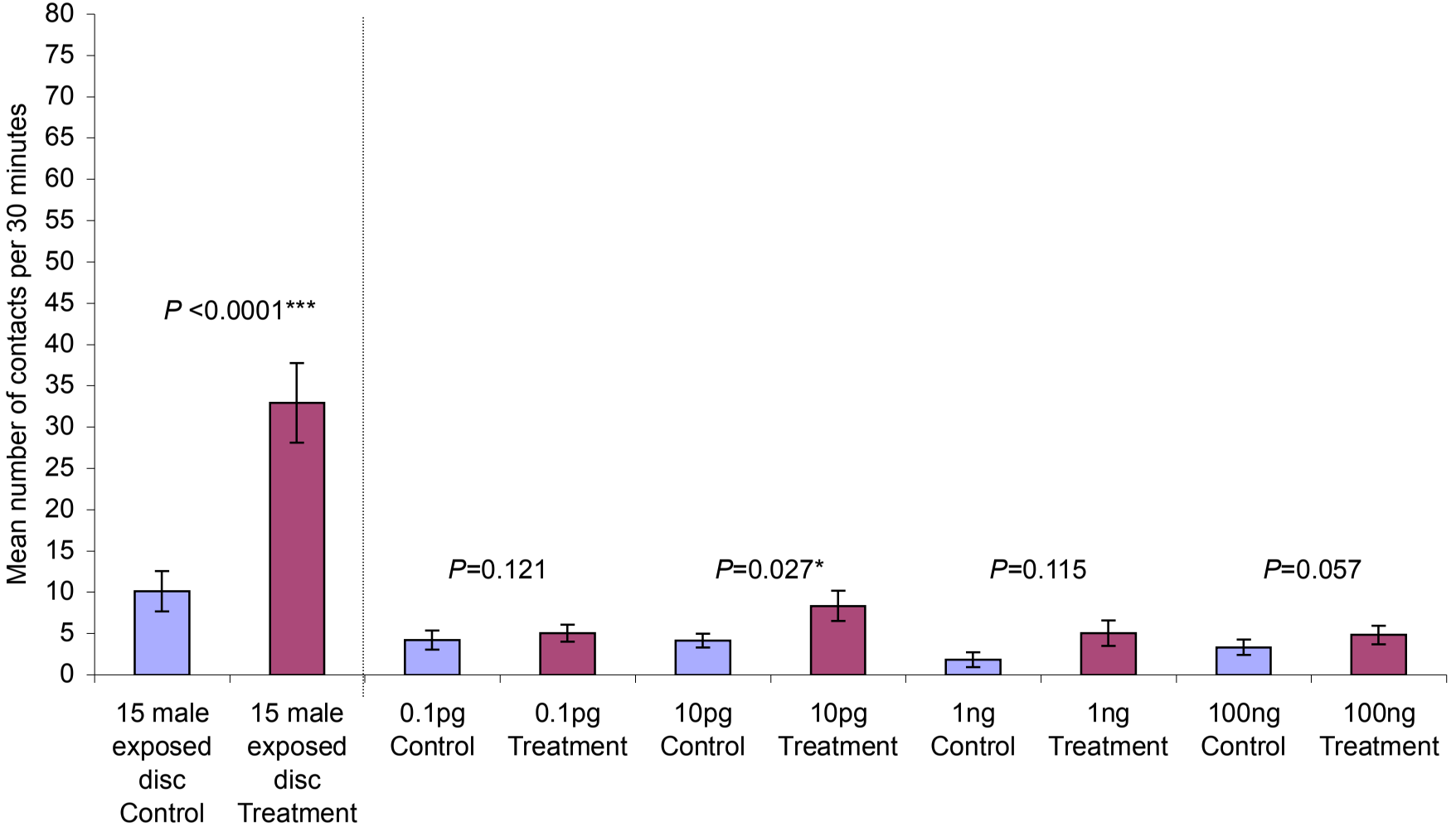

Figure 5.4: Filter disc binomial choice assay. Application of synthetic compounds to treatment filter discs. Mean number (±SEM) filter disc contacts made by 20 females in 30 min on a disc with 5 μl applied neryl (*S*)-2-methylbutanoate compared with hexane control discs (control), n=10.

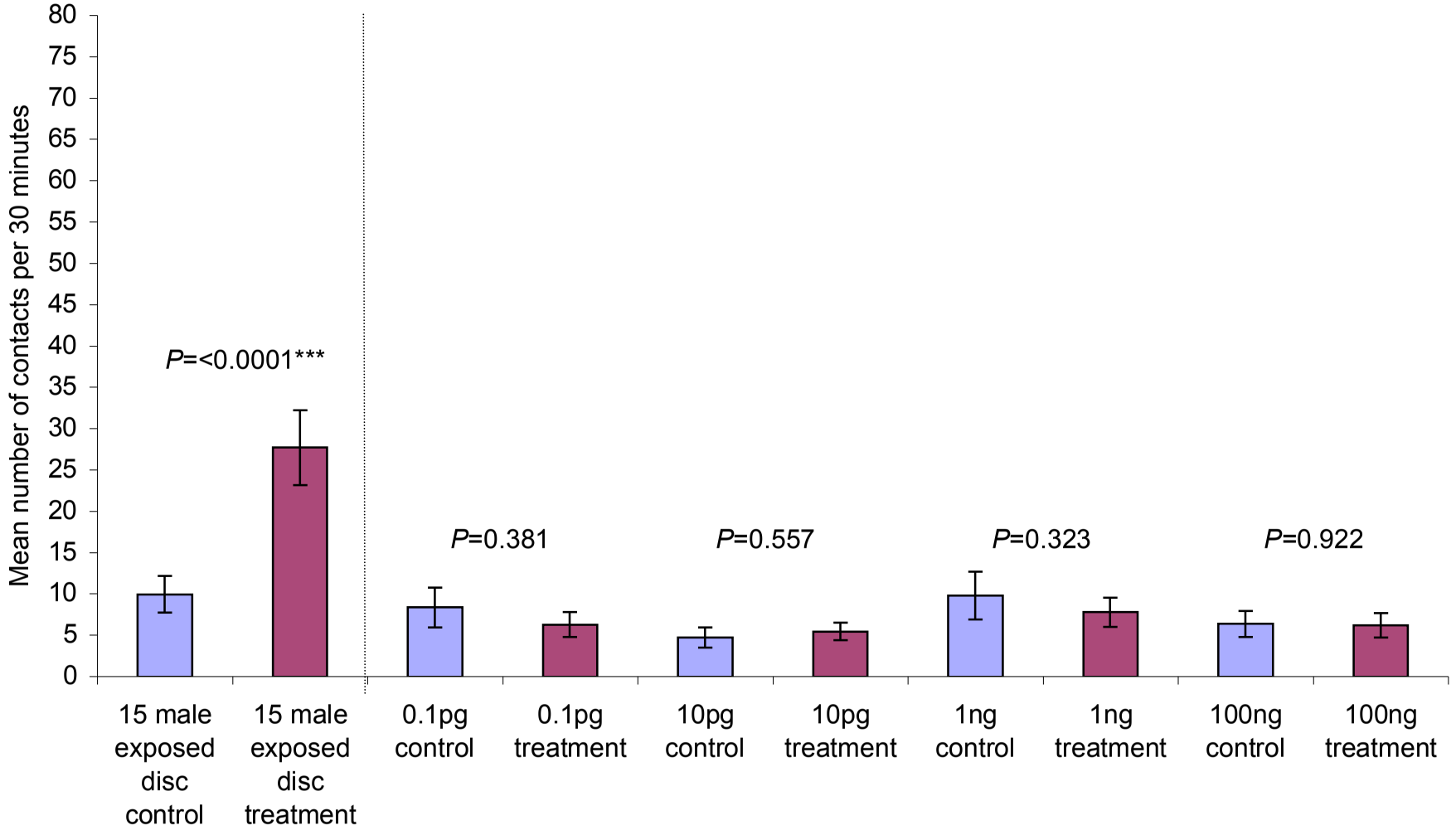

Figure 5.5: Filter disc binomial choice assay. Application of synthetic compounds to treatment discs. Mean number (±SEM) filter disc contacts made by 20 females in 30 min on a disc with 5 μl applied racemic lavandulyl acetate compared with hexane control discs (control), n=12.

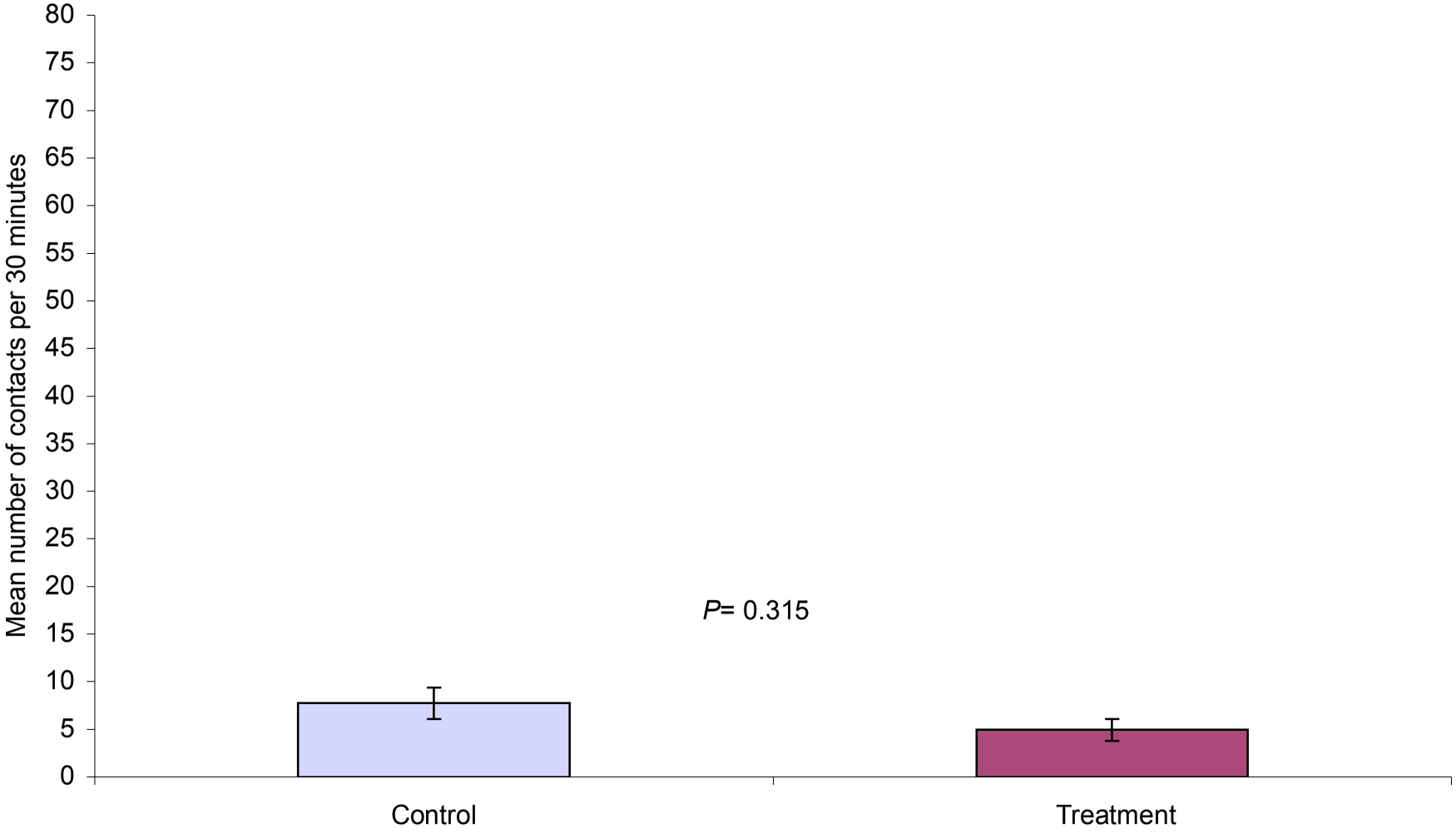

Figure 5.6: Filter disc binomial choice assay. Application of synthetic compounds to treatment discs. Mean number (±SEM) filter disc contacts made by 20 females in 30 min on a disc with 1 μl [10 ng μl^{-1}] applied geraniol compared with hexane control discs (control), n=10. For ease of comparison, the scale from figures 5.3 - 5.5 has been retained here.

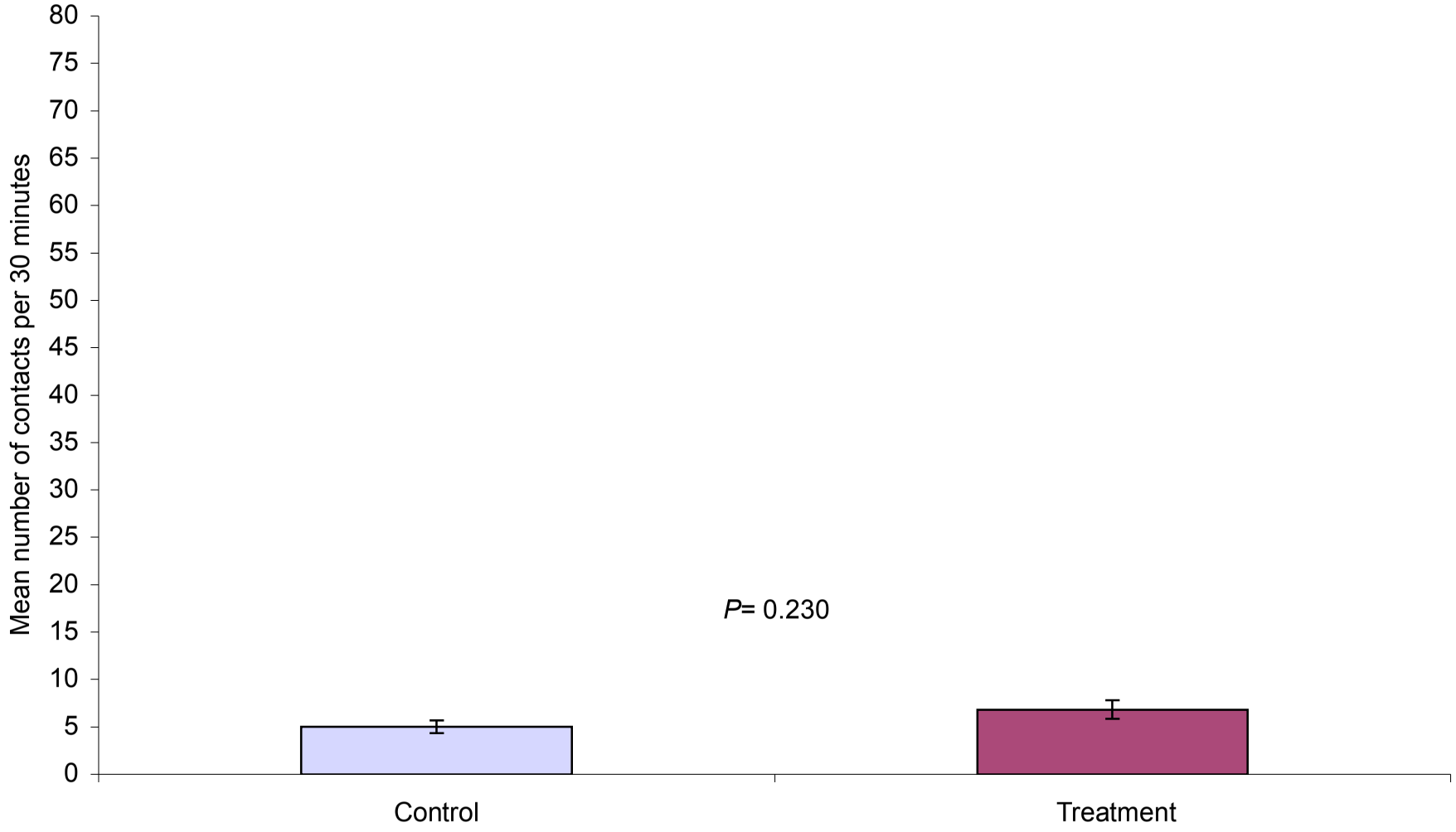

Figure 5.7: Filter disc binomial choice assay. Application of synthetic compounds to treatment discs. Mean number (±SEM) filter disc contacts made by 20 females in 30 min on a disc with 1 μl [10 ng μl^{-1}] applied *p*-anisaldehyde compared with hexane control discs (control), n=10. For ease of comparison, the scale from figures 5.3 - 5.5 has been retained here.

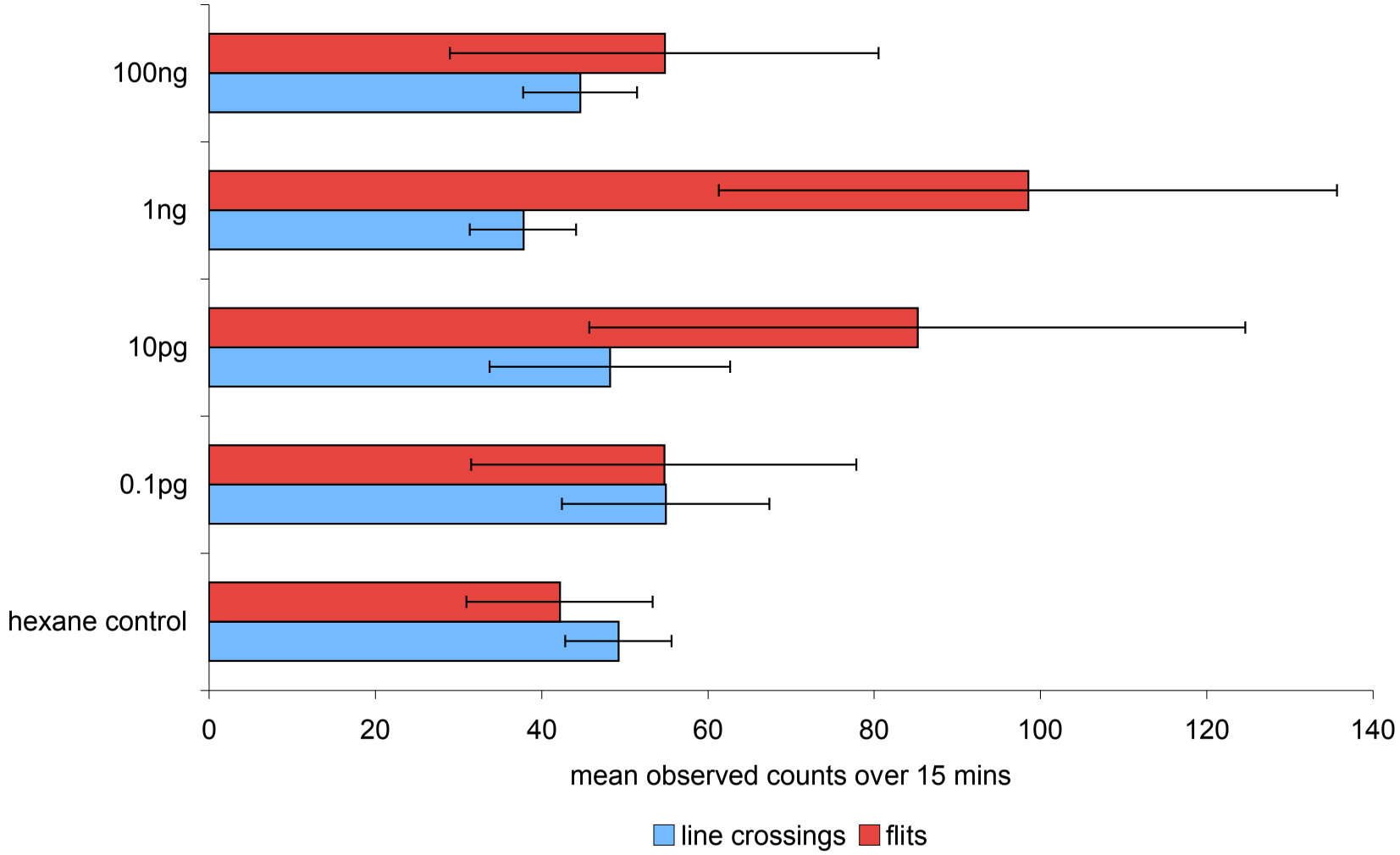

Figure 5.8: No-choice observation arena. Mean number (±SEM) of observed line crossings and flits over 15 min when presented with neryl (*S*)-2-methylbutanoate (n=14). Neither flits nor line crossings were significantly different from control across the concentrations assayed.

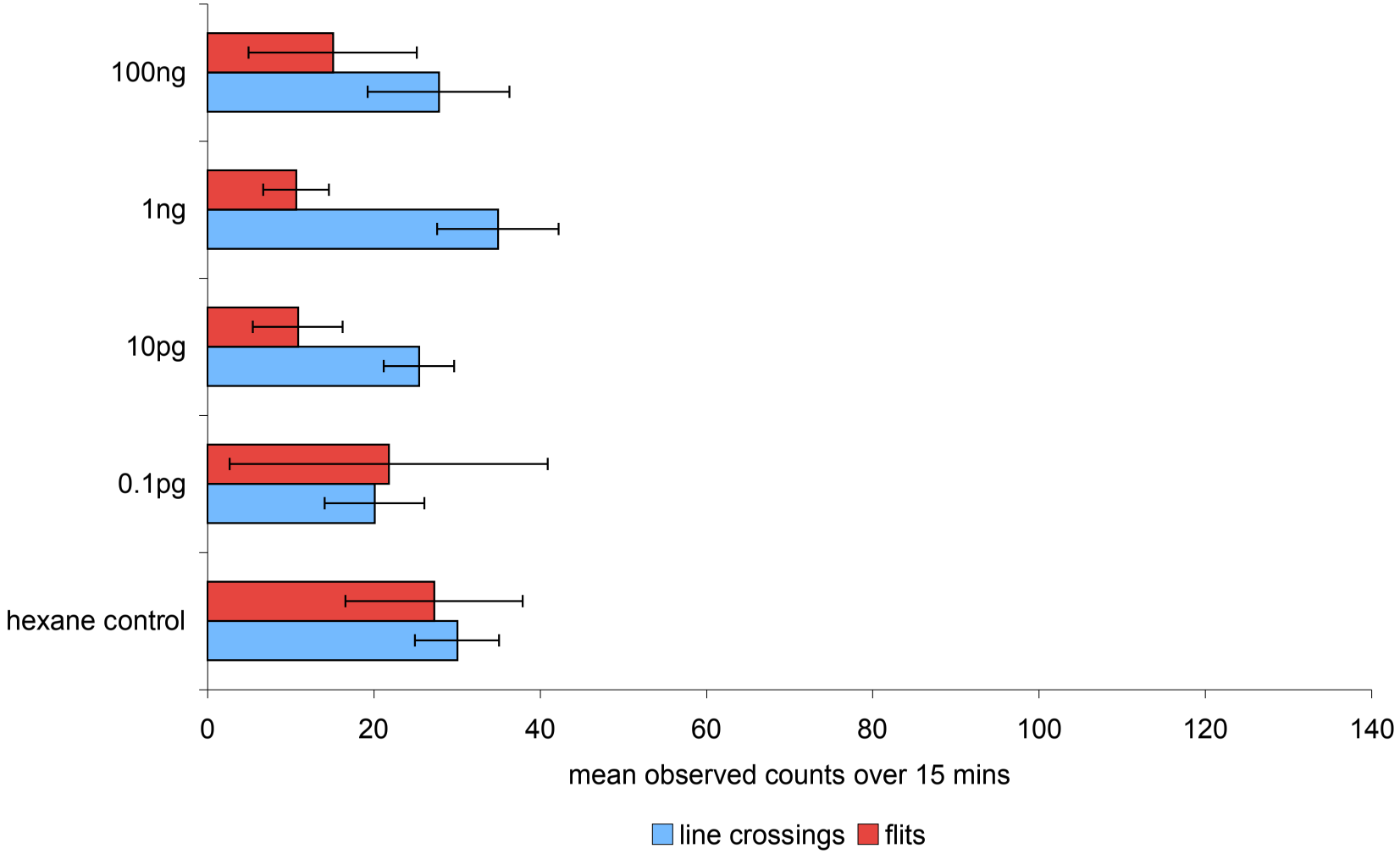

Figure 5.9: No-choice observation arena. Mean number (±SEM) of observed line crossings and flits over 15 min when presented with (racemic) lavandulyl acetate (n=14). Neither flits nor line crossings were significantly different from control across the concentrations assayed.

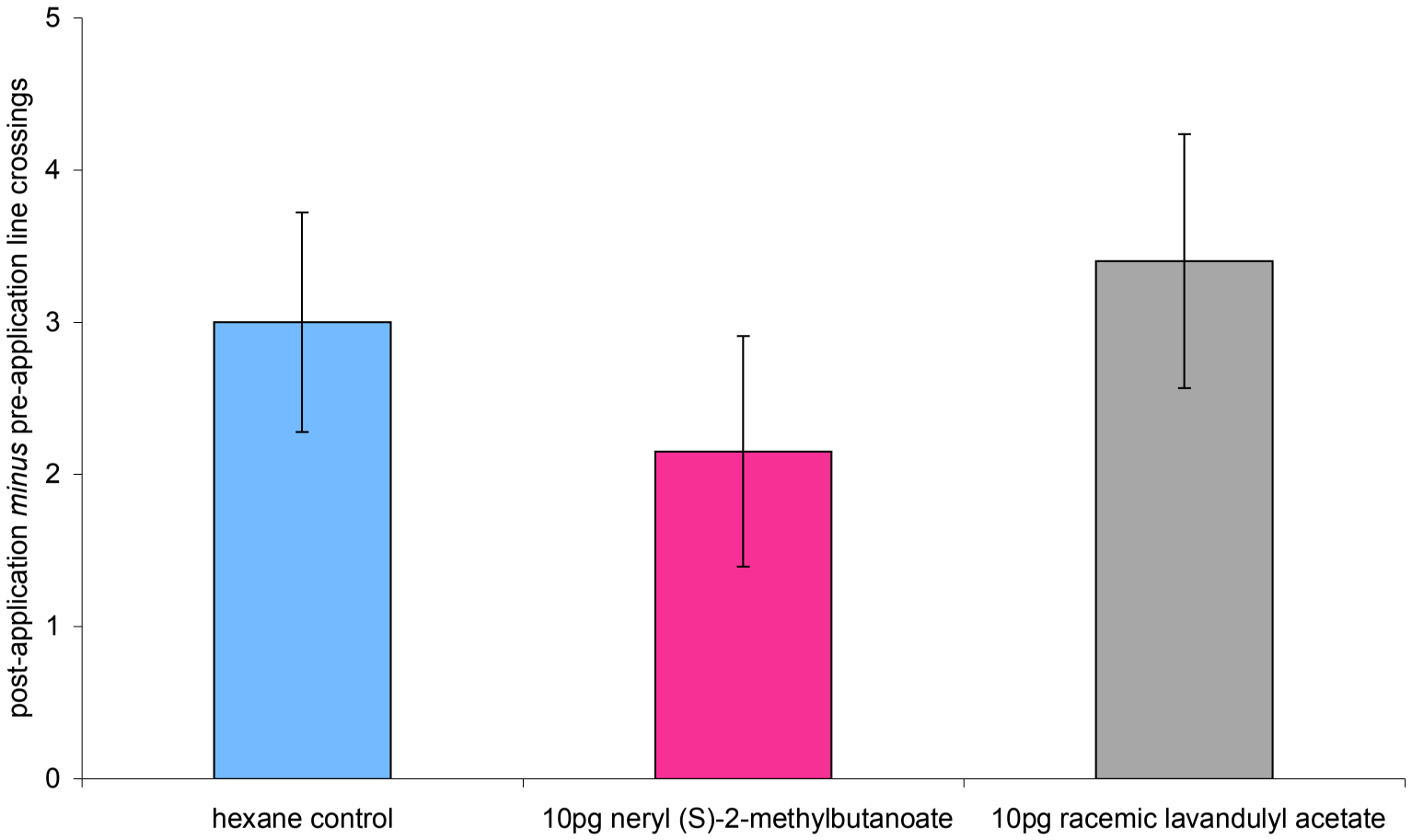

Figure 5.10: Straight-tube bioassay. Examination of activity when individuals are presented with neryl (*S*)-2-methylbutanoate, racemic lavandulyl acetate and a hexane (control) as a point odour source. Figure shows mean number (±SEM) post-application *minus* pre-application line crossings (n=60). Neither neryl (*S*)-2-methylbutanoate nor racemic lavandulyl acetate post application line crossings were significantly different from hexane control.

5.12 References

Dethier, V.G. (1960) The designation of chemicals in terms of the responses they elicit from insects. *Journal of Economic Entomology*, 53 134-136.

Dublon, I.A.N., Hamilton, J.G.C. and Kirk, W.D.J. (2008) Quantification of the release rate of the aggregation pheromone of the western flower thrips, *Frankliniella occidentalis* (Pergande), using solid-phase microextraction (SPME). *Acta Phytopathologica et Entomologica Hungarica*, 43 249-256.

Frey, J.E., Cortada, R.V. and Helbling, H. (1994) The potential of flower odours for use in population monitoring of western flower thrips *Frankliniella occidentalis* Perg. (Thysanoptera: Thripidae). *Biocontrol Science and Technology*, 4 177-186.

Gołębiowskia, M., Maliński, E., Nawrot, J., Szafranek, J. and Stepnowski, P. (2007) Identification of the cuticular lipid composition of the western flower thrips *Frankliniella occidentalis*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 147 288-292.

Gómez, M., García, F., GreatRex, R., Lorca, M. and Serna, A. (2006) Preliminary field trials with the synthetic sexual aggregation pheromone of *Frankliniella occidentalis* on protected pepper and tomato crops in south-east Spain. *IOBC/WPRS Bulletin*, 29 153-158.

Hamilton, J.G.C., Hall, D.R., Kirk, W.D.J. (2005) Identification of a male-produced aggregation pheromone in the western flower thrips *Frankliniella occidentalis*. *Journal of Chemical Ecology*, 31 1369-1379.

Howse, P., Stevens, I. and Jones, O. (1998) *Insect Pheromones and their Use in Pest Management*. Chapman & Hall, London, UK.

de Kogel, W.J., Koschier, E.H. and Visser, H. (1999) Y-tube olfactometer to determine the attractiveness of plant volatiles to western flower thrips. *Proceedings of the section Experimental and Applied Entomology*, 10 131-135.

Koschier, E.H., de Kogel, W.J. and Visser, J.H. (2000) Assessing the attractiveness of volatile plant compounds to western flower thrips *F. occidentalis*. *Journal of Chemical Ecology*, 26 2643-2655.

Sirugue, D., Bonnard, O., Le Quere, J.-L., Farine, J.-P. and Brossut, R. (1992) 2-methylthiazolidine and 4-ethylguaiacol, male sex pheromone components of the cockroach *Nauphoeta cinerea* (Dictyoptera, Blaberidae): a reinvestigation. *Journal of Chemical Ecology*, 18 2261-2276.

Tuelon, D.A.J., Hollister, B. and Cameron, E.A. (1993) Behavioural responses of western flower thrips to anisaldehyde, and implications for trapping in greenhouses. *IOBC/WPRS Bulletin*, 16 177-180.

Chapter 6

Flight responses of adult females to synthetic compounds

6.1 Introduction

6.1.1 Air-flow and insect in-flight behaviour

Flight bioassays carried out in the laboratory can provide an indication of the behavioural responses that may be exhibited in the field. Orientation in the field requires that the insect orient itself in the presence of numerous environmental exogenous factors. It has been argued that insects that behave anemotactically and fly upwind should be assayed in flight (Wright, 1958) though it is generally accepted that a walking response may provide a good indication of flight behaviours. No laboratory flight bioassay can truly reflect field flight conditions so it is important to perform field trials in addition.

In a Y-tube olfactometer when walking in wind towards an odour, orientation is

straightforward as it requires only mechanoreceptive input. Upwind flight is more complicated; positive anemotaxis may occur where the insect senses sufficient odour molecules to initiate flight. Identification of areas in the odour plume with the highest number of odour molecules occurs, enabling the insect to 'lock on' (Kennedy, 1983) to this stream. In some lepidopteran moths, complex casting behaviour may occur where the insect corrects its orientation to remain within the part of the plume with the highest concentration. In strong winds the insect must determine the winds direction by visually observing deflection from its heading (Murlis *et al.*, 1992). Orientation to the odour source thus results. See Murlis *et al.*, (1992) for a comprehensive review of in-flight odour orientation.

6.1.2 Flight responses in the laboratory

Flight bioassays for insects have traditionally relied on the use of large-scale windtunnels. Whilst such apparatus can be most effective when used to screen semiochemicals they are costly to manufacture and maintain.

Successful windtunnels have been created for Thysanoptera at Wageningen UR, Netherlands (W.J. de Kogel, personal communication, Wageningen 2004) and these have been used to assay *F. occidentalis*. This facility has been replicated in CFR, Christchurch, New Zealand (Teulon *et al.*, 1999, Teulon *et al.*, 2005) although construction difficulties were experienced (M.M. Davidson, personal communication, California 2005).

A vertical flight chamber, with a main artificial light source has been designed for phototactic insects (Blackmer & Phelan, 1991). In this system the take-off propensity of the nitidulid coleopteran *Carpophilus hemipterus* in the presence of food odours and the effects of odour on phototactic orientation were investigated.

Smaller-scale flight bioassays for *F. occidentalis* have been developed with constant air through-flow. A novel small-scale flight bioassay has been used to investigate odour and visual cues in *F. occidentalis* (Frey *et al.*, 1994). In this assay, thrips were placed into a 30 μm mesh cage connected to a fan containing treatment and control plants. Individuals were able to choose accordingly between semiochemicals dispensed from filter paper placed within the plants. Whilst accepted as a viable assay where a significant behavioural response was observed with geraniol, the methods employed are difficult to replicate. It is also possible that the behavioural responses to this compound were affected by extraneous and potentially bioactive plant volatiles present in this environment.

A key component of the Y-tube bioassay is the presence of an air current flowing against the direction of the thrips movement. It is unclear whether thrips would be likely to fly in a still-air environment; attempts were thus made to create a simple still-air closed flight arena where flight responses to semiochemicals could be assayed in the absence of air-flow.

As a result of their small size, it was unclear what behaviours would be exhibited by thrips in the flight bioassay. It is possible that the odour may saturate the air-space, inhibiting take-off where the concentration is incorrect. Alternatively, should take-off occur perhaps thrips would land in the general area of the semiochemical as opposed to landing directly on the precise odour source (Berry *et al.*, 2006).

6.1.3 Experimental aims

This chapter presents attempts at developing a small scale *F. occidentalis* flight bioassay, where individuals are able to distinguish between semiochemicals. Both still-air and moving-air bioassays were devised with the former being more simple in design and

thus easier to construct. It was hoped to obtain a reliable flight bioassay that would be capable of assaying the aggregation pheromone neryl (*S*)-2-methylbutanoate over a range of concentrations.

6.2 Materials and methods

6.3 The still-air flight bioassay

A still-air flight bioassay was created using pairs of thin layer chromatography (TLC) chambers. These are glass tanks formed from a single piece of glass with a detachable lid. A no-choice experiment was thus designed where within a pair of TLC chambers, one contained a scented trap and another a control trap. Trap catch was compared at successive timepoints. A choice experiment was not considered to be possible as the odour would affect both traps in the small space. See Figure 6.1. If the test compound elicited a bioactive response it was possible that more individuals would be caught on scented traps within the pair. Several TLC chambers were used in pilot experiments prior to the purchase of four identical Camag chambers and lids (022.5250, Camag, Switzerland) with similar dimensions (204 × 217 × 62 mm (h × l × w). These chambers featured clear transparent glass aiding thrips viewing and unlike the previous chambers they were not obsolete and thus could have been replaced had they broken during cleaning.

Temperature was regulated for pairs of chromatography chambers by placing them upon a slide heating tray (Weiss-Gallenkamp, Loughborough, UK) maintained at 25°C. A light rig (Philips TLD 18W/35, Philips, UK) providing approximately 6 Wm^{-2} was placed above the chambers. In order to prevent semiochemical odour filling the room whilst the chambers were being prepared, the entire experiment was carried out in a

fume cupboard with a flow rate of 0.5 ms^{-1}. During the experiments the chambers were sealed with their lids, so this air flow did not affect the experiments.

One trap per chamber was suspended vertically from the centre of the chamber lid with wire attached to the lid centre (Oecos Ltd, Kimpton, Hertfordshire, UK); 20 mm of a 50 mm metal wire with a 90° bend at 30 mm was adhered to the lid using a 15 mm length of Scotch tape (3M United Kingdom, Bracknell, UK). This in turn was connected to a hand cut 40 mm^2 square "wetstick" wet double sided yellow sticky trap (Oecos Ltd, Kimpton, Hertfordshire, UK) with a central 5 mm^2 cellulose filter paper (Grade 1, 42.5 mm, Whatman International Limited, Maidstone, UK) square semiochemical application site. The wire was threaded through a hole 3 mm from the edge of the trap. The placement of this hole was initially the top corner though after experimental observation of thrips escaping from the suspending wire, in the final design this was altered to the middle top of the trap. In an attempt to prevent thrips escaping from the trap via the wire, the wire was coated in wetstick glue. Despite using the wet traps thrips were routinely observed to walk over the wet trap surface. Two separate 10 μl hexane rinsed GC syringes (Hamilton, Reno, NV, USA) were used to apply test semiochemical or hexane control to individual filter paper squares in the centre of the traps.

Within pairs, the chambers were randomly designated as treatment or control with their positions and arrangement on the slide heating tray remaining constant. This reduced the effect of any directional bias. 25 mixed age female *F. occidentalis* were added to each chamber by placing a glass aspirator vessel directly underneath the trap in the centre of the chamber. Trap catches were recorded at 30 minute intervals for a period of 120 minutes (see Figure 6.1). 50 mixed-age female thrips were used at one time with 25 individuals per chamber. It was only possible to run two chambers at one time. One trial was run per day, with start time being kept constant (14:00 h).

To obtain a positive control and thus ensure the apparatus was allowing the thrips to discriminate between odours, geraniol (Gold Grade 98%, Sigma-Aldrich, UK) was selected as a test compound. 10% geraniol (in 1μl paraffin oil) has been shown to attract *F. occidentalis* in a Y-tube olfactometer (Koschier, *et al.*, 2000). The compound has also been shown to be biologically active with *F. occidentalis* in a flight bioassay (Frey *et al.*, 1994) and to increase trap catch in several species of flower-dwelling thrips (Kirk, 1985). As it was unclear at what concentration geraniol would be effective it was assayed in the first instance at 10 ng.

The male headspace component neryl (*S*)-2-methylbutanoate was also assayed.

Glassware was prepared following the general methodology detailed in section 2.4. The TLC chambers and corresponding lids were baked overnight in order to remove extraneous odour sources.

6.3.1 Still-air flight bioassay observations and statistical analysis

Trap catches in each chamber were determined at 30 min intervals for a period of two hours. 2-way ANOVA with pairs of chambers treated as blocks was used to compare treatment and control trap catch. Analysis was separate for each time-point. The SEM error bars on still-air flight bioassay graphs include day variation which was removed by the ANOVA so the displayed error bars exaggerate the variability when comparing treatment and control at any one time.

6.4 The moving-air twin chamber bioassay

A novel small-scale flight bioassay was constructed from two modified culture flasks. Using the design described below, it was envisaged that thrips would fly against an air-flow, towards a baited or unbaited semiochemical trap with the two traps being in two separate flasks. A no-choice experiment was thus designed where two culture flasks each contained one vertically suspended trap that was baited with either hexane control or the semiochemical being assayed. Thrips were released downwind and the numbers trapped in both chambers, recorded and compared at successive timepoints. A current of air from a cylinder source was supplied in order to create air through-flow within the chamber. It was envisaged that trap catch would be elevated in the presence of an attractant semiochemical. See Figure 6.2.

A Quickfit two litre culture flask (FV2L, Quickfit, Fisher Scientific, UK) was modified to incorporate an additional bespoke centralised female ground glass BL24/29 port at the base (Scientific Glass Ltd., Hanley, UK). Added to this port was an extra male BL24/29 ground glass insert with a hollow tube in the centre. This tube was 6 mm inside diameter on the side inserted into the culture flask port and in order to accommodate the external air supply it was narrowed to 3 mm inside diam. on the outermost side (Scientific Glass Ltd., Hanley, UK). See the lower illustration in Figure 6.3. A rolled-up cellulose 20 mm diam. semiochemical source filter paper (1001020 grade 1, Whatman International Limited, Maidstone, UK) was inserted, using clean forceps into the narrower part of the tube. When inserted the extra glass insert also provided a 5 mm protrusion into the main body of the culture flask upon which to suspend a sticky trap (Oecos, Kimpton, Hertfordshire, UK). Square 80 mm^2 wetstick traps were cut to size and a centralised hole was created using a cork borer (Weiss-Gallenkamp, Loughborough, UK). This enabled them to be suspended on the glass insert (8 mm diam.).

The culture flask was sealed using a corresponding Quickfit multi port culture flask injection head (MAF 2/32, Quickfit, Fisher Scientific, UK) secured to the culture flask using three Quickfit clamps (JC35, Quickfit, Fisher Scientific, UK). Two of the three smaller female ground glass BL19/26 ports on the injection head were stoppered with proprietary Quickfit BL19/26 ground glass stoppers. An exhaust tube (Tygon R-3603 Laboratory Tubing, Saint-Gobain Performance Plastics, Paris, France) was placed into one of the remaining unstoppered BL 19/26 ports and secured in-place with PTFE tape (Z104388, Sigma-Aldrich, UK). A custom made glass male BL24/29 insert (Scientific Glass Ltd., Hanley, UK) was placed into the remaining larger diam. female BL24/29 port. This provided both an interface to accommodate the aspirator vessel (containing the thrips) (170 mm in length, 22 mm inside diam. narrowing to 11 mm) and also a 23 mm protrusion into the main body of the culture flask. The protrusion ended in an edge thus encouraging the initiation of flight by the thrips. Thrips were aspirated directly into the aspirator vessel and introduced without delay. See Figures 6.2, 6.4 and 6.3.

Glassware was prepared following the general methodology detailed in 2.4.

In order to avoid contamination with air from the experimental room or from the exhaust air, a zero-grade air source (<1 ppm CO_2, <3 ppm (vol) H_2O and 20% O_2, British Oxygen Company, UK) was used to provide air throughflow from the filter paper odour source side. This air left the cylinder and was passed through a carbon trap (Alltech, IL, USA) and subsequently split using a Swagelok T connector (Swagelok, Warrington, UK). Air outputs were then passed through two flow-meters (PMRI-011426 Rotameter, Supelco, Bellefonte, PA, USA). A carboloy bead was used in these instruments to produce a constant output of 80 mm s^{-1} (with 40 mm s^{-1} entering each chamber, and decreasing as the flask sectional area increased). Thrips can fly against speeds of up to 100 mm s^{-1} (Teulon *et al.*, 1999). A flow rate of 100 mm s^{-1} has also been used to promote a walking response in a Y-tube olfactometer with air entering at 50 mm s^{-1}

from both arms (Kirk & Hamilton, 2004). Flow rates were periodically checked on both flow-meters using a manual bubble flow-meter (Supelco, Bellefonte, PA, USA). In an attempt to reduce extraneous odour sources, inert FEP grade tubing (Sigma-Aldrich, Poole, UK) and Swagelok compression fittings (Swagelok, Warrington, UK) were used throughout.

As thrips activity patterns often vary throughout the day (O'Leary & Kirk, 2005), experiments were carried out over the same time period, (commencing at 14:00 h) and in a constant temperature room (see 2.6). Overhead lighting was provided using a light-rig as described in 4.3.1.

6.4.1 Moving-air twin chamber bioassay observations and statistical analysis

Trap catch in each chamber was examined at 30 min intervals for a period of 150 minutes. As previously described in section 6.3.1, 2-way ANOVA was again used to compare treatment and control trap catch at successive timepoints. Treatment and control pairs were classed as block, with one block per day. As with the still-air bioassay the SEM error bars on moving-air flight bioassay graphs include day variation which was removed by the ANOVA so the displayed error bars exaggerate the variability when comparing treatment and control at any one time.

6.5 Results

6.6 Still-air flight bioassay initial experiments

As mentioned previously, in order to achieve a viable olfactometer design, several systems were tested in pilot experiments over several months. Subsequent refinements led to a finalised design and these data are presented in section 6.7.

6.6.1 Take-off in the absence of an air current

Initial experiments demonstrated that *F. occidentalis* females will fly in a still-air TLC chamber environment with no semiochemical stimulus. Several experiments were carried out with varying temperatures and time durations of 180-240 min (data not presented).

6.6.2 Trap catch with geraniol treatment

Geraniol was assayed in several types of still-air TLC chambers. See Table 6.1 for a summary.

In the first instance, in pilot experiments, 1μl [10 ng/μl] geraniol in hexane was administered to the treatment TLC chamber and trap catch compared against a corresponding hexane control chamber (internal dimensions: height 196 mm, length 222 mm, width 80 mm, Desaga 120100, Heidelberg, Germany). As is evident from Figure 6.5, the addition of 10ng/μl geraniol gave a significant increase in trap catch after 120 min (ANOVA, $F_{1,5}$=7.81, P=0.038). There was a significant difference in overall trap catch between blocks suggesting variability in response (ANOVA $F_{1,5}$=5.54, P=0.042).

6.6.3 Trap catch with geraniol treatment and extended time-frame

The time period was extended to examine potential effects on trap catch over a longer time period. This necessitated some minimal changes to the experimental environment as a different fume cupboard was needed due to the increased 4 hour duration. Observations were made every 30 minutes instead of every 5 minutes as this was more straightforward (Figure 6.6).

As is evident from Figure 6.6, whilst thrips contacts were elevated on the treatment trap when compared to the control, there was no significant increase in mean trap catch after 120 min (ANOVA, $F_{1,5}$=3.77, P=0.110) or 240 minutes (ANOVA, $F_{1,5}$=1.43, P=0.286). The lack of significance at 120 min contrasts with the previous result. There was once again a considerable variation in day-to-day response at both 120 and 240 min respectively (ANOVA, $F_{1,5}$=23.52, P=0.002, $F_{1,5}$=12.57, P=0.007).

The results of the initial and subsequent 120 minute experiments (Figures 6.5 and 6.6) are combined in Figure 6.7. There is a significant difference between trap catch in the combined data at 120 min (ANOVA, $F_{1,11}$=10.56, P=0.008) with a highly significant effect of day (ANOVA, $F_{1,11}$=11.74, P<0.0001).

6.7 Final still-air flight bioassay results

6.7.1 Trap catch with geraniol compared with control

1μl [10ng/μl] geraniol was assayed using the finalised, similar sized Camag TLC chambers. In previous observations, despite the presence of strong adhesive, thrips were

routinely observed walking over and sometimes departing the trap surface. As the suspending wire was less sticky it was considered possible that this was an easier method of escape in comparison to the trap surface. In an attempt to reduce numbers reaching the wire, trap orientation was altered slightly; traps were suspended with their lowest edge horizontal to the base of the chamber and not in a diamond shape as previously. It was hoped to lower the frequency of individuals encountering the wire by preventing two of the trap edges leading towards it.

As is evident from 6.8 there was no significant difference in thrips caught per trap at 120 minutes (ANOVA, $F_{1,11}$=0.02, P=0.898). This contrasts with the previous pilot geraniol experiments, performed under similar conditions. It is possible that this contrast can be explained by random variation in response. As was recorded previously, there was a significant day-to-day effect in trap catch (ANOVA, $F_{1,11}$=7.64, P=0.001).

6.7.2 Trap catch with neryl (*S*)-2-methylbutanoate compared with control

The male produced aggregation pheromone, neryl (*S*)-2-methylbutanoate was assayed at 1μl [10ng/μl] (Figure 6.9). There was no significant difference in thrips caught per trap at 120 minutes (ANOVA, $F_{1,9}$=0.43, P=0.589).

6.8 Moving-air twin chamber flight bioassay

6.8.1 Bias tests and pilot experiments

Bias tests were performed to detect possible variations in trap catch caused by differences in the flask position (i.e. left or right positional bias). There was no positional

bias detected after 120 minutes when left and right chamber trap catches were compared (ANOVA, $F_{1,4}$=2.04, P=0.226).

Over the course of several months, in an attempt to gain increased trap catch in the moving-air bioassay with a known attractant (geraniol) many variables were adjusted (data not shown). Time period was extended as it was noted that large numbers of thrips were not trapped after 120 minutes. Large numbers of thrips (50 per trap) were used as it was hoped that large differences in trap catch between treatment and control traps would increase the likelihood of detecting a significant difference, should one be present. Semiochemical volume was increased to 2 μl. It was hoped that by doubling the volume and in doing so doubling the concentration, there would be a higher likelihood of odour molecules reaching downwind of the test individuals. As trap catch after 120 min was comparatively low given the number of individuals released time period was further extended to 150 min. A large trap size was used in an attempt to provide a large visual stimulus that was visible from the inside edge of the thrips insertion port. It was hoped that having a large visible square 80 mm^2 trap would encourage take-off.

6.8.2 Trap catch when presented with geraniol

As is evident from Figure 6.10 there is a significant difference at 150 minutes between treatment and control mean trap catch when assaying 2 μl [10ng μl^{-1}] (ANOVA, $F_{1,11}$=7.05, P=0.022).

6.8.3 Trap catch when presented with neryl (*S*)-2-methylbutanoate

Due to time and thrips colony constraints, neryl (*S*)-2-methylbutanoate was only assayed in an earlier experiment using smaller square 40 mm^2 yellow traps and 25 thrips

per chamber. 1 μl [10ng μl^{-1}] did not result in a significant difference in trap catch after 120 minutes (ANOVA, $F_{1,6}$=1.00, P=0.356) (see Figure 6.11).

6.9 Discussion

The laboratory observed flight response of *F. occidentalis* in the apparatus used appears to be highly variable. Whilst it would appear that both assays show some promise they were both time consuming to design and thus remain an unreliable method of screening attractant semiochemicals in the laboratory. The moving-air twin chamber bioassay was also colony intensive, requiring 100 thrips per trial.

6.9.1 The still-air flight bioassay

It is clear that individual thrips will take off and land in the absence of an air-current. Individuals were observed to climb the sides of the aspirator vessel in which they were introduced and take-off from its edge. Whilst the still air bioassay was relatively easy to construct, requiring no custom glassware modifications, the trap response remained highly variable.

This assay provides an indication of trap arrival though it has a flaw in that any instances of arrival and subsequent departure that fall between measurements remain unaccounted for. Where traps record a large number of thrips arrivals this would ordinarily not present a problem but where treatment and control trap catches differ by only a few thrips, as in this instance, it becomes increasingly important. The trap used was of the "wetstick" variety and therefore more sticky than the "dry" traps ordinarily used in field trials. As mentioned previously despite the presence of strong adhesive, thrips were routinely observed walking over the trap surface. This observation

necessitated the change of trap orientation and attempts were made to video record the thrips whilst upon the trap surface. This proved impossible with our apparatus due to the small size of the thrips, the two sides of the trap and the interference caused by the optical qualities of the glass sides of the chambers.

Trap catch was statistically similar in the majority of treatment and control instances and it is likely that the visual stimulus of the colourful trap and additional random collisions may have contributed to this. Transparent traps would be needed in order to eliminate the effects of visual stimulus.

In all cases the traps were oriented with the trap face vertical, facing the long sides of the chamber. This meant that there was a visual stimulus from both the left and the right of the chamber base with the aspirator vessel containing the thrips being placed directly below the suspended traps. It was hoped that this would provide less of an immediate visual stimulus for those thrips that departed directly from the edge of the aspirator vessel thus reducing control catch and so allowing for a larger difference from treatment.

Whilst it is possible that individual thrips could have reached the trap by walking, it can be considered unlikely in that the individual would have been required to negotiate a large surface area of glass and would have needed to walk down the wire. No thrips were observed doing this.

The still-air flight bioassay uses glass chambers and so whilst strongly illuminated from the light rig above, the U.V. component would have been reduced beyond the glass lid. This was also the case for the moving-air twin chamber bioassay. Light is not necessary for take-off in *F. occidentalis* though UVA and human-visible white light are both able to stimulate take-off (O'Leary & Kirk, 2005). Construction of the assay lid with U.V. transmissible plastic would enable UVA transmission though it would further complicate the cleaning of the apparatus.

It is unclear why there were increased trap catches with 10 ng μl^{-1} geraniol when compared with control in one type of TLC chamber and why this effect was lost in the final chamber design. Both chambers were comparable in shape and volume and experiments were carried out in the same conditions and over a similar time period. In all instances there was considerable day-to-day variation in trap catch and this is likely to have affected reproducibility.

The aggregation pheromone, neryl (*S*)-2-methylbutanoate failed to elicit a detectable trap catch increase at the 10 ng μl^{-1} concentration assayed. As has been demonstrated previously (Chapter 5), this compound produces a significant contact response at a very low concentration (10 pg) though it is possible that this response may occur over a wider range including 10 ng. Further concentrations and replications are needed if this is to be confirmed.

6.9.2 The moving-air twin chamber bioassay

The moving-air twin chamber bioassay provided a novel small scale flight assay with regulated air through-flow. However, it was difficult to construct and once again proved to be an unreliable method of screening odours.

Whilst comparable in size to the still-air flight bioassay it is unclear if the moving-air twin chamber bioassay was large enough for a full flight-orientation response to occur in between time of odour detection by the thrips and arrival at source. Positive anemotaxis may be occurring (as occurs in a Y-tube olfactometer) though the short length of the bioassay may restrict this response. It is likely that when the thrips reaches the end of the insertion tube it is able to detect odour molecules, dispersed downwind of the source. Dry ice smoke was used in initial trials to gain an insight into air-flow within the system; smoke plumes were not restricted to the centre of the

chamber and appeared to reach the edge of the inserted tube (to which thrips would be able to take-off from). Where these molecules exceed a certain concentration, upwind flight should result where the insect maintains its flight within the active space (Murlis *et al.*, 1992). The thrips is likely to be able to detect wind direction prior to take off though it is doubtful if a sophisticated moderation of in-flight movement to remain within the active space occurs. Such complex casting responses have not been observed in Thysanoptera and whilst response time is almost instantaneous in male *Grapholitha molesta* moths (∼0.15 s)(Baker & Haynes, 1987) it is unlikely to occur over such a short distance. It is also possible that over the 150 min period the chamber becomes overly saturated with odour reducing the possibility of on-source landing.

The trap orientation in the moving-air twin chamber bioassay differs from that of the still-air flight bioassay where a visual stimulus is in this instance presented in front of the thrips. As with the still-air flight bioassay it is possible that a strong visual response was also being obtained alongside an odour response. A larger trap size was used and this would suggest that a greater visual stimulus was present.

As had occurred previously with the still-air flight bioassay, thrips were once again able to escape from the trap surface if they had not been properly adhered to the trap. Arrival and subsequent departure occurring between measurements would thus not have been recorded.

As a result of the significant response obtained with geraniol at 2 μl 10 ng μl^{-1} (20 ng) it was hoped to perform the same experiment with and without air through-flow. Time and culture constraints prevented this from occurring. Such an experiment would provide valuable information into the role of an air current in such an assay.

6.9.3 Flight activity

As indicated by combined catch in both bioassays, general activity levels of the thrips varied from day-to-day but remained relatively consistent between the two chambers. Detected activity within the still-air flight bioassay was proportionately more than detected activity within the moving-air twin chamber bioassay. The number of trapped *F. occidentalis* in the treatment still-air flight bioassay at 120 min was often comparatively high (reaching over 50% in some instances) in comparison to the numbers caught in the treatment moving-air twin chamber bioassay at the same time point (ordinarily under 40% catch). No firm conclusions can be drawn from this due to the difference in design and volume between the two assays combined with the difference in initial population. It appears though that though the visual stimulus was larger and more apparent in the moving air bioassay, fewer thrips reached the trap. The reasons for this remain unclear.

Where an effect appeared to be present in both assays it was infrequently pronounced with mean treatment and control catches ordinarily being different by a few individuals.

Geraniol [10ng]					
chamber	location	trap orientation	120 min ANOVA	240 min ANOVA	Figure
Desaga	fume cupboard 1	diamond shape	$F_{1,5}$=7.81, P=0.038*	-	6.5
Desaga	fume cupboard 2	diamond shape	$F_{1,5}$=3.77, P=0.110	$F_{1,5}$=1.43, P=0.286	6.6
Desaga	combined results of above	diamond shape	$F_{1,11}$=10.56, P=0.008**	-	6.7
Camag	fume cupboard 1	horizontal	$F_{1,11}$=0.02, P=0.898	-	6.9

Table 6.1: Still-air flight bioassay. Female activity when exposed to 1μl geraniol compared with hexane control. Dimensions for Desaga chamber were (mm) 196 × 222 × 80. Dimensions of Camag chamber were (mm) 204 × 217 × 62.

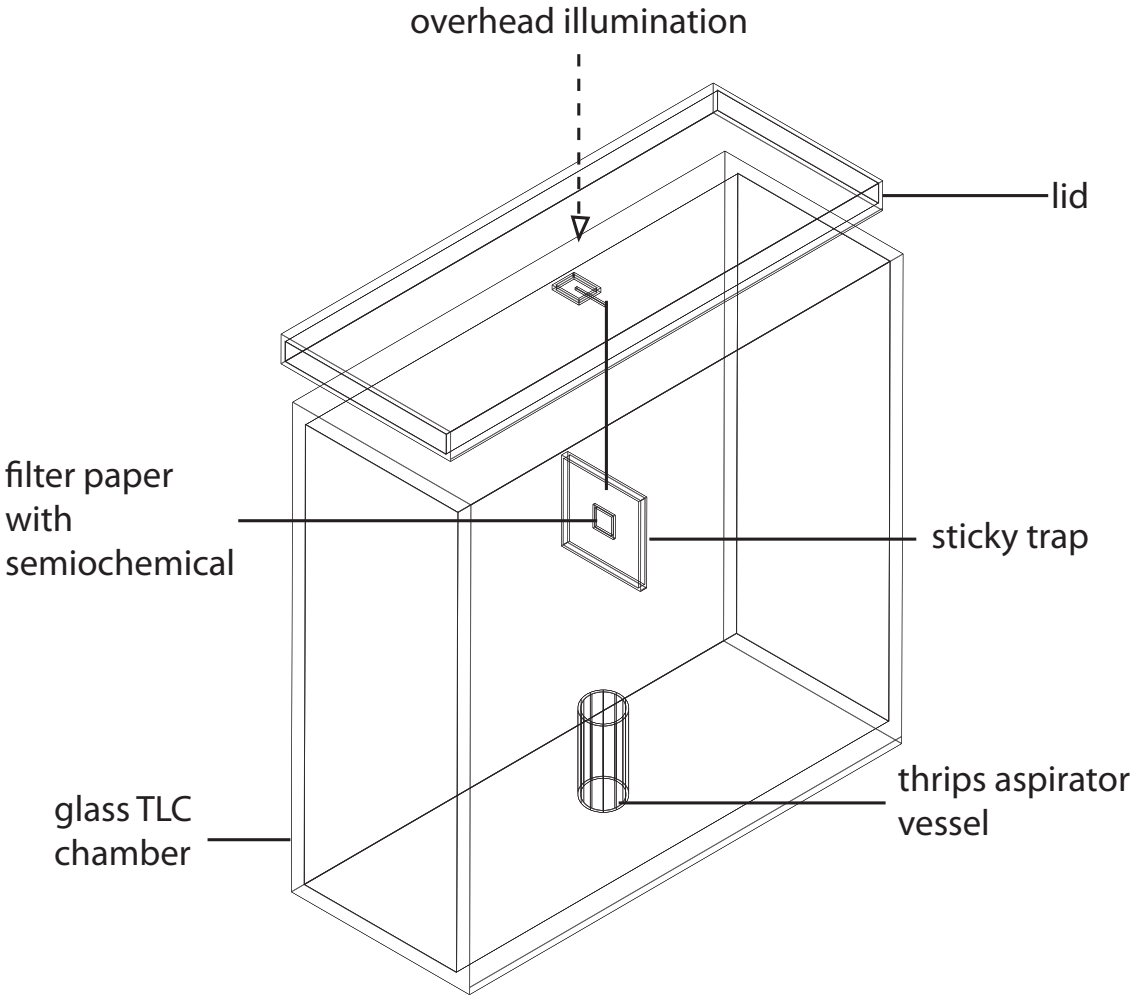

Figure 6.1: Still-air flight bioassay, isometric-left, exploded view

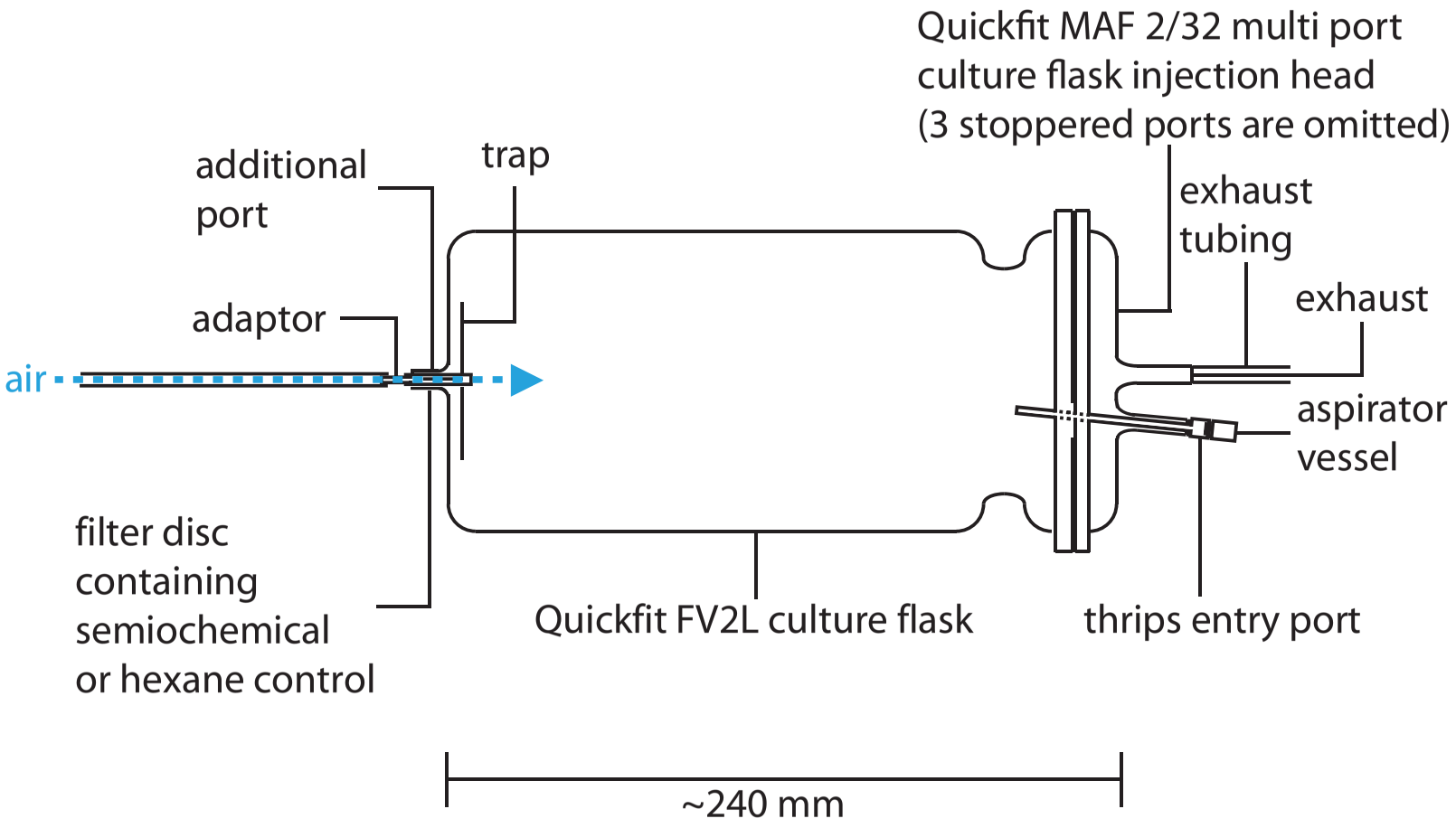

Figure 6.2: Moving-air twin chamber bioassay, single chamber side view

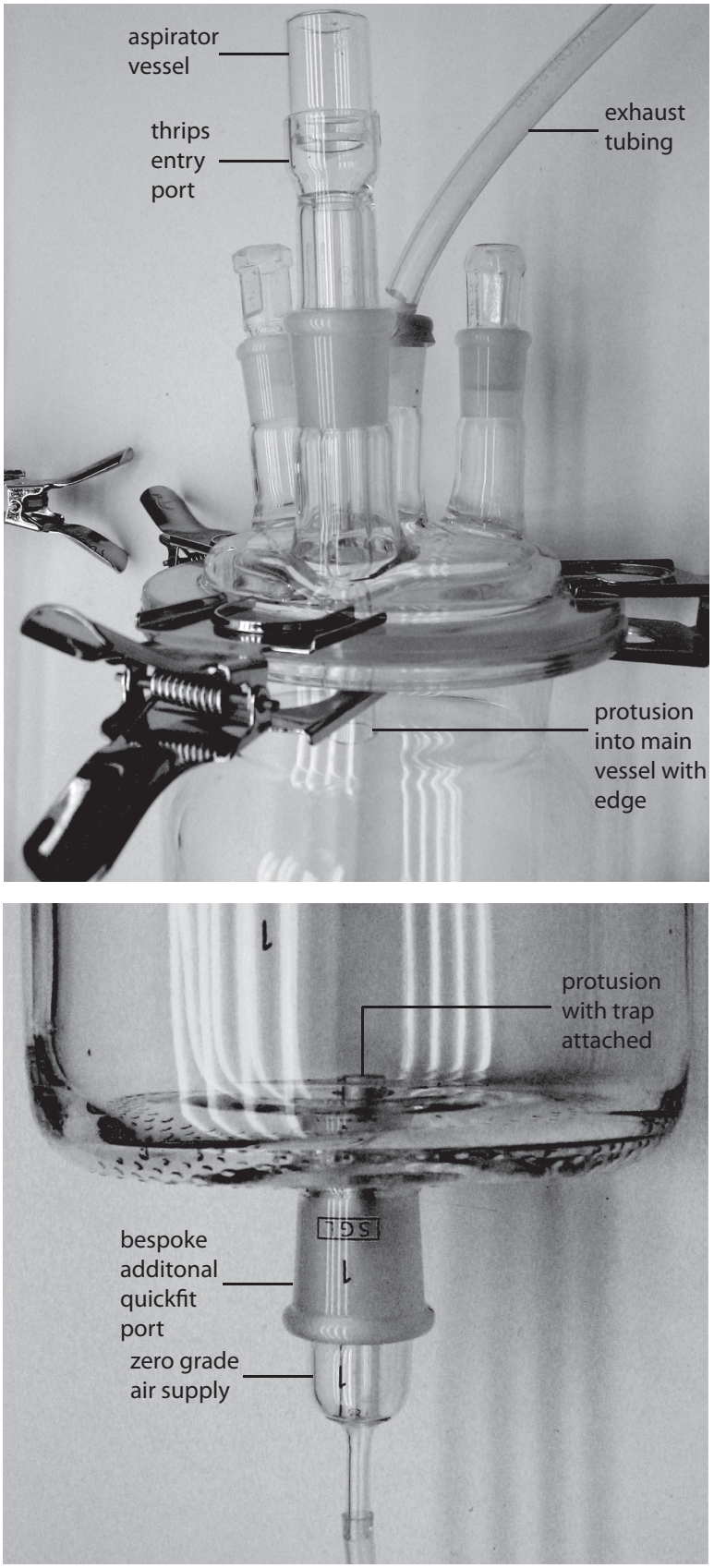

Figure 6.3: Photograph showing moving-air twin chamber bioassay ports. View from above. The top photo shows thrips entry part and exhaust. The lower photo shows air intake [All photos: Dublon, 2006].

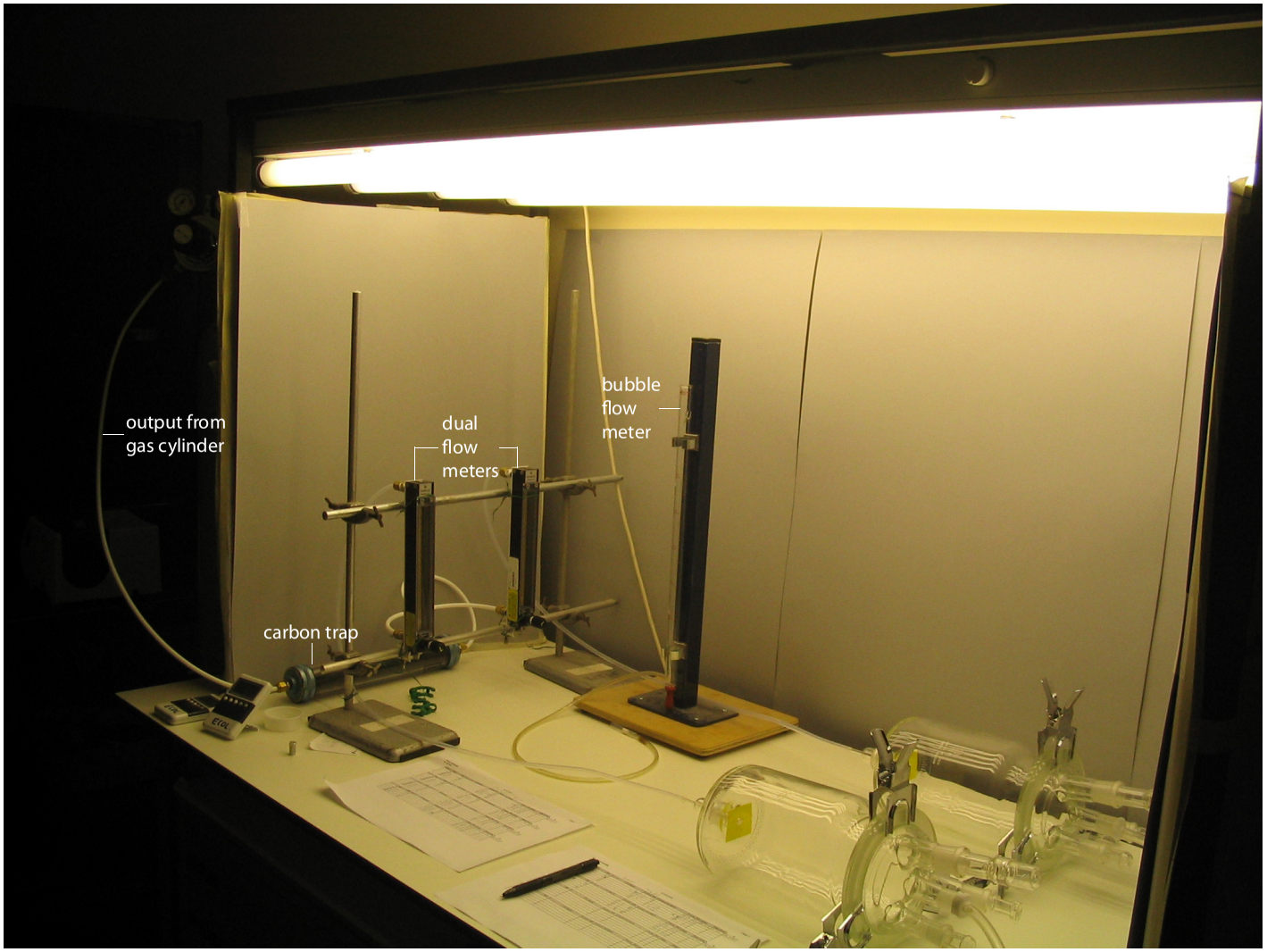

Figure 6.4: Photograph showing moving-air twin chamber bioassay apparatus *in situ* [Photo: Dublon, 2006].

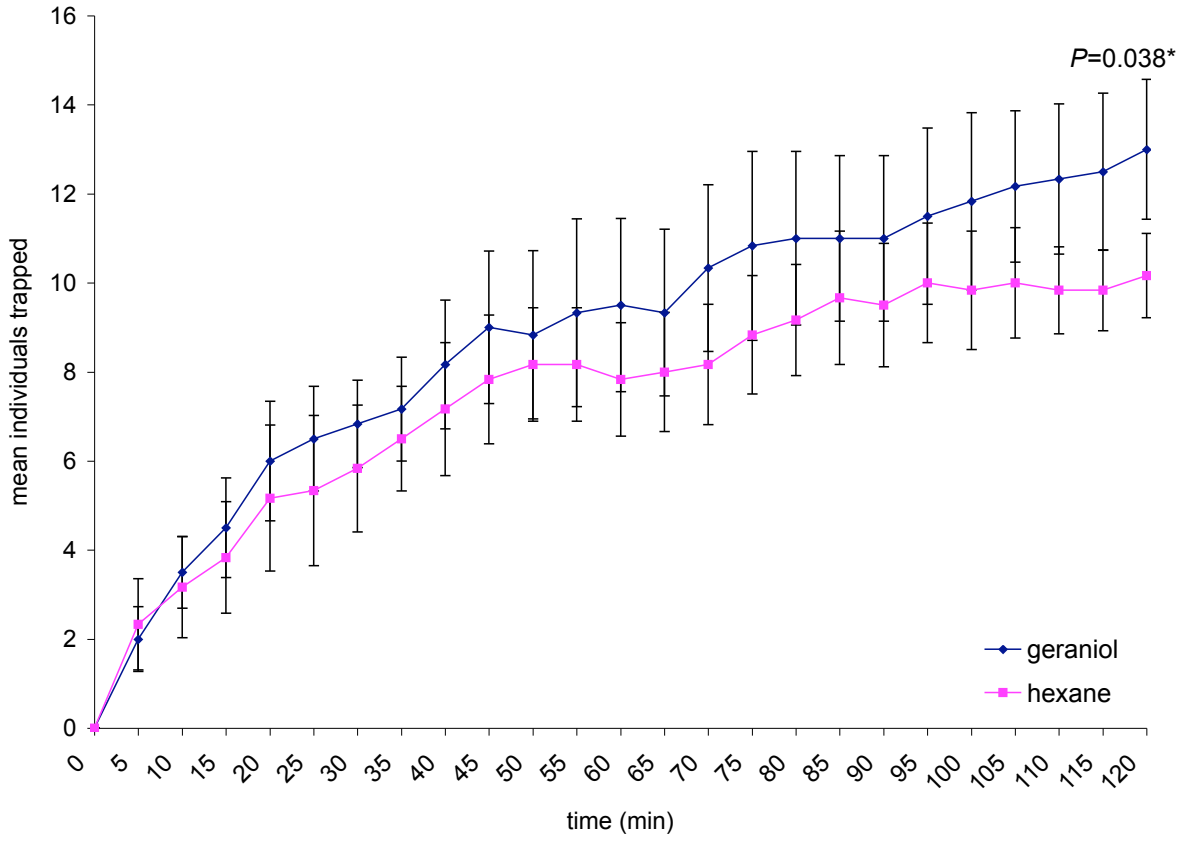

Figure 6.5: Still-air flight bioassay initial pilot experiment. Mean cumulative number (±SEM) of thrips caught per trap with 1μl [10ng/μl] geraniol compared with hexane control, n=6.

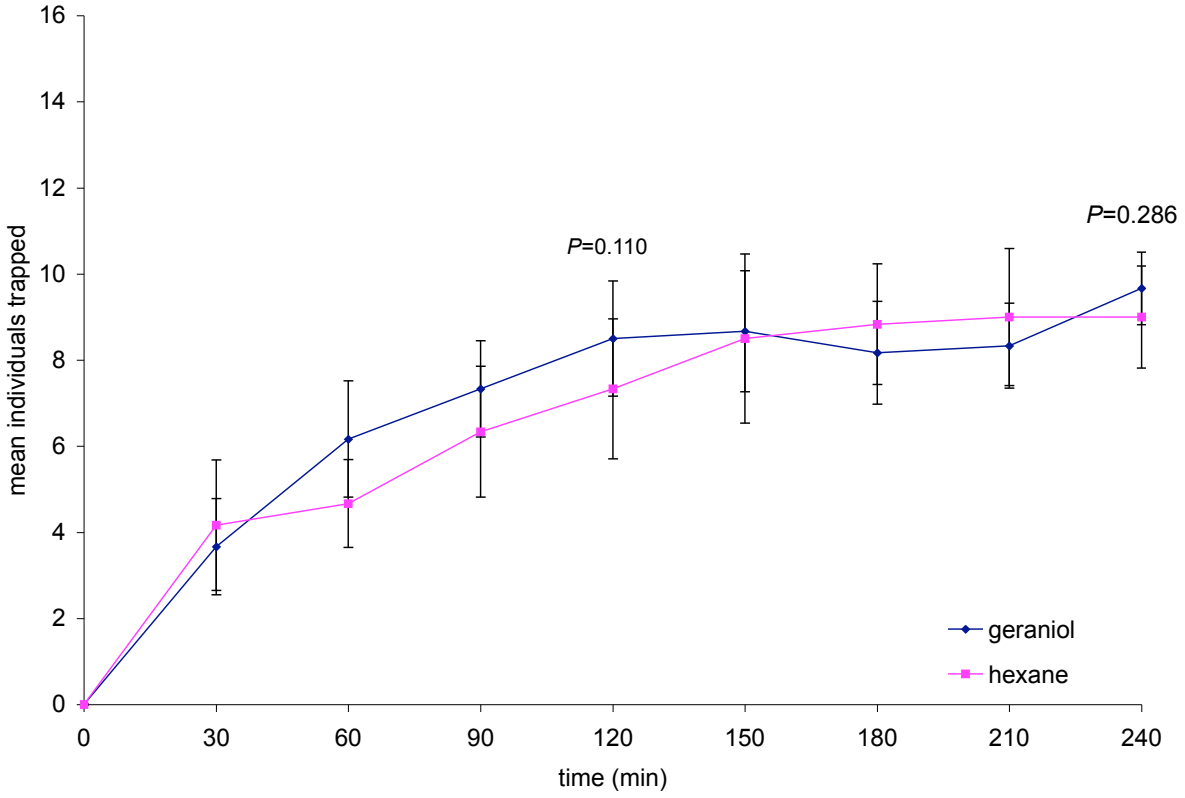

Figure 6.6: Still-air flight bioassay initial pilot experiment. Mean cumulative number (±SEM) of thrips caught per trap with 1μl [10ng/μl] geraniol compared with hexane control and extended duration, n=6.

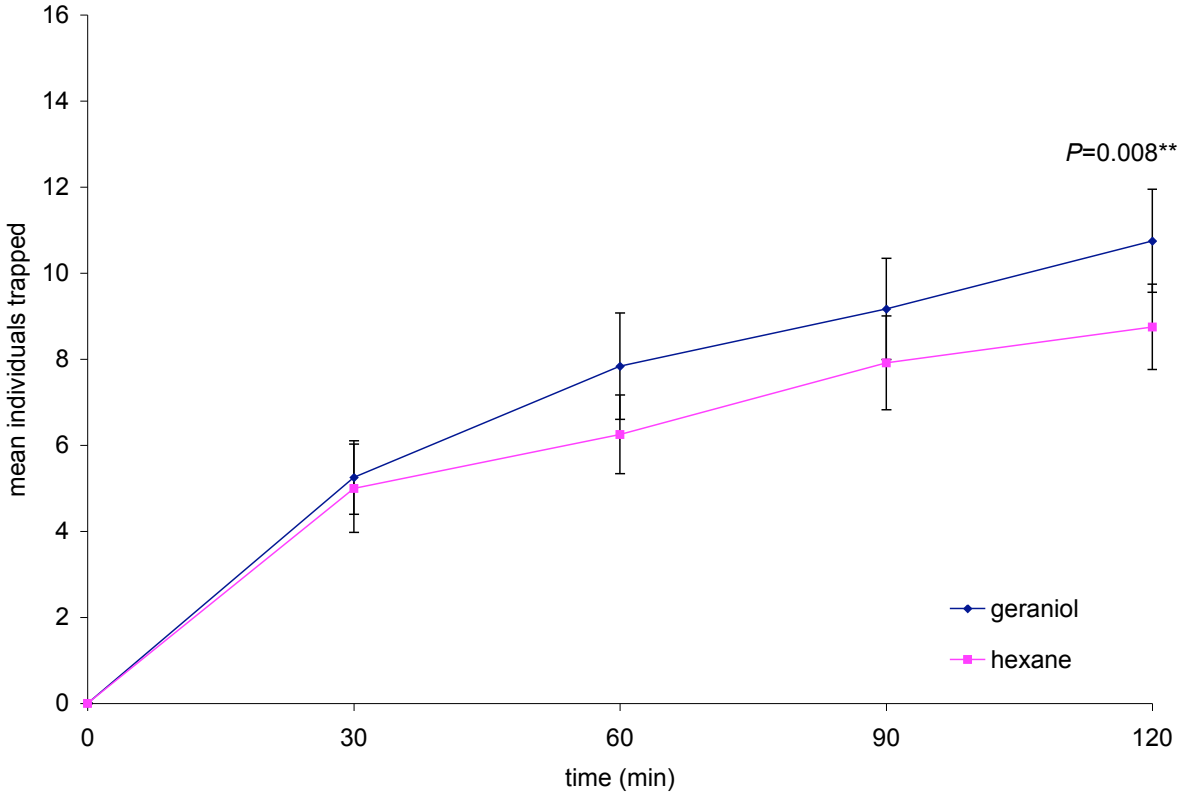

Figure 6.7: Still-air flight bioassay initial pilot experiment. Mean cumulative number (±SEM) of thrips caught per trap with 1μl [10ng/μl] geraniol compared with hexane control. Combined results from Figures 6.5 and 6.6 up to 120 minutes , n=12.

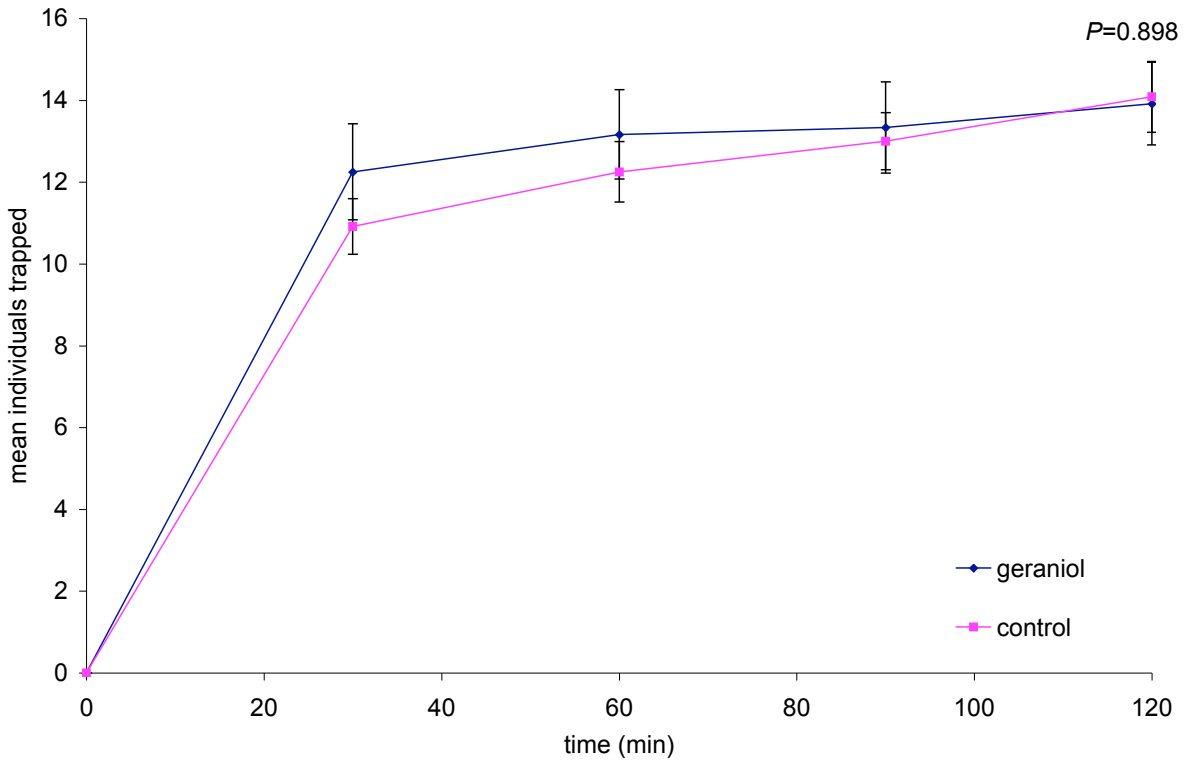

Figure 6.8: Still-air flight bioassay finalised design. Mean cumulative number (±SEM) of thrips caught per trap with 1μl [10ng/μl] geraniol compared with hexane control, n=12.

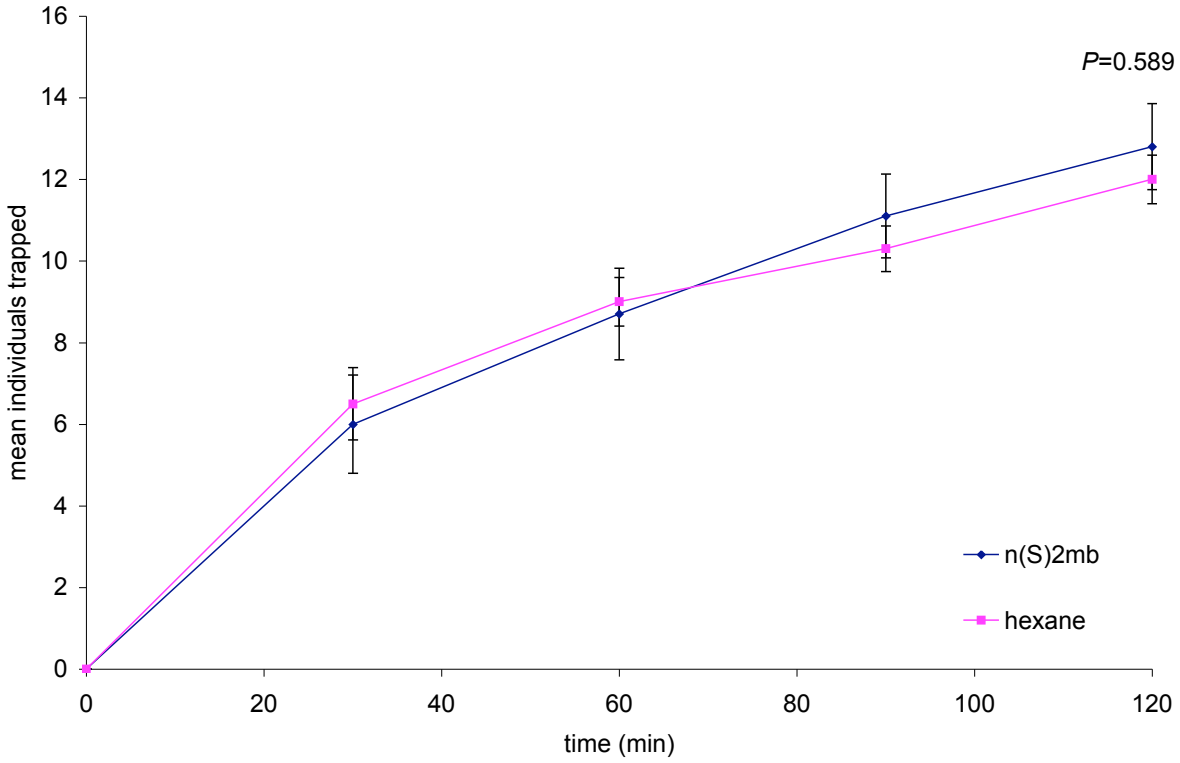

Figure 6.9: Still-air flight bioassay finalised design. Mean cumulative number (±SEM) of thrips caught per trap with 1μl [10ng/μl] neryl (*S*)-2-methylbutanoate compared with hexane control, n=10.

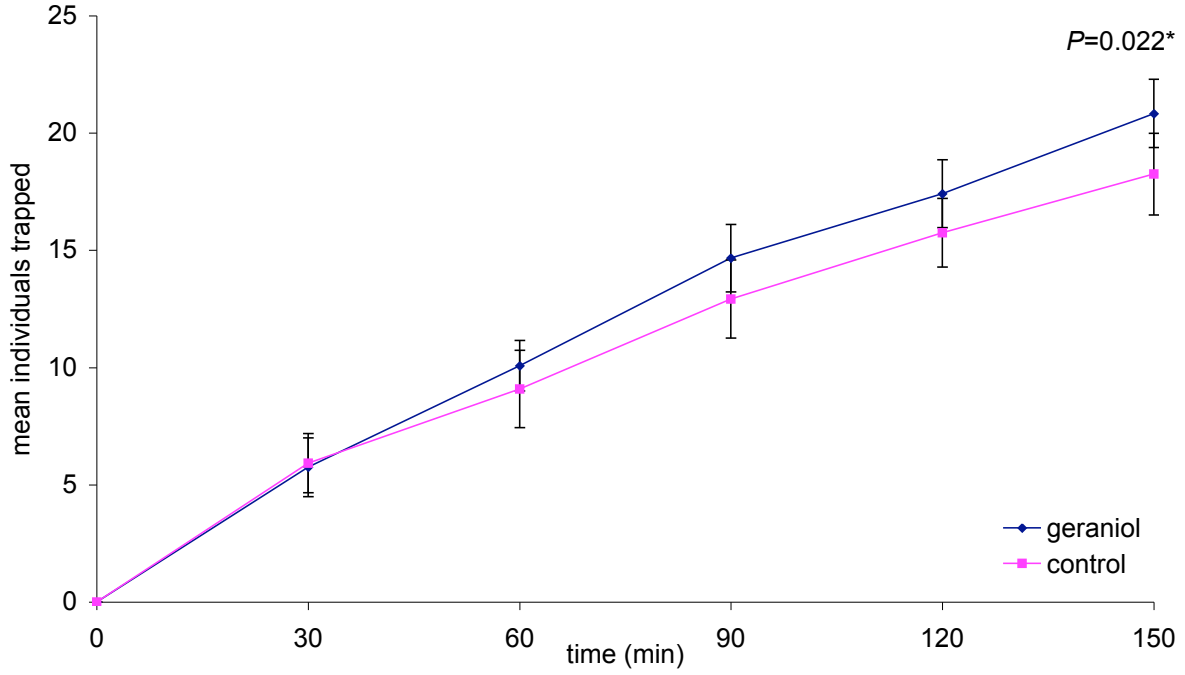

Figure 6.10: Moving-air twin chamber bioassay. Mean cumulative number (±SEM) of thrips caught per trap with 2μl [10ng/μl] geraniol treatment compared with hexane control, n=12.

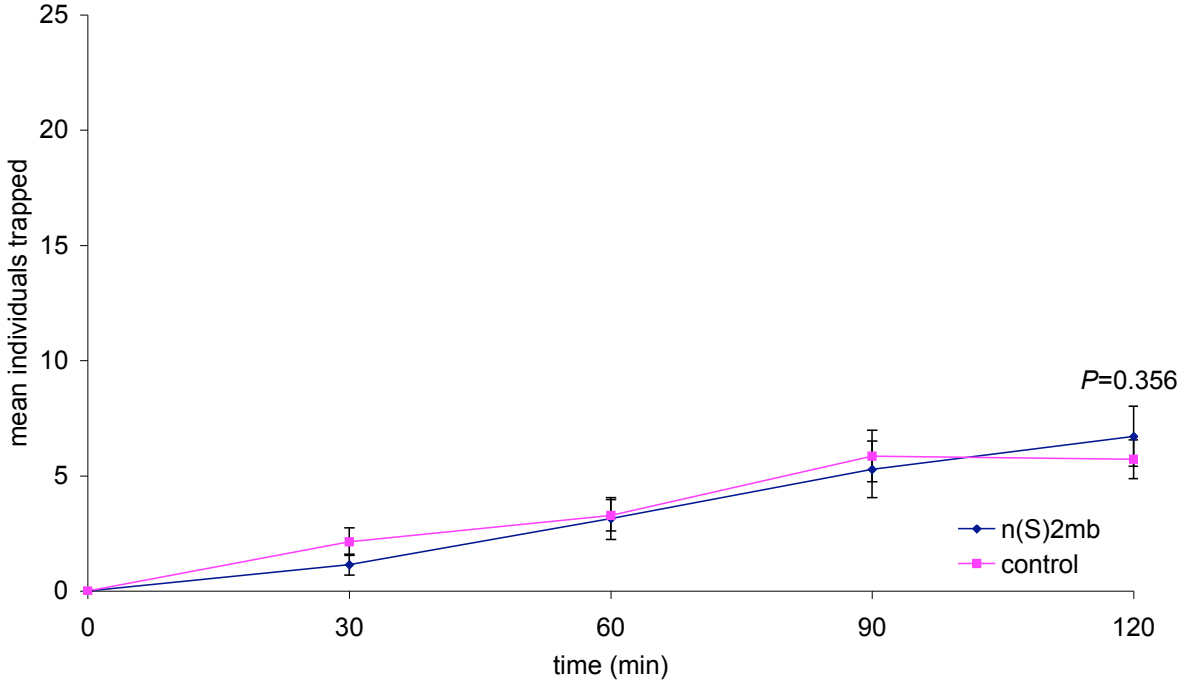

Figure 6.11: Moving-air twin chamber bioassay. Mean cumulative number (±SEM) of thrips caught per trap with 1μl [10ng/μl] neryl (*S*)-2-methylbutanoate treatment compared with hexane control, n=7.

6.10 References

Baker, T.C. and Haynes, K.F. (1987) Manoeuvres used by flying male oriental fruit moths to relocate a sex pheromone plume in an experimentally shifted wind-field. *Physiological Entomology*, 12 263-279.

Blackmer, J.L. and Phelan, P. L. (1991) Behavior of *Carpophilus hemipterus* in a vertical flight chamber: transition from phototactic to vegetative orientation. *Entomologia Experimentalis et Applicata*, 58 137-148.

Berry, N., Butler, R.C. and Teulon, D.A.J. (2006) Responses of New Zealand flower thrips (*Thrips obscuratus*) to synthetic and natural stimuli (odour and colour) in a wind tunnel. *New Zealand Journal of Crop and Horticultural Science*, 34 121-129.

Frey, J.E., Cortada, R.V. and Helbling, H. (1994) The potential of flower odours for use in population monitoring of western flower thrips *Frankliniella occidentalis* Perg. (Thysanoptera: Thripidae). *Biocontrol Science and Technology*, 4 177-186.

Kennedy, J.S. (1983) Zigzagging and casting as a programmed response to wind-borne odour: a review. *Physiological Entomology*, 8 109-120.

Kirk, W.D.J. (1985) Effect of some floral scents on host finding by thrips (Insecta: Thysanoptera). *Journal of Chemical Ecology*, 11 35-43.

Kirk, W.D.J. and Hamilton, J.G.C. (2004) Evidence for a male-produced sex pheromone in the western flower thrips *Frankliniella occidentalis*. *Journal of Chemical Ecology*, 30 167-174.

Koschier, E.H. de Kogel, W.J. and Visser, J.H. (2000) Assessing the attractiveness of volatile plant compounds to western flower thrips *Frankliniella occidentalis*. *Journal of Chemical Ecology*, 26 2643-2655.

Murlis, J., Elkinton, J.S. and Cardé, R.T. (1992) Odor plumes and how insects use them. *Annual Review of Entomology*, 37 505-532.

O'Leary, A.T. and Kirk, W.D.J. (2005) The effect of wavelength on take-off in the western flower thrips. *Acta Phytopathologica et Entomologica Hungarica*, 39 123-129.

Teulon, D.A.J., Hollister, B., Butler, R.C. and Cameron, E.A. (1999) Colour and odour responses of flying western flower thrips: wind tunnel and greenhouse experiments. *Entomologia Experimentalis et Applicata*, 93 9-19.

Teulon, D.A.J., Davidson, M.M., James, D. and Butler, R. (2005) Plant volatile and thrips: are attractants arresting? Poster presentation in: *Thrips and Tospoviruses: Proceedings of the 8th International Symposium on Thysanoptera* (`http://www2.biologie.uni-halle.de/org/thripsnet/conferences/Asilomar/pdf-files/Poster01.pdf`).

Wright, R.H. (1958) The olfactory guidance of flying insects. *The Canadian Entomologist*, 90 81-89.

Chapter 7

Flight responses of adult females and males in the field

7.1 Introduction

F. occidentalis is a major glasshouse pest and is often present in vast numbers in protected glasshouses and polytunnels (Smith *et al.*, 1997).

Screens and barriers can be used to help isolate protected crops from infestation, and traps can be used to reduce the number of thrips entering the glasshouse. Traps can also be used to monitor the population of *F. occidentalis* in a crop and can also provide quantitative data on thrips activity patterns (Pearsall, 2002).

Within the crop, correct trap positioning is crucial to ensure maximal trap catch as is the type of trap used. When selecting a trap its colour is important; in a test of twenty different colours a specific shade of blue (No. 257, Rias, Roskilde, Denmark, DK-4000, resembling Pantone 279) was found to trap the greatest numbers of *F. occidentalis*

(Brødsgaard, 1989). Known thrips attractants such as *p*-anisaldehyde (Teulon *et al.*, 1999) can be added at specific concentrations to a lure placed on the trap surface. This has been shown to be an effective method of increasing trap rate for a variety of Thysanoptera. Pheromonal lures can also be added to traps in order to increase their efficacy. Such lures are readily available for numerous lepidopteran pests with the diversity of identified and synthetic lures increasing over the last decade (Copping, 2004).

Plastic house field trials were conducted in May 2004 by J.G.C. Hamilton and W.D.J. Kirk in two Spanish locations with the aim of examining the effects of (*R*)-lavandulyl acetate and neryl (*S*)-2-methylbutanoate on trap catch.

After careful trap counting by me and subsequent statistical analysis by W.D.J. Kirk, the attractive effect of neryl (*S*)-2-methylbutanoate on yellow traps was demonstrated and the results presented in Hamilton *et al.*, (2005). As a result, neryl (*S*)-2-methylbutanoate has been released under license as the commercial product, Thripline$_{ams}$ (Syngenta Bioline, Essex, UK), a pheromonal lure intended for *F. occidentalis* population monitoring (Hamilton & Kirk, 2003).

Additional experiments were devised, designed and conducted at this time by J.G.C. Hamilton and W.D.J. Kirk. They experiments are presented and analysed below. They investigated the effect of neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate on *F. occidentalis* trap catch on blue sticky traps. Whilst I took no part in the initial design and deployment of the traps, I counted the trap catches and analysed the data. These analyses are presented here.

7.1.1 Background

The concentration at which any semiochemical is deployed is likely to affect its trap catch. It was therefore important to test neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate at multiple concentrations in order to find the best concentration.

In addition when using active semiochemical traps (i.e. traps that due to their colour are likely to attract insects without additional semiochemical stimuli being applied) it is important to examine interactions between visual and olfactory responses. Odour at the correct concentration may induce landing at the trap or induce a visual response in individuals (Kirk, 1985). In this instance, the size of the trap and thus the size of the visual stimulus affects landing. Conversely where the response is only to odour, trap size may be of less importance. The effect of trap size on catch appears to increase with trap area and moreover be directly proportional to trap perimeter length in some species (Kirk, 1987; Carrizo, 2008). It was important to examine the role of the visual component when testing neryl (*S*)-2-methylbutanoate. Treatment and control trap catch were thus analysed on a reduced size trap.

As both neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate are chiral in structure and some insects are able to discern between enantiomers (see 1.6.1) it was important to test racemic mixtures to determine if the presence of the other enantiomer affected trap catch. Racemic mixtures are often easier to commercially synthesise and if equally biologically active, a racemic mixture would reduce lure production costs.

Whilst neryl (*S*)-2-methylbutanoate has been shown to increase trap catch for both male and females it was important to further examine male and female trap catch frequencies. If the role of neryl (*S*)-2-methylbutanoate is primarily sexual aggregation as suggested previously (Kirk & Hamilton, 2004), then it is possible that females may be less responsive to the compound if engaged in other non-breeding related activities.

This would suggest a lower treatment:control ratio for females than males for traps containing neryl (*S*)-2-methylbutanoate.

Spatial distribution of thrips across the surface of traps was unknown and is of interest because it may give further clues about how thrips respond to the semiochemicals. It was not clear if the thrips would land in close proximity to the lure. It has been observed that *Thrips imaginis* are often distributed near trap edges on horizontal traps and whilst this may result from the thrips approaching from the side where they first encounter the trap there may be orientation towards the edge (Kirk, 1987). It has been found that *F. occidentalis* and whitefly (*Trialeurodes vaporariorum*) are often clustered on the sides and upper edge of greenhouses sticky traps (in this instance the traps were orientated with the face vertical and short edge horizontal) (Steiner *et al.*, 1999). Patches of higher density, termed here as "clumps" may be non-random in distribution with their detection and designation being affected by the size of sampling unit (Greig-Smith, 1983).

It was not known if neryl (*S*)-2-methylbutanoate or (*R*)-lavandulyl acetate would increase trap catch for other species of Thysanoptera. Pheromones, as opposed to general plant derived attractants such as *p*-anisaldehyde, are highly species specific though it is possible that these compounds may affect the behaviour of other species. Additional studies were thus undertaken to examine potential effects on trap catch of neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate on additional species of Thysanoptera where present.

Analysing field trial results such as the ones presented in Hamilton *et al.* (2005) and the ones presented in this chapter with full trap counting can be very time-consuming. It is therefore useful to examine potential ways to increase trap count efficiency. Several methods are described in the literature which necessitate only partial trap counting with a view to obtaining the same or a similar statistical result as a whole trap count. In

one such study only 20% of the trap area was counted to produce an accurate estimate of total trap catch for thrips (Heinz *et al.*, 1992).

As mentioned previously intra-trap clumping effects are interesting from a biological perspective and determining potential patterns of clumping is essential if a model for partial trap counting is sought. Such clumping effects may affect the validity of any derived partial trap analysis models. Clumping is likely to result from environmental effects (such as sunlight on one particular side) and not from an active response of thrips to each other.

7.2 Experimental aims

Field trial experiments were carried out by Hamilton and Kirk with the aim of evaluating the effects of neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate on *F. occidentalis* trap catch. In addition a blend of these compounds was tested with the aim of detecting possible synergism.

In addition racemic neryl 2-methylbutanoate was tested with the aim of evaluating the effect of the enantiomeric blend on *F. occidentalis* trap catch.

Reduced size blue traps were also used in one experiment with the aim of determining the effects of reducing the lures visual component on *F. occidentalis* trap catch.

Trap catch was analysed for two other thysanopteran species present on the traps with the aim of evaluating the biological activity of neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate.

Efforts were undertaken to examine intra-trap distribution with the aim of discovering potential clumping of individuals caused by thrips responding to other thrips.

Whole trap count data was examined with the aim of obtaining a partial trap catch model that would produce similar statistical conclusions as those produced by whole trap data analysis.

7.3 Materials and methods

7.3.1 Trap placement and in-block randomisation

All field experiments described in this chapter were carried out by Kirk and Hamilton over the period 25th - 30th of May 2004 in two Spanish locations. Detailed methods describing the field conditions and materials used are provided in Hamilton *et al.*, (2005).

In summary two plastic houses were selected due to large size and ease of accessibility. One was at the Zamora site near Torre Pacheco, Murcia. This was a 6,500 m^2 multitunnel plastic house containing *Capsicum* sweet pepper crops var. Habana. House one was situated at: N 37° 42.993 W 0°C 57.735 and contained plants grown hydroponically in cocopeat to a crop height of 110 cm. One house was at the Carrillo site, near La Palma, Murcia, Spain (N 37° 42.325 W 0° 58.457). This was again a multitunnel plastic house 22,000 m^2 containing *Capsicum* crops var. Herminio grown in soil to a crop height of 110 cm.

Numbered blue sticky traps (Takitrap 8052-01, dry-sticky trap, 100 mm × 250 mm, Syngenta Bioline Ltd., Essex) were used for all experiments with full size traps. Smaller, less visually obvious traps were produced for Experiment 5 (section 7.4.6) by removing all but the top 50 mm from a regular trap. Traps were suspended vertically with the base of the trap about 10 cm above crop height by attaching them to vertical strings. This provided visual stimulus in an area likely to intercept in-flight thrips. A

randomised block design was used for trap placement in each experiment.

In all experiments, a solvent cleaned, 6.3 mm diameter × 10.8 mm length rubber septum (100706, Sigma-Aldrich, UK) was stuck to the middle of the slightly convex side of the trap, using the sticky surface of the trap. Traps were oriented so that the septum faced north thus avoiding direct sunlight on the septum. As a result of suspending the traps using string, there was some inevitable movement so it is possible that this orientation was not maintained.

After deployment, traps were collected and returned to the Keele laboratory for analysis. In order to retain any thrips loosely adhered to the trap surface, prior to collection, the entire trap was covered in transparent plastic on both sides. A transparent plastic freezer bag pre-cut open along its length was used for this. Prior to analysis traps were stored in a freezer at -17°C to prevent them becoming malodourous.

7.3.2 Semiochemical sources

Semiochemicals were obtained as described in Hamilton *et al.*, (2005). In summary, neryl (*S*)-2-methylbutanoate (enantiomeric excess of 97.8%) and racemic neryl 2-methylbutanoat were synthesised at NRI, University of Greenwich, along with (*R*)-lavandulyl acetate (enantiomeric excess of 98.2%).

7.3.3 Semiochemical trials

Blue traps were deployed in the plastic house and either baited with semiochemical septa, blends of semiochemicals on septa or septa with hexane only.

The properties of (*R*)-lavandulyl acetate and neryl (*S*)-2-methylbutanoate were

examined in Experiments 1 and 2. Both compounds were tested at [30 μg] (with 30 μl of 1 μg μl^{-1} being applied) and compared with a blend of the two compounds at [60 μg] and control traps (septa only 30 μl hexane). 25 traps were used per treatment, with four treatments per block. Each trap was exposed for a 24 h period from the 25th to the 26th of May 2004 in the Carrillo site plastic house. Traps were spaced 3.6 m apart within rows and 6 m between rows. Temperatures ranged between 15 - 35°C and relative humidity was 29 - 94%.

This experiment was repeated (Experiment 2) in the same plastic house on the 26th to the 27th of May 2004. Trap spacing was kept constant with temperature remaining comparable (13 - 33°C). Traps were re-randomised within blocks.

In Experiment 3 the effect on *F. occidentalis* trap catch when using neryl (*S*)-2-methylbutanoate at an increased concentration [3 mg] was investigated and compared with control. It was not known if neryl (*S*)-2-methylbutanoate would be ineffective at such an elevated concentration.

27 traps were used per treatment, with two treatments per block. Each trap was was exposed for a 24 h period from the 27th to the 28th of May 2004 at the Carrillo site plastic house. Traps were spaced 7.2 m apart within rows and 6 m between rows. Temperatures ranged between 15 - 35°C and relative humidity was 36 - 94%.

In Experiment 4 the effect of the *R* enantiomer of neryl 2-methylbutanoate on *F. occidentalis* trap catch was investigated and compared with neryl (*S*)-2-methylbutanoate and blank control traps. Trap catch results are presented for traps containing neryl (*S*)-2-methylbutanoate [30 μg], racemic neryl 2-methylbutanoate [60 μg] and blank control traps. It was not known if enantiomeric specificity would be demonstrated by *F. occidentalis*, resulting in a possible loss of response to the compound. When testing the racemate concentration was doubled to 60 μg, meaning that the lure contained 30 μg of each enantiomer.

20 traps were used per treatment, with three treatments per block. Each trap was exposed for a 24 h period from the 29th to the 30th of May 2004 in the Zamora site plastic house two. Traps were spaced 3.4 m apart between rows and 15 m between rows. Temperatures ranged between 15 - 36°C and relative humidity was 34 - 95%.

In Experiment 5, the effect of visual stimulus on trap catch with neryl (*S*)-2-methylbutanoate scented traps was investigated in order to examine the role of the lures visual component. Cut-down, reduced visual stimulus blue traps were used to compare neryl (*S*)-2-methylbutanoate [30 μg] with blank control traps. The importance of the visual component in affecting *F. occidentalis* trap catch was not known and it was possible that reducing the trap size would result in a directly proportional decrease in catch.

As trap size was reduced and there were only two treatments it was decided to increase the number of traps deployed. 50 traps were thus used per treatment, with two treatments per block. Each trap was exposed for a 24 h period from the 28th to the 29th of May 2004 in the Carrillo site plastic house. Traps were spaced 3.6 m apart between rows and 6 m between rows. Temperatures ranged between 15 - 36°C and relative humidity was 32 - 95%.

7.3.4 Semiochemical field trials for additional species

In some instances *Thrips tabaci* and *T. angusticeps* (identified using the methods described in 2.2.3) appeared to be present in sufficient numbers to examine the effect of neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate on their respective trap catches. *T. tabaci* is a major pest species as both a phytophagous pest and a tospovirus vector (Lewis, 1997). *T. angusticeps* whilst not an identified tospovirus vector is classed as a pest thrips and has been recorded as a pest of leguminous plants (Gough, 1955).

The presence of these species was examined in the dataset with the largest numbers of additional Thysanoptera. This was Experiment 1, i.e. the trial testing neryl (*S*)-2-methylbutanoate, (*R*)-lavandulyl acetate, a blend of the two and control traps.

Aeolothripidae were present on the traps though the numbers were considered insufficient for statistical analysis.

7.3.5 Trap counting and analysis

As a result of the large *F. occidentalis* numbers present on some of the traps, and in order to gain information on intra-trap thrips distributions, a grid system was implemented during counting where the trap face and reverse were each split into eight square cells by means of a transparent acetate sheet overlay. It was possible to use an overlay as the traps remained in their plastic bag during the counting process. In the case of normal full size traps, eight square cells, A-H were created per side where cells were arranged alphabetically from left to right and then top to bottom. This meant that each trap was split into 16 square cells (eight at the front and eight at the back). For trap cell designation see Table 7.1.

The number of male and female *F. occidentalis* present on all of the field trial traps broken down by square cell was examined using a dissecting microscope in the first instance (Wild M5A, Wild AG, Heerbrugg, Switzerland). Where *in situ* identification proved ambiguous, specimens were carefully removed and mounted up and viewed using a compound microscope (Leica ATC 2000, Leica, UK or Reichert 323-783, Reichert Inc, Austria).

All cells on the trap included a trap edge. Individual thrips found on the very left edges of the trap face were counted along with the face counts (face cells A,C,E and G). If extra thrips were found on the very right edges of the trap face they were included

in the reverse counts (back cells A,C,E and G).

Whilst designed to be as uniform as possible the surface coating of trap adhesive varied somewhat where the two uppermost and the two lowermost trap cells per side often had less adhesive area. These were cells A, B, G and H on both front and back.

7.3.6 Data analysis for full trap catch

Traps were counted in full using the grid cell system method described in 7.3.5. Male, female and combined trap catch were examined for each treatment and control in order to discover potential sex-specific effects. Data were tested for normality using Anderson-Darling normality tests and a $log_{10}(x+1)$ transformation applied to normalise variance. A $log_{10}(x)$ transform was not applied as some cells contained no individual thrips. Where parametric, ANOVA was used in each experiment to determine if treatment trap catch differed from control. Dunnett's multiple comparisons were used where appropriate to provide comparisons with the control.

Where the data remained non-parametric after $log_{10}(x + 1)$ transformation, non-parametric Friedman 2-way analysis of variance tests were used.

For trap catch comparison with additional species of Thysanoptera, Wilcoxon signed-rank tests were used where data were non-parametric. In this instance the median trap catch with the respective treatments are compared with the median of the control trap.

7.3.7 Partial trap counting as a predictor of full trap counting result

Entire trap counting of side edge and middle grid cells was undertaken with a view to determining if a partial counting model could be obtained that would predict full trap counts. Linear regression was used to produce four models in which 50% of the trap area was used to predict the total trap catch for females, males and total trap catch. As light, breeze and other extraneous factors were likely to affect thrips placement within the trap, cells with low random variability but which were most closely proportional to trap catch were sought. It was not intended to select cells on the basis of them having the highest thrips numbers.

Should breezes cause horizontal or vertical gradients across the trap surface, it could be expected that central trap catch would be less variable. In addition numbers of thrips may have been reasonably high if thrips had landed near the septa and moved within this area. Therefore In model *A*, the middle four grid cells (cells C,D,E and F) on each trap face were used to predict total observed trap catch for front and back trap faces.

Models B - D were developed around the idea that thrips may have been present in large numbers along vertical and horizontal edges, perhaps due to variations in sunlight or breeze. As mentioned previously having very high thrips numbers in the counted cells is not necessarily desirable as whilst it may increase the models predictive value, it will also necessitate that more individuals are counted. In model B the top and bottom two grid cells (cells A,B,G and H) on each trap face were used to predict total observed trap catch for front and back. Model C used the left vertical edge when viewed from the front on both trap faces (cells A,C,E and G) on each trap face. Model *D* used the same edge cells A,C,E and G on the trap front but the alternative vertical cells on the back trap face (B,D,F and H). This was done to allow for thrips that had perhaps

landed on the vertical front edge but had walked around to the reverse cell edge or *vice versa*. The reliability of these four models was compared.

7.3.8 Intra-trap distributions

In order to examine intra-trap *F. occidentalis* distribution and determine if the distribution was uniform or clumped, multiple *G*-tests (Sokal & Rohlf, 1995) were carried out on the grid cells on the front of the control traps in the first and the last 10 blocks where each trap contained one or more thrips. One or more thrips was needed as the G test equation requires computation of the natural log. Data from experiment 1 was used for this analysis as it provided a large number of control traps with 1 or more thrips in every cell.

In order to reduce the chances of making a type 1 error Williams correction was used to obtain a better approximation of the chi-square distribution (Williams, 1976; Sokal & Rohlf, 1995).

In order to account for trap orientation movement after suspension, for the purposes of this analysis and to ensure that the dataset was being interpreted in a uniform way, the front of the trap was deemed to be the side with the highest trap catch. Each grid cell was thus treated as a separate sample and its observed value compared with its expected. As it was not known if certain cells on the front of the selected control traps contained more thrips than others, expected values for each cell were calculated as the total cumulative thrips across the 8 cells divided by the number of cells (8).

7.4 Results

Error bars for Experiments 1-5 denote ±SEM and are obtained from ANOVA performed on untransformed data and are therefore indicative only. The effects of block are removed by the ANOVA and so displayed error bars reflect this.

7.4.1 Experiment 1: neryl (*S*)-2-methylbutanoate, (*R*)-lavandulyl acetate and a blend on blue sticky traps

Neryl (*S*)-2-methylbutanoate significantly increased trap catch for both males and females on scented traps (overall ANOVA $F_{1,24}$=14.41, P<0.0001) (Figure 7.1 and Table 7.2). This increase though significant is only around a ×1.3 increase between treatment and control.

In this instance (*R*)-lavandulyl acetate reduces trap catch (P=0.044) by around × 0.8 for both sexes (Table 7.2). This contrasts with previous experiments where (*R*)-lavandulyl acetate had no significant effect on trap catch, though resulted in lower mean catch (Hamilton *et al.*, 2005). It is possible that this compound may affect behaviour differently at alternative concentrations and ratios.

There was no significant effect on trap catch in traps containing a blend of neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate (P=0.993), and no significant interaction between the two compounds (P=0.330). It is possible here that (*R*)-lavandulyl acetate is counter-acting the effects of neryl (*S*)-2-methylbutanoate.

When counting *F. occidentalis* it appeared that there were instances when some grid cells contained high numbers of individuals (over 30) in close proximity to each other.

7.4.2 Experiment 2: repeat of Experiment 1 (neryl (*S*)-2-methylbutanoate, (*R*)-lavandulyl acetate and a blend on blue sticky traps)

Whilst overall trap catch was reduced from the results of Experiment 1, the observations described there are repeated here (Figure 7.2 and Table 7.3).

A significant interaction between the compounds was found for male (P=0.037) and combined trap catch (P=0.014) and this interaction was nearly significant for females in Experiment 1 (P=0.052). This suggests that in instances where the two compounds are combined the decrease in male and combined trap catch caused by (*R*)-lavandulyl acetate exceeds the increase caused by neryl (*S*)-2-methylbutanoate.

7.4.3 Combined analysis of Experiment 1 and 2

As a result of large differences in trap catch numbers between days, when combining the data from Experiments 1 and 2 (Figure 7.3 and Table 7.4) it was decided to sum the catches between the two days. Neryl (*S*)-2-methylbutanoate increased trap catch for both sexes (P<0.0001) and (*R*)-lavandulyl acetate decreased trap catch for both sexes (P=0.037). A significant interaction between compounds was observed for females (P=0.006) (as was nearly the case in Experiment 1) and combined females and males (P=0.04). The lack of interaction with males (P=0.398) contrasts with Experiment 2 so should be interpreted with care. It would appear that there is a significant but weak interaction between the compounds for combined trap catch. When the two compounds are combined in this instance the decrease in combined trap catch caused by (*R*)-lavandulyl acetate exceeds the increase caused by neryl (*S*)-2-methylbutanoate.

7.4.4 Experiment 3: large doses of aggregation pheromone. Neryl (*S*)-2-methylbutanoate [3 mg] compared with control on blue sticky traps

There was no significant effect on trap catch when neryl (*S*)-2-methylbutanoate was presented at an increased dose of 3 mg with traps spaced at twice the distance to reduce possible cross contamination of treatment and control traps (ANOVA $F_{1,26}$=0.83, P=0.371) (Figure 7.4 and Table 7.5). As a result of increased trap spacing it is unlikely that the observed lack of effect results from control traps gaining increased contacts from residual neryl (*S*)-2-methylbutanoate odour.

7.4.5 Experiment 4: enantiomeric specificity of aggregation pheromone. Neryl (*S*)-2-methylbutanoate [30 μg] and racemic neryl 2-methylbutanoate [60 μg] compared with control on blue sticky traps

Apparent enantiomeric specificity is displayed in this experiment where the racemic mixture reduces trap catch in comparison to the control (Figure 7.5 and Table 7.6). For combined trap catch, numbers are reduced by around $\times$0.8 (P<0.0001). As previously, neryl (*S*)-2-methylbutanoate caused significantly increased trap catch for females (P=0.009) though not for males (P=0.972) or combined trap catch (P=0.150) . Response to neryl (*S*)-2-methylbutanoate for combined males and females is lower than in previous experiments; a mean increase in trap catch of around $\times$1.1 is observed. It is possible that this decrease in response is caused by contamination of the *R* enantiomer being present in the *S* enantiomer. Alternatively residual *R* enantiomer from racemic scented traps within the block may have affected trap catch.

As the racemic mixture was presented at a different and increased dose, it is possible that this may have contributed to the observed reduced trap catch.

7.4.6 Experiment 5: reduced visual stimulus and the effect of aggregation pheromone. Neryl (*S*)-2-methylbutanoate [30 μg] compared with control on cut-down blue sticky traps

Reduced size blue traps caught far fewer thrips than full-size blue traps (Figure 7.6 and Table 7.7). The aggregation pheromone, neryl (*S*)-2-methylbutanoate is effective on traps with a reduced visual component as significantly more males and females were caught on traps containing neryl (*S*)-2-methylbutanoate. The increase of around $\times$1.375 between control and treatment was similar to the increase obtained with full size traps of around $\times$1.32 (Experiment 1).

7.4.7 Effects of neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate on other species of Thysanoptera

T. angusticeps was caught in sufficient numbers (393 individuals) in Experiment 1 to allow for examination of possible effects of neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate on trap catch (Figure 7.7, Table 7.9). *T. tabaci* numbers in the same experiment were very low (37 females). Results were analysed and are presented for completeness. Whilst the test is still valid numbers are too low to have any confidence in the result (Figure 7.8, Table 7.8).

The aggregation pheromone, neryl (*S*)-2-methylbutanoate did not enhance trap

catch for either species. Whilst mean *T. angusticeps* trap catch was elevated with neryl (*S*)-2-methylbutanoate by a factor of ×1.3 this difference was not significant (Tables 7.8 and 7.9). Following transformation, the data for both *T. angusticeps* and *T. tabaci* remained non-parametric so a less powerful non-parametric, Wilcoxon signed-rank test was used instead. As ANOVA was not performed error bars therefore denote ±SEM in this graph and therefore include variations caused by blocks.

(*R*)-lavandulyl acetate appeared to increase mean *T. tabaci* trap catch by a factor of ×3 but this was not significant (Tables 7.8). Control and (*R*)-lavandulyl acetate trap catch means were 0.2 and 0.6 respectively. These were very low and should thus be interpreted with care.

7.4.8 Intra-trap distribution

Intra-trap mean distribution of individual thrips is presented in Table 7.10. Two sets of Williams corrected G test values were obtained, for the first ten control, front trap faces (Figure 7.9). All of these trap faces had one or more thrips per cell (a prerequisite for G tests). It was hoped to use the last ten front trap faces but unfortunately only five traps contained one or more thrips per cell so these were used (Figure 7.10). In the first set of traps which had the highest density only traps 2 and 3 had detectable clumping where there was a significant difference between expected and observed χ^2 value. Williams adjusted G values equalled 32.40 and 45.78 respectively against an χ^2 for P=0.05 of 14.07 at 7 d.f.

In the last five traps with one or more thrips in each front segment only trap 12 appeared to be clumped. In this instance Williams adjusted G equalled 26.747 at 7 d.f.

It appears that where clumping occurs it is infrequent with instances in only 3 out of 15 traps. Upon close inspection of clumped traps it appears that thrips are generally

more likely to clump on the left front edge cells (i.e. A,C,E and G). Of these cells, cell C has a high distribution of thrips in all clumped traps. This reflects mean recorded trap catch (Table 7.10).

7.4.9 Partial trap catch as a predictor for full trap catch

Linear regression was used to produce four models for female, male and combined trap catch. In order to normalise variances regression was performed on $log_{10}(x+1)$ data. Model A used total trap catch for males, females and combined sexes in cells C,D,E and F on front and back. Model B used total trap catch for males, females and combined sexes in cells A,B,G and H on front and back. Model C used total trap catch for males, females and combined sexes in cells A,C,E and G on front and back. Model D used total trap catch for males, females and combined sexes in cells A,C,E and G on the trap front and the associated cells on the back i.e cells B,D,F and H. Trap catch within each cell was added and a $log_{10}(x+1)$ transformation undertaken to normalise residuals. Four separate regressions on transformed data compared observed total female, male and combined trap catch with predicted trap catch using the four methods described above. Regressions were carried out for females, males and combined trap catch to see if the model was more accurate at predicting one sex over another. As there was considerable observed variation in trap catch totals between blocks it was necessary to remove the effect of blocks in the regression. R^2 values were obtained from the counts data by dividing sequential regression sums of squares by total regression sums of squares. See Tables 7.11, 7.12 , 7.13 and 7.14.

Two of these models, A and D, predict the observed trap catch with good predictive ability (all R^2 values exceed 89%). Model B's residuals remained non parametric for males leading to deviation from the parametric distribution. Likewise model C's residuals remained non parametric for combined trap catch. The predictions from these

two models should therefore be interpreted with care.

Of the two models with parametric residuals, model A has the best predictive ability for females, males and combined female and male catch respectively predicting 94.8, 90.8 and 94.9% of the variance in total trap catch. This model was tested where ANOVA was performed on the $log_{10}(x + 1)$ transformed counts from cells CDEF as described in section 7.3.6 (Table 7.15). Neryl (*S*)-2-methylbutanoate significantly increased combined trap catch on scented traps (Overall ANOVA $F_{1,24}$=10.72 P<0.0001). Neryl (*S*)-2-methylbutanoate did not increase trap catch for males when using this model (P=0.059).

When using the model A catch data, (*R*)-lavandulyl acetate does not reduce trap catch in any instance. Care needs to be taken when interpreting partial trap catch data for model A male trap catch as it remained non-parametric after transformation.

7.5 Discussion

Whilst there is potential for odours from one trap affecting nearby traps (specifically where more than one treatment scent is used), it is clear that neryl (*S*)-2-methylbutanoate is an effective compound for increasing trap catch for both male and female *F. occidentalis*. This broadly confirms the data published on this topic (Hamilton *et al.*, 2005; Gómez *et al.*, 2006). The increase of around ×1.3 is however less than the ×3 previously reported on blue traps (Gómez *et al.*, 2006). The reasons for this difference in treatment:control ratio remain unknown and cannot be easily explained by differences in trap type and deployment. It may be that the thrips were merely more responsive in the Gómez trial.

(*R*)-lavandulyl acetate does not increase *F. occidentalis* trap catch when deployed at

the concentrations tested. It is unlikely that such a compound would be synthesised by male *F. occidentalis* for no reason so it is possible that this compound has a pheromonal role but at a different concentration. In previous trials, this compound did not affect trap catch when deployed at two different concentrations (Hamilton *et al.*, 2005). It is possible that the observed effect (i.e. a reduction in trap catch) is variable or is apparent only as a result of the additional replication. Where this compound reduces trap catch it is less efficient than the reduction gained from application of *F. occidentalis* alarm pheromone to blue sticky traps (MacDonald *et al.*, 2002) so its potential for use as a repellant is limited. As mentioned in section 5.11 it is possible that (*R*)-lavandulyl acetate has an alternative pheromonal role that may or may not contribute synergistically to the neryl (*S*)-2-methylbutanoate response.

Whilst neryl (*S*)-2-methylbutanoate increased trap catch it did not do so at a level that is as high as might be envisaged. The known, floral attractant *p*-anisaldehyde has been shown to increase trap catches of *F. occidentalis* on blue sticky traps by $\times 1.7$ (Brødsgaard, 1990). Trap catch in glass houses can be exceptionally variable so it is important to take this into consideration when comparing the results of this trial with that of Gómez *et al.*, 2006.

The aggregation pheromone neryl (*S*)-2-methylbutanoate attracts both males and females in a similar ratio. Assuming that the main role of the compound is sexual attraction and as females may not always be interested in copulating, this was somewhat unexpected and it is possible that virgin *F. occidentalis* females would respond differently. Should neryl (*S*)-2-methylbutanoate odour facilitate an odour-induced visual response in both sexes this may make the brightly coloured scented traps more attractive, thus increasing trap catch.

Many insects have been shown to perceive and respond to visual targets, often using contrast of a dark pattern against a light background (Bernays & Chapman, 1994). It

is unlikely that the presence of randomly distributed individuals upon the blue traps can be visually observed by a thrips in flight though should this be possible, it is likely that it would result in increased females and competing males.

The percentage increase in trap catch is similar on full size traps when compared with smaller traps though trap catch numbers are reduced in proportion to reduced trap area. *F. occidentalis* therefore do not appear to be concentrated on the smaller trap.

If neryl (*S*)-2-methylbutanoate induces landing in airborne thrips, as is often the case in water traps where individuals land on trap sides if not the trap itself, it may be possible that more thrips would land in the general area of the lure source. If landing was in the general area of the semiochemical, with smaller treatment traps, we might thus assume similar observed treatment to control trap catch ratios where thrips landing in the semiochemical vicinity miss the treatment trap face altogether.

As the odour component remains constant in both full and cut-down traps it is likely that *F. occidentalis* would sense neryl (*S*)-2-methylbutanoate odour over the same distance but with reduced trap size, the visual stimulus may not be as apparent from a distance. The colour contrast provided by the trap, despite its small size may be enough to result in it becoming a visual target and result in orientated movement towards it with more individuals being drawn to neryl (*S*)-2-methylbutanoate traps as a result.

As the treatment:control ratio between smaller and larger traps was around the same, and trap catch numbers decrease with visual stimulus, this suggests that a clear plastic neryl (*S*)-2-methylbutanoate scented passive trap would catch very little extra thrips in comparison to a similar transparent control. A clear plastic trap could thus be tested alongside full and reduced size blue traps if this experiment were to be repeated.

As neryl (*S*)-2-methylbutanoate adds to trap catch on the blue trap it is suitable for population monitoring, where early stage detection of *F. occidentalis* is necessary. Elucidation of the behavioural role of (*R*)-lavandulyl acetate and identification of minor male *F. occidentalis* produced headspace components as potential synergists would perhaps increase the effectiveness of this compound.

Whilst clumping was seemingly apparent when counting total trap catch in Experiment 1, the intra-trap distribution of the thrips on control traps appears to be mostly unclumped in distribution. It is of course possible that clumps are present but that they are smaller than the cell size used in the analysis and hence undetected.

Where clumping occurs on the front of control traps it is most often along the leftmost front edge, towards the middle. It is not possible to state with certainty if this results from environmental extraneous factors such as sunlight upon trap face or from thrips responding to each other.

A partial trap analysis model has been derived which allows for an accurate predictor of trap catch with only 50% of the trap area being counted. This model is effective though it did not detect reduced trap catch with (*R*)-lavandulyl acetate. This model counts the middle area on the front and back of the traps and proves to be an effective method of predicting total observed combined trap catch and catch for each sex. The number of individuals within the cells requiring counting account for roughly 60% of the total observed thrips. Whilst this counting percentage is still higher than might be hoped for, where trap catch is in the thousands such as in this instance (7178 combined individual *F. occidentalis*) it would make a large difference in improving trap counting time efficiency.

front		back	
a	b	a	b
c	d	c	d
e	f	e	f
g	h	g	h

Table 7.1: Counting cell placement on front and back of field traps

	female P	male P	combined P
overall $F_{1,24}$=	10.76, P<0.0001***	-	-
	-	8.91, P<0.0001***	-
	-	-	14.41, P<0.0001***
n(S)-2-mb	0.0001***	0.0246*	0.0004***
(R)-la	0.662	0.040*	0.044*
blend	0.878	0.999	0.993

Table 7.2: Plastic house trials: Experiment 1. Neryl (S)-2-methylbutanoate (n(S)-2-mb) [30 μg], (R)-lavandulyl acetate ((R)-la) [30 μg] and a blend of neryl (S)-2-methylbutanoate [30 μg] combined with (R)-lavandulyl acetate [30 μg] compared with control on blue sticky traps. Table of P-values.

	female P	male P	combined P
overall $F_{1,24}$=	15.25, P<0.0001***	-	-
	-	6.52, P<0.0001***	-
	-	-	14.14, P<0.0001***
n(S)-2-mb	0.0001***	0.008**	0.0001***
(R)-la	0.486	0.808	0.552
blend	0.426	0.864	0.993

Table 7.3: Plastic house trials: Experiment 2. Repeat of Experiment 1. Neryl (S)-2-methylbutanoate (n(S)-2-mb) [30 μg], (R)-lavandulyl acetate ((R)-la) [30 μg] and a blend of neryl (S)-2-methylbutanoate [30 μg] combined with (R)-lavandulyl acetate [30 μg] compared with control on blue sticky traps. Table of P-values.

	female P	male P	combined P
overall $F_{1,49}$=	26.07, P<0.0001***	-	-
	-	14.36, P<0.0001***	-
	-	-	28.37, P<0.0001***
n(S)-2-mb	0.0001***	0.0002***	0.0001***
(R)-la	0.291	0.058	0.037*
blend	0.374	0.968	0.981

Table 7.4: Plastic house trials: Experiments 1 and 2 combined dataset. Neryl (S)-2-methylbutanoate (n(S)-2-mb) [30 μg], (R)-lavandulyl acetate [30 μg] and a blend of neryl (S)-2-methylbutanoate [30 μg] combined with (R)-lavandulyl acetate ((R)-la) [30 μg] compared with control on blue sticky traps. Combined results from previous two trials. Table of P-values.

	female P	male P	combined P
ANOVA $F_{1,27}$=	2.42, P=0.132	-	-
	-	0.07, P=0.799	-
	-	-	0.83, P=0.371

Table 7.5: Plastic house trials: Experiment 3. High concentration, [3 mg] of neryl (*S*)-2-methylbutanoate (n(*S*)-2-mb) compared with control on blue sticky traps. Table of *P*-values.

	female P	male P	combined P
overall $F_{1,19}$=	21.36, P<0.0001***	-	-
	-	9.78, P<0.0001***	-
	-	-	20.94, P<0.0001***
n(*S*)-2-mb	0.009**	0.972	0.150
rac n 2-mb	0.002**	0.0007***	0.0001***

Table 7.6: Plastic house trials: Experiment 4. Neryl (*S*)-2-methylbutanoate (n(*S*)-2-mb) [30 μg] and racemic neryl 2-methylbutanoate (rac n 2-mb) [60 μg] compared with control on blue sticky traps. Table of *P*-values.

Friedman 2-way analysis of variance	P
Females	
neryl (*S*)-2-methylbutanoate compared with control	0.009**
Males	
neryl (*S*)-2-methylbutanoate compared with control	0.048*
Combined females and males	
neryl (*S*)-2-methylbutanoate compared with control	0.005**

Table 7.7: Plastic house trials: Experiment 5. Neryl (*S*)-2-methylbutanoate [30 μg] compared with control on reduced size blue sticky traps. Table of *P*-values.

Wilcoxon signed-rank test	P
control median compared with:	
traps containing neryl (*S*)-2-methylbutanoate	1.000
traps containing (*R*)-lavandulyl acetate	0.266

Table 7.8: Plastic house trials: Experiment 1. Traps containing neryl (*S*)-2-methylbutanoate [30 μg] and traps containing (*R*)-lavandulyl acetate [30 μg] compared with control on blue sticky traps. Table of *P*-values for *Thrips tabaci* female trap catch.

Wilcoxon signed-rank test	P
Combined trap catch	
control median compared with:	
traps containing neryl (*S*)-2-methylbutanoate	0.808
traps containing (*R*)-lavandulyl acetate	0.422

Table 7.9: Plastic house trials: Experiment 1. Traps containing neryl (*S*)-2-methylbutanoate [30 μg] and traps containing (*R*)-lavandulyl acetate [30 μg] compared with control on blue sticky traps. Table of *P*-values for *Thrips angusticeps*.

Females

front		back	
2.19(a)	1.51(b)	0.76(a)	1.19(b)
4.43(c)	3.11(d)	1.51(c)	2.33(d)
3.93(e)	2.56(f)	1.12(e)	1.87(f)
2.87(g)	2.14(h)	0.96(g)	1.79(h)

Males

front		back	
3.67(a)	2.49(b)	1.14(a)	2.43(b)
5.20(c)	3.07(d)	1.77(c)	3.01(d)
3.71(e)	2.02(f)	0.95(e)	2.16(f)
2.24(g)	1.46(h)	0.89(g)	1.30(h)

Combined

front		back	
5.86(a)	4.00(b)	1.90(a)	3.62(b)
9.63(c)	6.18(d)	3.28(c)	5.34(d)
7.64(e)	4.58(f)	2.07(e)	4.03(f)
5.11(g)	3.60(h)	1.85(g)	3.09(h)

Table 7.10: Intra-trap distribution of individual thrips. Trap cell layout is as illustrated in Table 7.1 and counting grid cells are represented in parentheses. Mean *F. occidentalis* trap catch per grid segment in the largest field trial data set (means from 100 traps).

	P
Females	
Analysis of variance $F_{1,99}$=1014.06	<0.0001***
Regression equation:	
log_{10}(total females+1)=	
0.470 + 0.843 $log_{10}(femaleA+1)$ - 0.00499 blocks	
R^2 for $log_{10}(femaleA+1)$=94.8%	
predictor:	
constant	<0.0001***
$log_{10}(femaleA+1)$	<0.0001***
blocks	0.001**
Males	
Analysis of variance $F_{1,99}$=580.03	<0.0001***
Regression equation:	
log_{10}(total males+1) =	
0.571 + 0.796 $log_{10}(maleA+1)$ - 0.00640 blocks	
R^2 for $log_{10}(maleA+1)$ =90.8%	
predictor:	
constant	<0.0001***
$log_{10}(maleA+1)$	<0.0001***
blocks	<0.0001***
Combined females and males	
Analysis of variance $F_{1,99}$=1057.47	<0.0001***
Regression equation:	
log_{10}(total combined+1) =	
0.552 + 0.831 $log_{10}(combinedA+1)$ - 0.00519 blocks	
R^2 for $log_{10}(combinedA+1)$ =94.9%	
predictor:	
constant	<0.0001***
$log_{10}(combinedA+1)$	<0.0001***
blocks	<0.0001***

Table 7.11: Partial trap counting: Model A. Linear regression where the middle grid cells C,D,E and F on each trap face were summed for observed females, males and combined trap catch. These values were then used to predict total female, male and combined trap catch. Table of P-values.

	P
Females	
Analysis of variance $F_{1,99}$=362.91	<0.0001***
Regression equation:	
log_{10}(total females+1) =	
0.674 + 0.826 $log_{10}(femaleB+1)$ - 0.00862 blocks	
R^2 for $log_{10}(femaleB+1)$=86.5%	
predictor:	
constant	<0.0001***
$log_{10}(femaleB+1)$	<0.0001***
blocks	<0.0001***
Males	
Analysis of variance $F_{1,99}$=299.68	<0.0001***
Regression equation:	
log_{10}(total males+1) =	
0.754 + 0.745 $log_{10}(maleB+1)$ - 0.00717 blocks	
R^2 for $log_{10}(maleB+1)$=84.3%	
predictor:	
constant	<0.0001***
$log_{10}(maleB+1)$	<0.0001***
blocks	0.001**
Combined females and males	
Analysis of variance $F_{1,99}$=569.19	<0.0001***
Regression equation:	
log_{10}(total combined+1) =	
0.634 + 0.868 $log_{10}(combinedB+1)$ - 0.00509 blocks	
R^2 for $log_{10}(combinedB+1)$=91.5%	
predictor:	
constant	<0.0001***
$log_{10}(combinedB+1)$	<0.0001***
blocks	0.006**

Table 7.12: Partial trap counting: Model B. Linear regression where the top and bottom grid cells ABGH on each trap face were summed for observed females, males and combined trap catch. These values were then used to predict total female, male and combined trap catch. Table of P-values.

	P
Females	
Analysis of variance $F_{1,99}$=526.32	<0.0001***
Regression equation:	
log_{10}(total females+1) =	
0.469 + 0.880 $log_{10}(femaleC+1)$ - 0.00436 blocks	
R^2 for $log_{10}(femaleC+1)$=91.1%	
predictor:	
constant	<0.0001***
$log_{10}(femaleC+1)$	<0.0001***
blocks	0.036*
Males	
Analysis of variance $F_{1,99}$=363.05	<0.0001***
Regression equation:	
log_{10}(total males+1) =	
0.463 + 0.880 $log_{10}(maleC+1)$ - 0.00347 blocks	
R^2 for $log_{10}(maleC+1)$=87.9%	
predictor:	
constant	<0.0001***
$log_{10}(maleC+1)$	<0.0001***
blocks	0.090
Combined females and males	
Analysis of variance $F_{1,99}$=525.69	<0.0001***
Regression equation:	
log_{10}(total combined+1) =	
0.472 + 0.897 $log_{10}(combinedC+1)$ - 0.00313 blocks	
R^2 for $log_{10}(combinedC+1)$=91.3%	
predictor:	
constant	<0.0001***
$log_{10}(combinedC+1)$	<0.0001***
blocks	0.111

Table 7.13: Partial trap counting: Model C. Linear regression where the vertical grid cells ACEG on each trap face were summed for observed females, males and combined trap catch. These values were then used to predict total female, male and combined trap catch. Table of P-values.

	P
Females	
Analysis of variance $F_{1,99}$=685.01	<0.0001***
Regression equation:	
log_{10}(total females+1) =	
0.477 + 0.861 $log_{10}(femaleD+1)$ - 0.00804 blocks	
R^2 for $log_{10}(femaleD+1)$=91.8	
predictor:	
constant	<0.0001***
$log_{10}(femaleD+1)$	<0.0001***
blocks	<0.0001***
Males	
Analysis of variance $F_{1,99}$=499.41	<0.0001***
Regression equation:	
log_{10}(total males+1) =	
0.468 + 0.854 $log_{10}(maleD+1)$ - 0.00696 blocks	
R^2 for $log_{10}(maleD+1)$=89.4%	
predictor:	
constant	<0.0001***
$log_{10}(maleD+1)$	<0.0001***
blocks	<0.0001***
Combined females and males	
Analysis of variance $F_{1,99}$=525.69	<0.0001***
Regression equation:	
log_{10}(total combined+1) =	
0.514 + 0.859 $log_{10}(combinedD+1)$ - 0.00742 blocks	
R^2 for $log_{10}(combinedD+1)$=91.9%	
predictor:	
constant	<0.0001***
$log_{10}(combinedD+1)$	<0.0001***
blocks	<0.0001***

Table 7.14: Partial trap counting: Model D. Linear regression where the vertical grid cells ACEG on the front trap face and BDFH on the reverse face of each trap were summed for observed females, males and combined trap catch. These values were then used to predict total female, male and combined trap catch. Table of P-values.

	female P	male P	combined P
overall $F_{1,24}$=	8.37, P<0.0001***	-	-
	-	6.73, P<0.0001***	-
	-	-	10.72, P<0.0001***
n(S)-2-mb	0.0007***	0.059	0.0035**
(R)-la	0.768	0.088	0.068
blend	0.763	0.964	0.982

Table 7.15: Partial trap counting using model A: Experiment 1 dataset. Neryl (S)-2-methylbutanoate (n(S)-2-mb) [30 μg], (R)-lavandulyl acetate ((R)-la) [30 μg] and a blend of neryl (S)-2-methylbutanoate [30 μg] combined with (R)-lavandulyl acetate [30 μg] compared with control on blue sticky traps. Results from ANOVA performed on trap catch in cells C,D,E and F. Table of P-values.

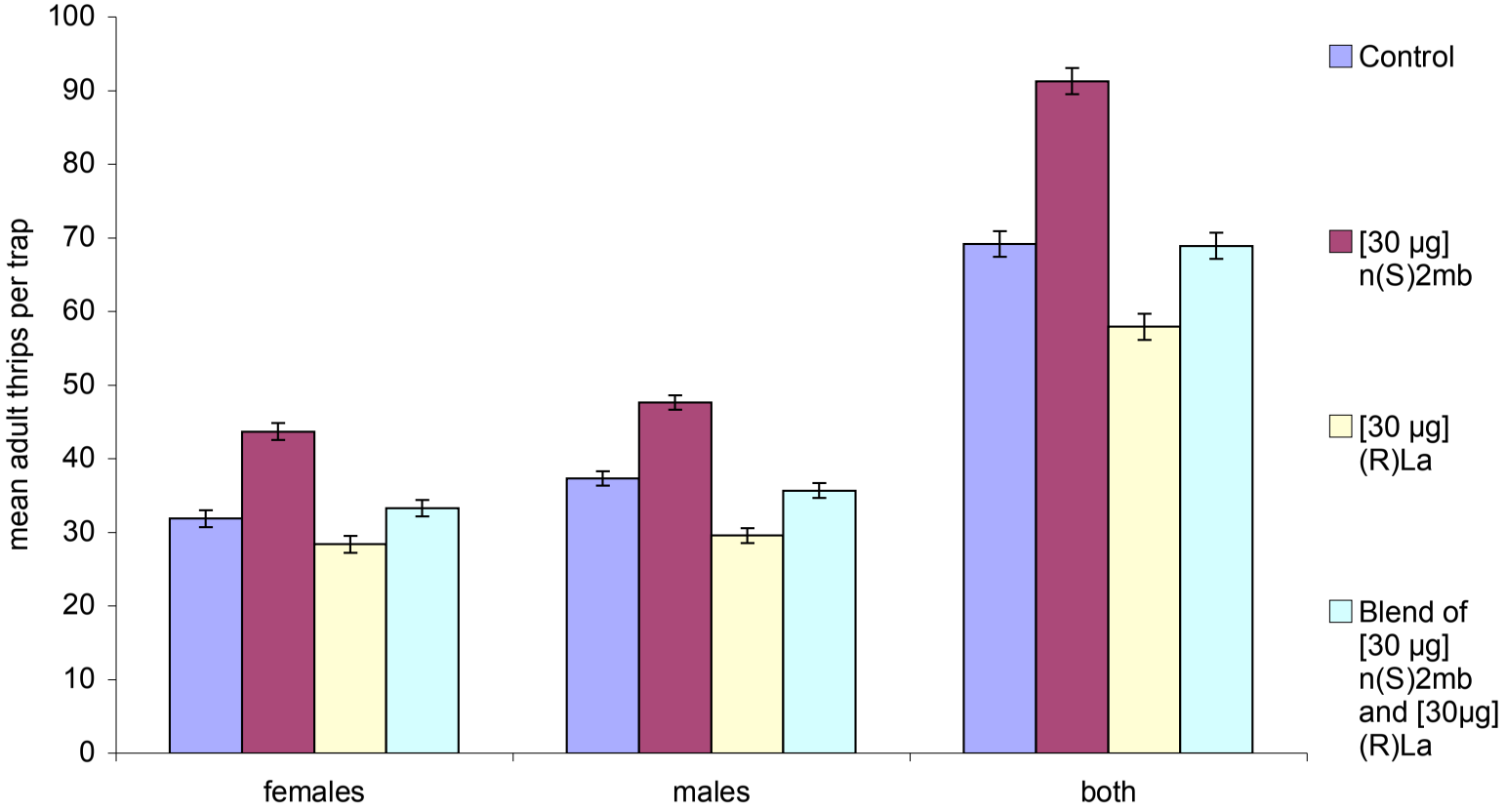

Figure 7.1: Plastic house trials: Experiment 1. Neryl (*S*)-2-methylbutanoate [30 μg], (*R*)-lavandulyl acetate [30 μg] and a blend of neryl (*S*)-2-methylbutanoate [30 μg] combined with (*R*)-lavandulyl acetate [30 μg] compared with control on blue sticky traps.

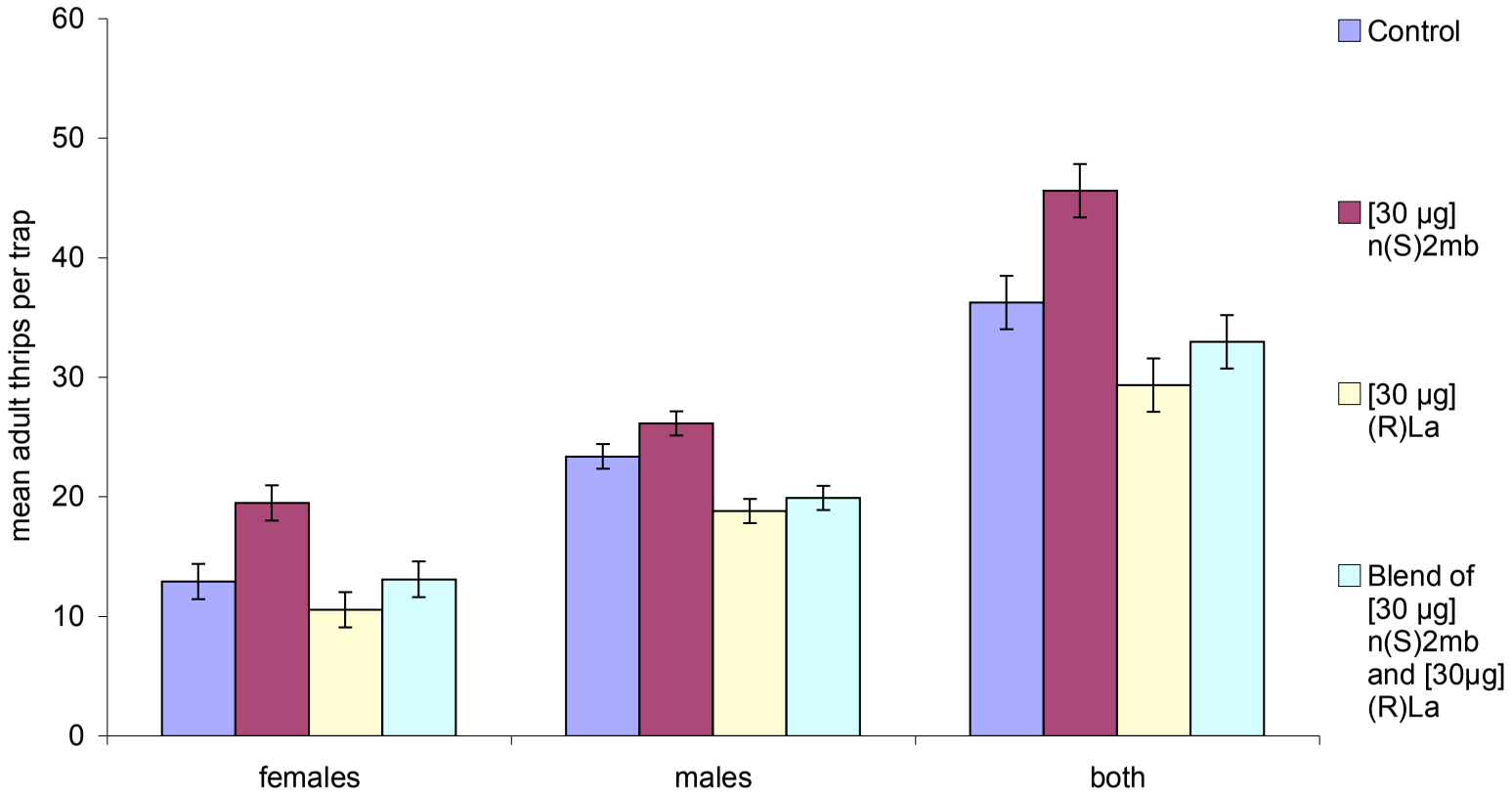

Figure 7.2: Plastic house trials: Experiment 2. Repeat of Figure 7.1. Neryl (*S*)-2-methylbutanoate [30 μg], (*R*)-lavandulyl acetate [30 μg] and a blend of neryl (*S*)-2-methylbutanoate [30 μg] combined with (*R*)-lavandulyl acetate [30 μg] compared with control on blue sticky traps.

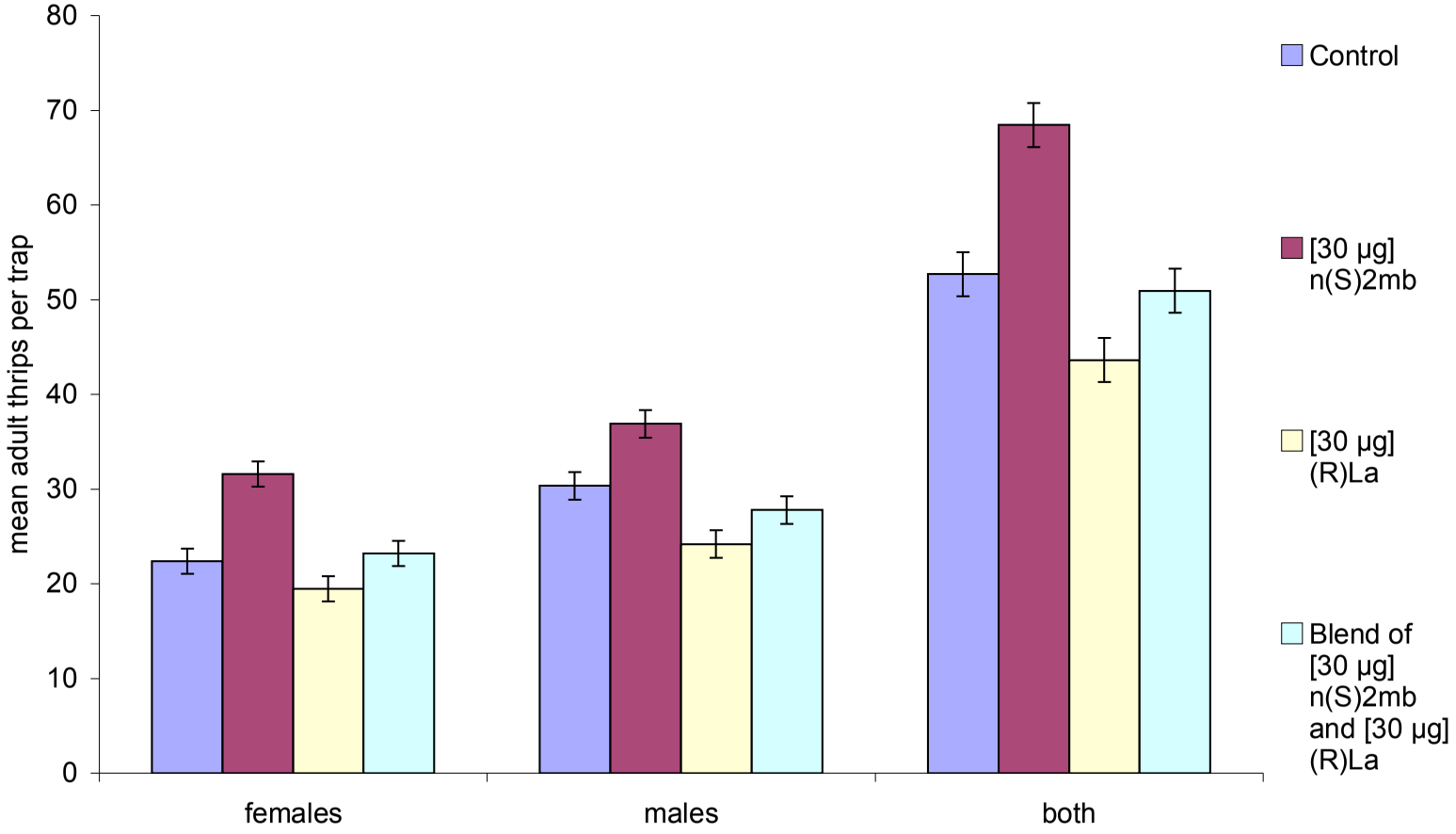

Figure 7.3: Plastic house trials: Experiment 1 and 2. Trap catch data from Figure 7.1 combined with trap catch data from Figure 7.2. Neryl (*S*)-2-methylbutanoate [30 μg], (*R*)-lavandulyl acetate [30 μg] and neryl (*S*)-2-methylbutanoate [30 μg] combined with (*R*)-lavandulyl acetate [30 μg] compared with control on blue sticky traps.

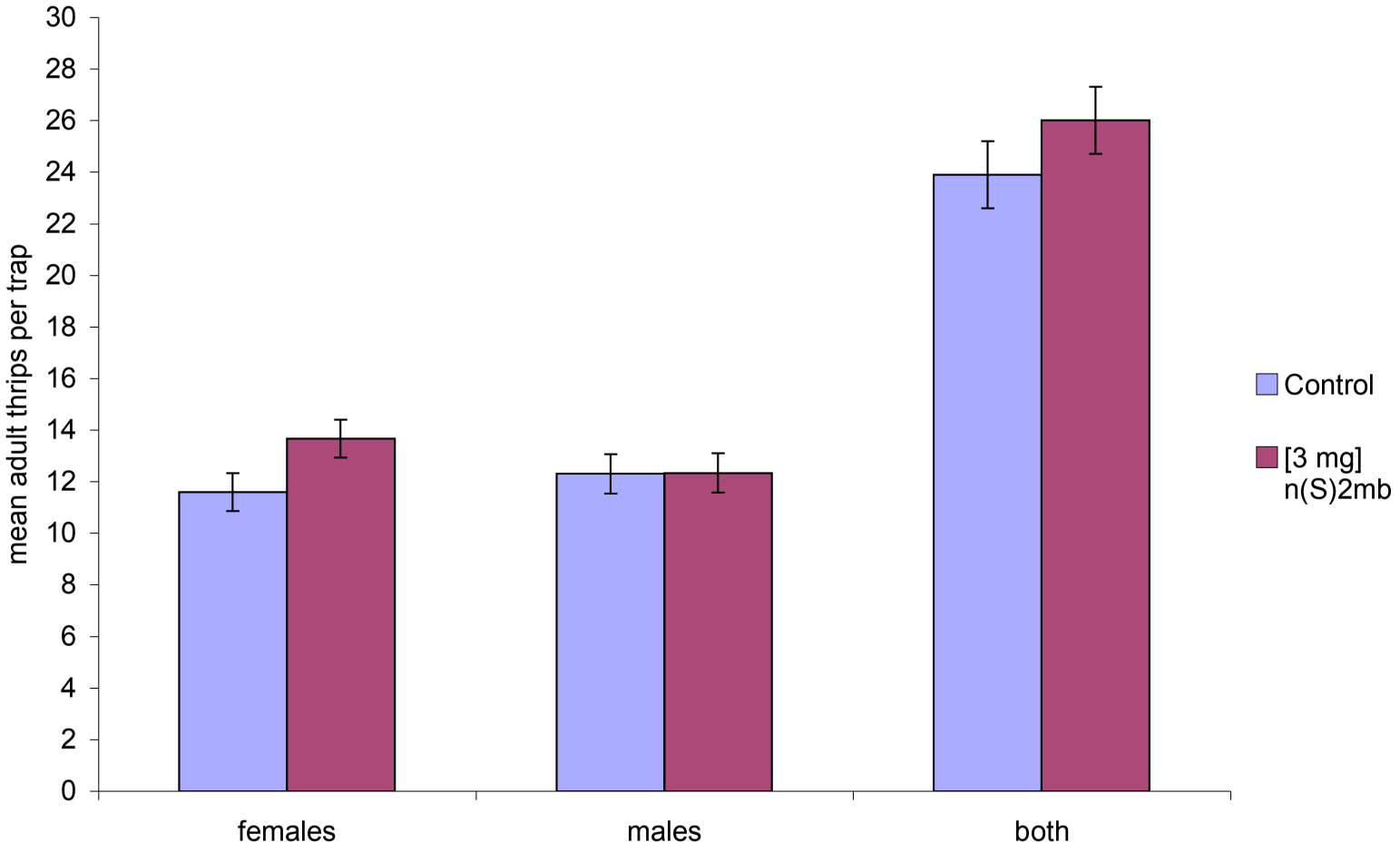

Figure 7.4: Plastic house trials: Experiment 3. High concentration, [3 mg] of neryl (*S*)-2-methylbutanoate compared with control on blue sticky traps.

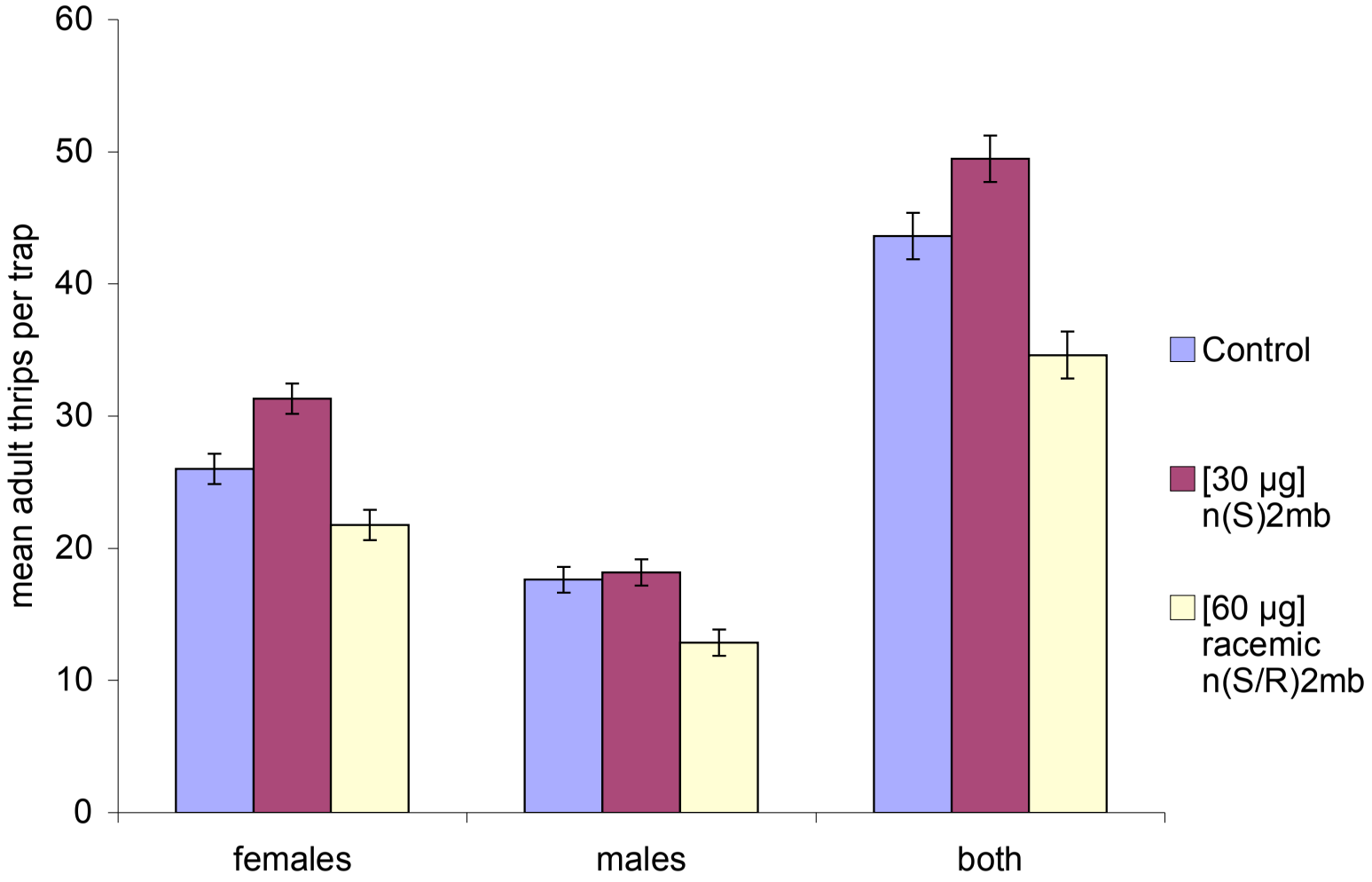

Figure 7.5: Plastic house trials: Experiment 4. Neryl (*S*)-2-methylbutanoate [30 μg] and racemic neryl 2-methylbutanoate compared with control on blue sticky traps.

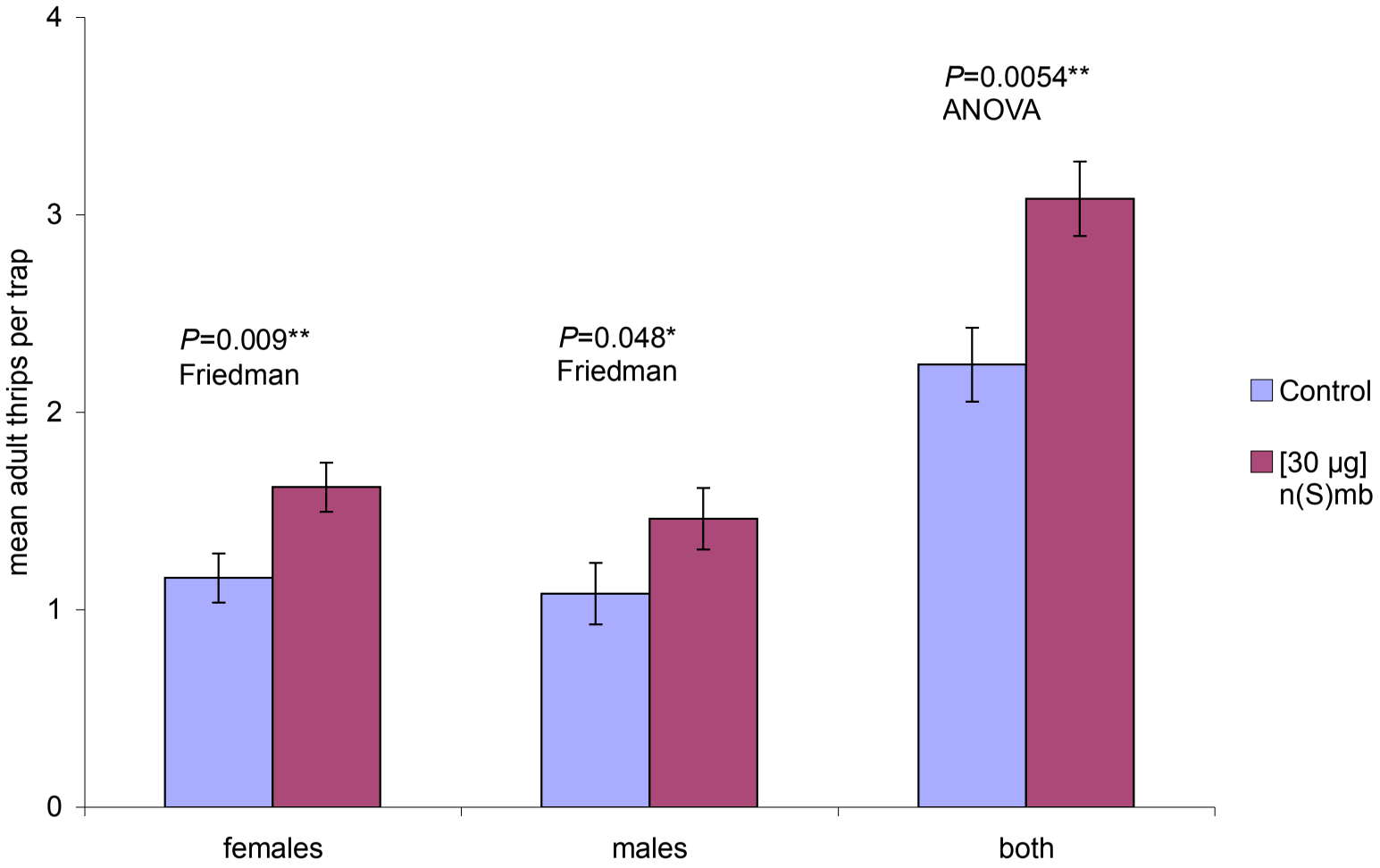

Figure 7.6: Plastic house trials: Experiment 5. Neryl (*S*)-2-methylbutanoate [30 μg] compared with control on reduced size blue sticky traps.

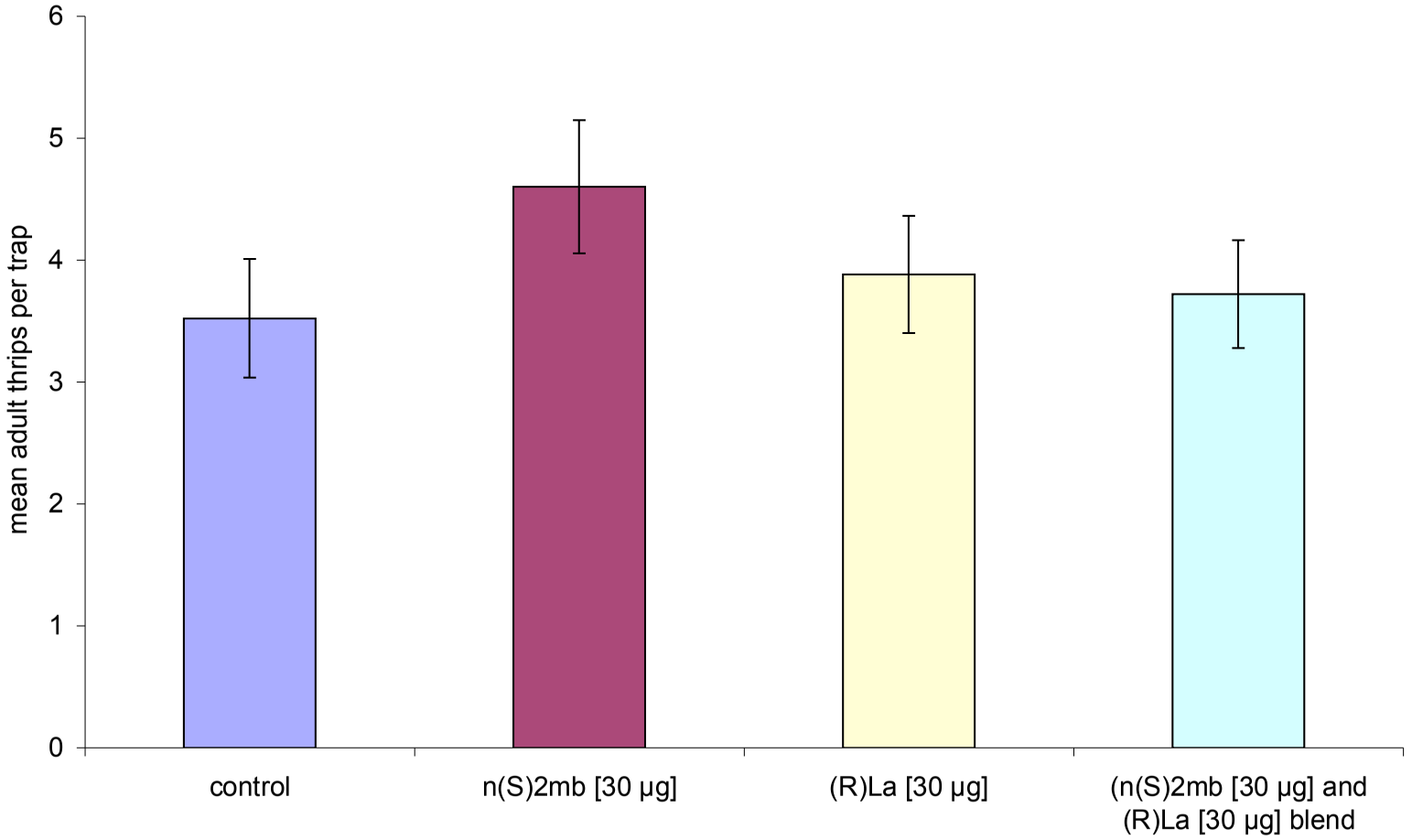

Figure 7.7: Trap catch of *Thrips angusticeps* in plastic house trials: Experiment 1. Neryl (*S*)-2-methylbutanoate [30 μg], (*R*)-lavandulyl acetate [30 μg] and a blend of neryl (*S*)-2-methylbutanoate [30 μg] combined with (*R*)-lavandulyl acetate [30 μg] compared with control on blue sticky traps. Error bars represent ±SEM. No significant difference in trap catch was found between treatments.

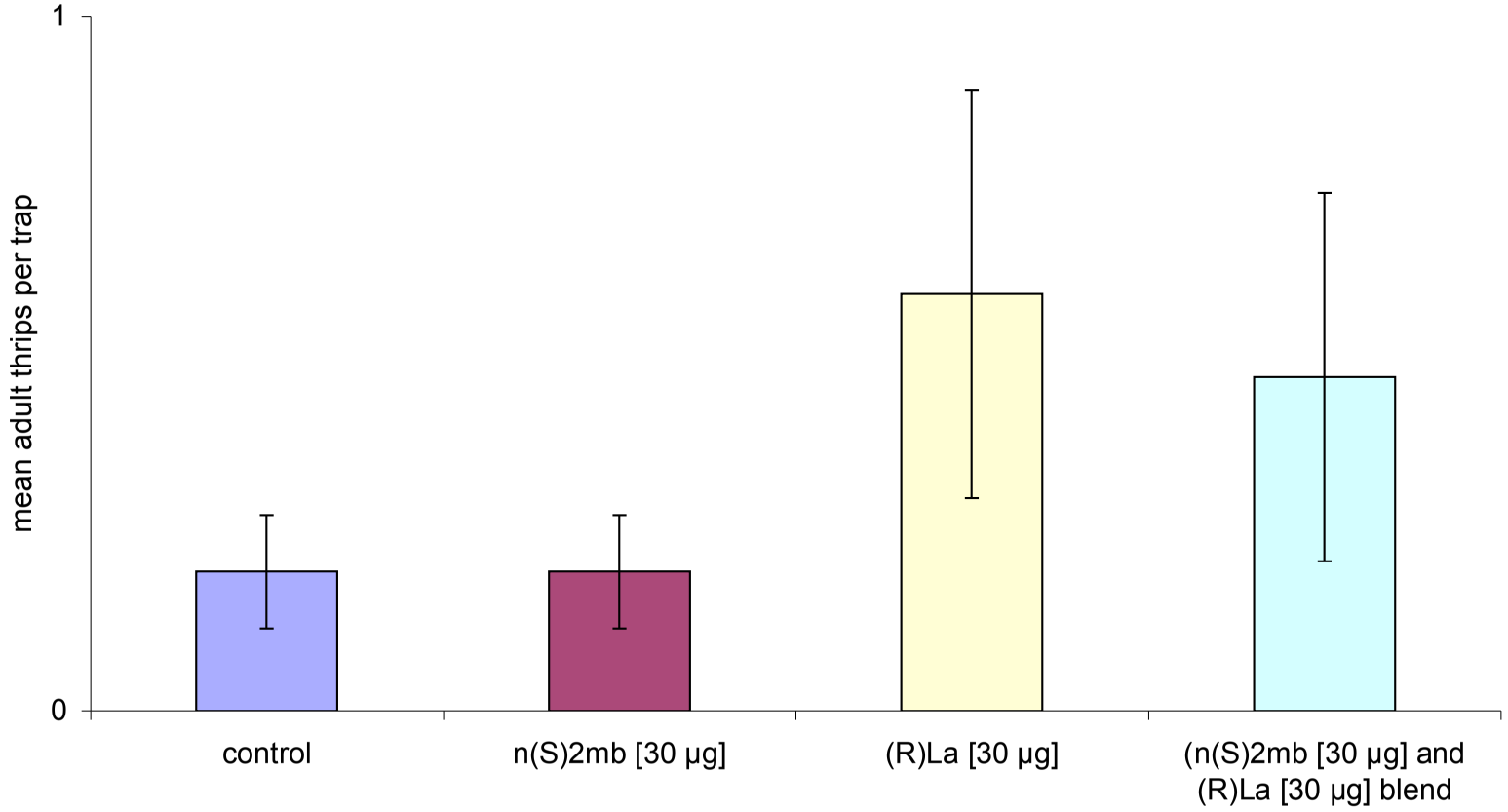

Figure 7.8: Trap catch of *Thrips tabaci* in plastic house trials: Experiment 1. Neryl (*S*)-2-methylbutanoate [30 μg], (*R*)-lavandulyl acetate [30 μg] and a blend of neryl (*S*)-2-methylbutanoate [30 μg] combined with (*R*)-lavandulyl acetate [30 μg] compared with control on blue sticky traps. Error bars represent ±SEM. No significant difference in trap catch was found between treatments.

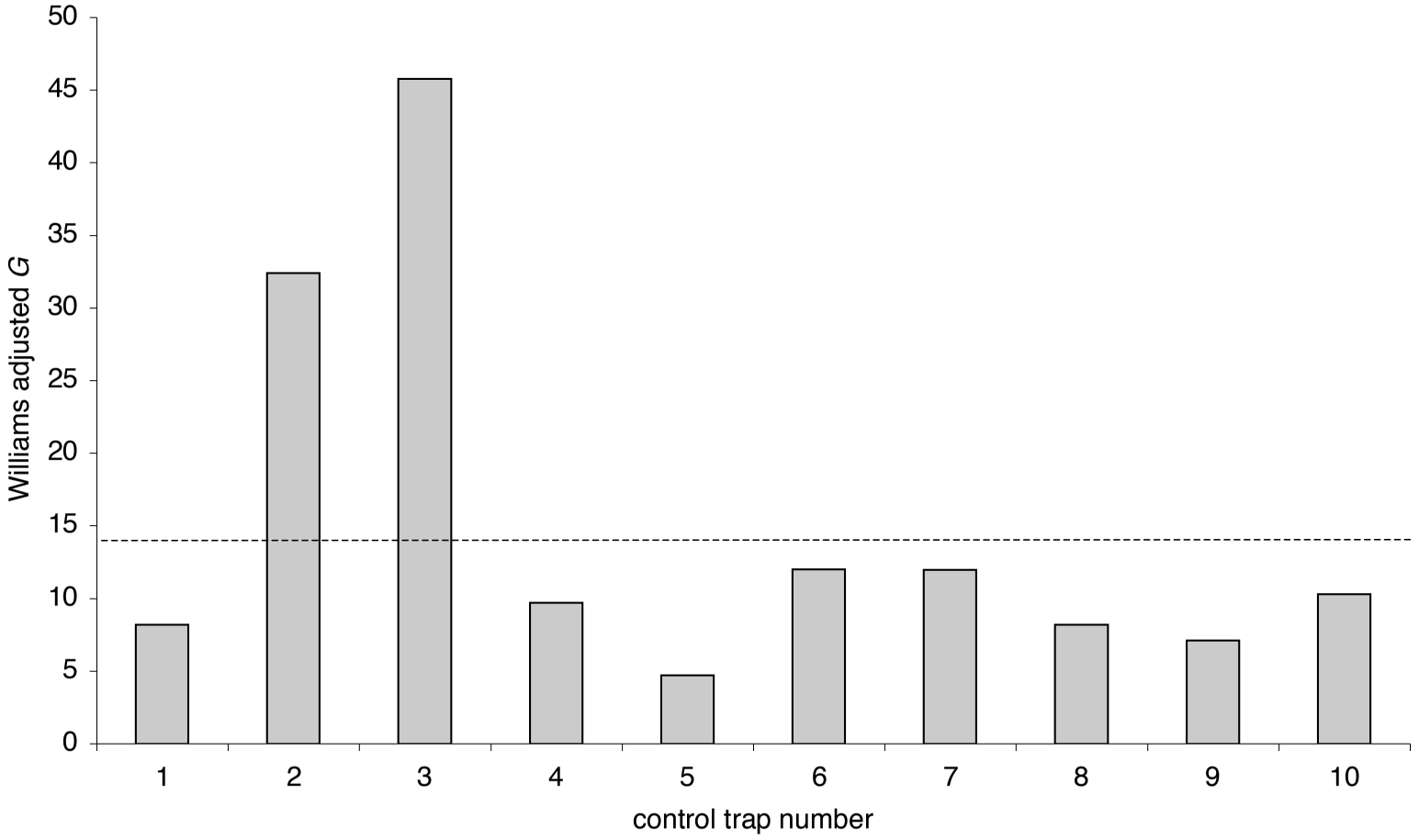

Figure 7.9: Williams adjusted G-test values for control trap front for the first 10 traps. The dotted line displays a critical value of χ^2 for 7 degrees of freedom (14.067), at the P=0.05 confidence level.

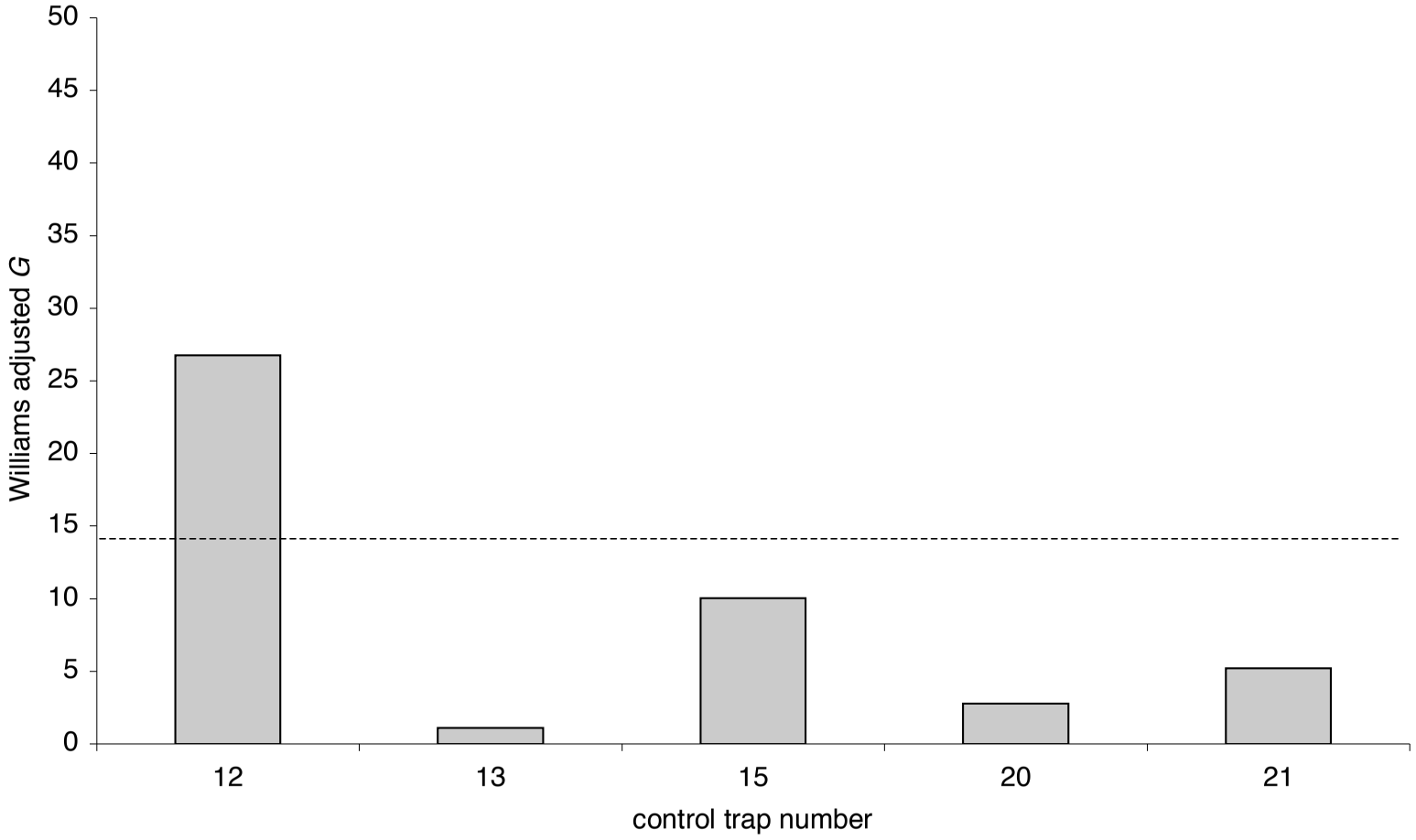

Figure 7.10: Williams adjusted G-test values for control trap front of the last five traps with $\geq$1 thrips per grid segment. The dotted line displays a critical value of χ^2 for 7 degrees of freedom (14.067), at the P=0.05 confidence level.

7.6 References

Bernays, E.A. and Chapman, R.F. (1994) *Host-plant selection by phytophagous insects.* Chapman and Hall, New York, USA.

Brødsgaard, H.F. (1989) Coloured sticky traps for *Frankliniella occidentalis* (Pergande) (Thysanoptera, Thripidae) in glasshouses. *Journal of Applied Entomology*, 107 136-140.

Brødsgaard, H.F. (1990) The effect of anisaldehyde as a scent attractant for *Frankliniella occidentalis* (Thysanoptera: Thripidae) and the response mechanism involved. *SROP/WPRS Bulletin*, 13 36-38.

Carrizo, P. (2008) Effects of yellow trap size on sampling efficiency for flower thrips (*Frankliniella occidentalis* from pepper plants (*Capsicum annum*) grown in greenhouses. *Ciencia e Investigación Agraria, Latinamerican Journal of Agricultural and Environmental Sciences*, 35 155-160.

Copping L.G. (2004) Semiochemicals. Chapter 4. In *The manual of biocontrol agents. Third edition of the biopesticide manual.* Edited by: L.G. Copping. British Crop Protection Council, Alton, UK. pp. 442-525.

Gómez, M., Garca, F., GreatRex, R., Lorca, M. and Serna, A. (2006) Preliminary field trials with the synthetic sexual aggregation pheromone of *Frankliniella occidentalis* on protected pepper and tomato crops in south-east Spain. *IOBC/WPRS Bulletin*, 29 153-158.

Gough, H.C. (1955) *Thrips angusticeps* Uzel attacking peas. *Plant Pathology*, 4 53.

Grieg-Smith, P. (1983) *Quantitative plant ecology.* 3rd edition. Blackwell Scientific Publications, Oxford, UK.

Hamilton, J.G.C. and Kirk, W.D.J. (2003) Method of monitoring/controlling thrips. Priority date: 21 December 2001. International patent application published under the Patent Cooperation Treaty WO 03/055309. World Intellectual Property Organization, Geneva.

Hamilton, J.G.C., Hall, D.R., Kirk,W.D.J. (2005) Identification of a male-produced aggregation pheromone in the western flower thrips *Frankliniella occidentalis*. *Journal of Chemical Ecology*, 31 1369-1379.

Heinz, K.M., Parrella, M.P. and Newman, J.P. (1992) Time-efficient use of yellow sticky traps in monitoring insect populations. *Journal of Economic Entomology*, 85 2263-2269.

Kirk, W.D.J. (1985) Effect of some floral scents on host finding by thrips (Insecta: Thysanoptera). *Journal of Chemical Ecology*, 11 35-43.

Kirk, W.D.J. (1987) Effects of trap size and scent on catches of *Thrips imaginis* Bagnall (Thysanoptera: Thripidae). *Journal of the Australian Entomological Society*, 26 299-302-

Kirk, W.D.J. and Hamilton, J.G.C. (2004) Evidence for a male-produced sex pheromone in the western flower thrips *Frankliniella occidentalis*. *Journal of Chemical Ecology*, 30 167-174.

Lewis, T. (1997) Pest Thrips In Perspective. Chapter 1. In: *Thrips as crop pests.*

Edited by: T. Lewis. CAB International, Wallingford, UK. pp. 1-13.

MacDonald, K.M., Hamilton, J.G.C., Jacobson, R. and Kirk, W.D.J. (2002) Effect of alarm pheromone on landing and take-off by adult western flower thrips. *Entomologia Experimentalis et Applicata*, 103 279-282.

Pearsall, I.A. (2002) Daily flight activity of the western flower thrips (Thysanoptera, Thripidae) in nectarine orchards in British Columbia, Canada. *Journal of Applied Entomology*, 126 293-302.

Smith, I. M., McNamara, D. G., Scott, P. R. and Holderness, M. (Eds) (1997). *Frankliniella occidentalis.* In: *Quarantine Pests for Europe. 2nd edition.* CAB International, Wallingford, UK. pp. 267-272.

Sokal, R.R. and Rohlf, F.J. (1995) *Biometry: the principles and practice of statistics in biological research.* 3rd edition. W.H. Freeman and Company, New York, USA.

Steiner, M.Y., Spohr, L.J., Barchia, I. and Goodwin, S. (1999) Rapid estimation of numbers of whiteflies (Hemiptera: Aleurodidae) and thrips (Thysanoptera: Thripidae) on sticky traps. *Australian Journal of Entomology*, 38 367-372.

Teulon, D.A.J., Hollister, B., Butler, R.C. and Cameron, E.A. (1999) Colour and odour responses of flying western flower thrips: wind tunnel and greenhouse experiments. *Entomologia Experimentalis et Applicata*, 93 9-19.

Williams, D.A. (1976) Improved likelihood ratio tests for complete contingency tables. *Biometrika*, 63 33-37.

Chapter 8

General discussion

The main aim of this thesis was to examine neryl (*S*)-2-methylbutanoate, a compound thought to have aggregation pheromone properties with respect to *F. occidentalis*, with a view to determining how this compound could be used to monitor and control the pest in protected crops (aim 1). Additional aims included developing new bioassays to investigate the properties of male-produced compounds (aim 2), undertaking studies to reveal the dynamics of sex pheromone release from *F. occidentalis* males (aim 3), attempting to identify the minor male-produced headspace compounds (aim 4) and to analyse field data from a trial using compounds thought to have aggregation pheromone properties with respect to *F. occidentalis* (aim 5).

8.1 Key findings

8.1.1 Male *F. occidentalis* produced compounds

Aggregation pheromone production dynamics were investigated using headspace SPME in conjunction with GC-FID and GC-MS and an estimate of production was obtained. Male *F. occidentalis* produce neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate (Hamilton *et al.*, 2005). Neryl (*S*)-2-methylbutanoate is produced on-demand at an estimated rate of 0.1-0.3 ng male^{-1} h^{-1} and these results have also been published elsewhere (Dublon *et al.*, 2008). The effects of density on male *F. occidentalis* headspace production were investigated though no firm conclusions were drawn. As occurs in *Ips pini* beetles (Ginzel *et al.*, 2007), it was hypothesised that a down-regulation of neryl (*S*)-2-methylbutanoate may be observed as density increases. On the contrary, production rate appeared to increase with increasing male density though subsequent experiments suggested that rapid adsorption to the SPME fibre of newly produced neryl (*S*)-2-methylbutanoate may have meant that individual males were unable to detect elevated levels of this compound in headspace. In addition, as many males were present in a small area at the higher density, it is possible that close proximity of individuals may have resulted in elevated aggressive interactions and perhaps increased neryl (*S*)-2-methylbutanoate production. The presence of a female in the entrainment process did not appear to affect production rates.

(*R*)-lavandulyl acetate is produced by male *F. occidentalis* (Hamilton *et al.*, 2005) and whilst its production rate remains unquantified, it is ordinarily produced in a lower amount than neryl (*S*)-2-methylbutanoate. As a chiral molecule, synthesised on demand by male *F. occidentalis*, it seems likely that it has a role in communication or defence. As enantiomeric specificity appears to be present in flight responses to neryl (*S*)-2-methylbutanoate it is possible that female *F. occidentalis* are also able to

discern between enantiomers of lavandulyl acetate where only (*R*)-lavandulyl acetate is biologically active. Further examination of the behavioural role of this compound is essential before its potential for exploitation in *F. occidentalis* control can be ruled out (see below).

Whilst it was not possible to confirm the identity of the minor male produced headspace compounds, comparison of standards with headspace entrainments using GC-MS enabled specific compounds to be excluded. Limonene was confirmed in SPME headspace entrainment though it is not clear if this compound results from residual chrysanthemum material that may have been present in the entrainment. Confirmation of male produced headspace compounds was made difficult by the small and variable amounts detected in male entrainments.

Male, and to a lesser extent, female-exposed filter discs elicit a biological response in newly introduced females where more contacts with the treatment disc are made. The response of females to male-exposed filter discs is greater in magnitude than the response obtained with synthetic neryl (*S*)-2-methylbutanoate, applied in isolation (see 8.1.2). This result is exciting and as it suggests that neryl (*S*)-2-methylbutanoate is not the sole cause of observed increased contacts, it may be possible that a new and hitherto undiscovered compound or set of compounds is being produced in males.

In the case of male *F. occidentalis* this compound or compounds were extractable from male-exposed filter discs and thus transferrable to new discs. This compound or set of compounds were not detectable in filter paper solvent extracts using GC-MS nor TLC. It is possible therefore that the substance(s) present on the filter disc are produced at a level below the detection threshold. Detection threshold varies significantly according to instrument or plate performance and operating conditions, though parallels can be drawn with detection limits of neryl (*S*)-2-methylbutanoate standard. Both systems were able to detect 10 ng neryl (*S*)-2-methylbutanoate.

It is possible that the 'extra' compound that appears to result in a strong biological contact response in females when presented with male-exposed discs is infact (*R*)-lavandulyl acetate where this compound acts synergistically and at low concentrations with neryl (*S*)-2-methylbutanoate. As females are not known to produce (*R*)-lavandulyl acetate nor neryl (*S*)-2-methylbutanoate, this fails to account for female-exposed discs eliciting a weak biological response. Incidences of female insects 'attracting' females are rare and ordinarily non-advantageous from an evolutionary perspective.

The presence of a male derived compound on a filter disc was in itself somewhat unexpected as it was considered likely that volatile headspace semiochemicals would not be retained on such a substrate. In carnation thrips *Taeniothrips dianthi* it has been suggested that males use abdominal sternal glands to deposit a compound with a calming effect upon the female (Pelikán, 1954). It is possible in *F. occidentalis* that a compound released from male sternal glands may be laid down in this way or alternatively an anal droplet may be produced. As during the entrainment process, the thrips were able to make direct contact with the SPME fibre it is possible that neryl (*S*)-2-methylbutanoate and (*R*)-lavandulyl acetate are directly laid down upon the fibre. Such an event has not thus far been observed.

It is possible that the *F. occidentalis* mating system uses neryl (*S*)-2-methylbutanoate as an aggregation pheromone and several additional compounds (including (*R*)-lavandulyl acetate) each with differing communication roles, perhaps over different distances. As mentioned in section 5.11 multiple roles can be associated with multiple male produced pheromones as is the case with male *Nauphoeta cinerea*. Males in this species release two compounds at low concentration that are highly volatile attracting females from a distance and two compounds that cause females to remain close, acting as either an attractant, a sexual stimulant or an arrestant (Sirugue *et al.*, 1992).

8.1.2 Laboratory behaviours

New laboratory bioassays have been developed in order to investigate the biological activity of the above compounds with the filter disc binomial-choice assay proving an effective, reliable and time efficient method of screening compounds and concentrations for *F. occidentalis* biological activity. Due to the simplicity of this bioassay it was not possible to separate possible attraction from arrestment and therefore the precise mechanism of the contact response was not elucidated. Unsuccessful attempts were made to further examine the activity walking behaviours exhibited in two additional bioassays. Unfortunately day-to-day variation in activity was too high to draw any firm conclusions from these experiments.

The aggregation pheromone, neryl (*S*)-2-methylbutanoate has been shown, to have biological activity in the laboratory where it causes increased contacts in mixed-age females. Whilst this effect is present at a low concentration, 10 pg μl^{-1} it is likely that it also works over a the range 10 pg - 100 ng.

An attempt was made at developing a small-scale flight bioassay to observe *F. occidentalis* flight responses when presented with semiochemicals. With the benefit of hindsight, this proved to be most time-consuming and the final two bioassay designs had reduced utility, as there was considerable variation in the flight response. The aggregation pheromone, neryl (*S*)-2-methylbutanoate did not increase trap catch in either of these assays at any of the concentrations tested. As demonstrated by the walking responses obtained in the filter-disc binomial choice assay the concentration of neryl (*S*)-2-methylbutanoate being assayed is likely to be of critical importance.

The racemic mixture of lavandulyl acetate elicited no response in the laboratory bioassays used. It is possible that this compound is ineffective if *F. occidentalis* is able to distinguish between enantiomers.

8.1.3 Glasshouse behaviours

The aggregation pheromone, neryl (*S*)-2-methylbutanoate caused a 35-54% increase in female and male *F. occidentalis* trap catch when applied to a synthetic lure. This confirms the initial plastic house trials findings (Hamilton *et al.*, 2005). Enantiomeric specificity appears to be displayed where the racemic mixture does not increase trap catch.

The percentage increase in trap catch is similar on full size traps when compared with smaller traps though trap catch numbers are reduced in proportion to the reduction in trap area. A 35-54% increase was recorded on full size blue traps and a similar 35-40% increase on reduced size blue traps. At the sizes tested, trap catch is proportional to trap size and therefore even with a pheromonal component being present, the visual component of the lure is thus of key importance when attempting to improve trap efficiency. Perhaps thrips are induced to land by the the initial visual stimulus and the odour stimulus is a secondary response. No conclusions can be drawn as to whether the thrips are arrested or attracted to the trap and as departure was not measured it is unclear if neryl (*S*)-2-methylbutanoate merely retains more thrips after they have landed.

(*R*)-lavandulyl acetate reduced trap catch by -16% at the concentration used. This contrasts with previous findings where the compound neither increased nor decreased trap catch (Hamilton *et al.*, 2005). Alternative concentrations need to be assayed before firm understandings about the biological effects of this compound can be made.

Neither neryl (*S*)-2-methylbutanoate nor (*R*)-lavandulyl acetate increased trap catch in *Thrips tabaci* nor *T. angusticeps*. Numbers caught were too low to draw negative conclusions about these compounds commercial utility when seeking to trap these species.

Individuals upon traps whilst appearing clumped were, when subjected to statistical

analysis infrequently clumped in distribution. It is likely however, that the grid cell size used in counting was too large to detect all of the clumps. Clumping and thrips position within traps can be affected by environmental factors such as sunlight and breeze. Thrips appeared to be numerous on the vertical front trap middle edge, and the reasons for this are unclear.

A method of partial trap counting, utilising the middle grid cells on each trap has been obtained with a view to increasing future trap counting efficiency. Using this method 50% of the trap area can be used to predict total trap catch with adequate predictive ability.

8.2 Future directions

It is essential to examine the role of (*R*)-lavandulyl acetate in the communication system of *F. occidentalis*. Due to a lack of *R* enantiomer it was not possible to test the walking response of females to this compound in the binomial choice assay. Testing this compound in the laboratory at a range of concentrations would provide valuable information on its potential as an 'attractant' and when assayed in conjunction with neryl (*S*)-2-methylbutanoate, a synergist. Whilst this compound may reduce catch in plastic houses, it is possible that this would be reversed at a lower concentration. It is apparent, from headspace entrainments that, male produced (*R*)-lavandulyl acetate occurs at a lower concentration than neryl (*S*)-2-methylbutanoate. It would therefore also be wise to assay this compound in the field at a reduced concentration.

It is possible that an entirely different (perhaps higher molecular weight and less volatile) compound is laid down upon filter discs by male *F. occidentalis*. This is most exciting and identification of this compound could be significant with respect to our understanding of *F. occidentalis* communication. If the extraction efficiency of male-

exposed filter discs could be improved it may be possible to detect these compounds using GC.

In parallel, male headspace entrainments should be carried out with SPME fibres capable of detecting higher molecular weight compounds. This would be a simple and comparatively inexpensive method of detecting potential additional compounds.

Elucidation of the male-produced minor compounds and confirmation of the presence of limonene may be of value with a view to increasing trap catch. Unfortunately production levels of the minor compounds appear to vary quite substantially and as a result it is unclear if these compounds would necessarily increase trap catch when added as a synergist. Identifying potential sources of limonene in chrysanthemum leaves and petals could easily be achieved using solvent plant extraction and GC-MS. This would help in deciding if this compound was present as a contaminant.

8.3 References

Dublon, I.A.N., Hamilton, J.G.C. and Kirk, W.D.J. (2008) Quantification of the release rate of the aggregation pheromone of the western flower thrips, *Frankliniella occidentalis* (Pergande), using solid-phase microextraction (SPME). *Acta Phytopathologica et Entomologica Hungarica*, 43 249-256.

Ginzel, M. D., Bearfield, J. C., Keeling, C. I., McCormack, C. C., Blomquist, G. J. and Tittiger, C. (2007) Antennally mediated negative feedback regulation of pheromone production in the pine engraver beetle, *Ips pini*. *Naturwissenschaften*, 94 61-64.

Hamilton, J.G.C., Hall, D.R., Kirk, W.D.J. (2005) Identification of a male-produced aggregation pheromone in the western flower thrips *Frankliniella occidentalis*. *Journal of Chemical Ecology*, 31 1369-1379.

Pelikán, J. (1951) O třásněnce karafiátové. [On carnation thrips, *Taeniothrips dianthi* Priesner]. *Entomologické Listy*, 14 5-38.

Sirugue, D., Bonnard, O., Le Quere, J.-L., Farine, J.-P. and Brossut, R. (1992) 2-methylthiazolidine and 4-ethylguaiacol, male sex pheromone components of the cockroach *Nauphoeta cinerea* (Dictyoptera, Blaberidae): a reinvestigation. *Journal of Chemical Ecology*, 18 2261-2276.

Appendix A:

Peer-reviewed publications resulting from this thesis:

i) Dublon *et al.*, 2008;

Dublon, I.A.N., Hamilton, J.G.C. and Kirk, W.D.J. (2008) Quantification of the release rate of the aggregation pheromone of the western flower thrips, *Frankliniella occidentalis* (Pergande), using solid-phase microextraction (SPME). *Acta Phytopathologica Entomologica et Hungarica*, 43 249-256.

Digital Object Identifier (DOI): 10.1556/APhyt.43.2008.2.6

Article available on-line from Saturday, November 22, 2008.

URL: http://www.akademiai.com/content/007772v0260623n7

Acta Phytopathologica Entomologica et Hungarica

Akadémiai Kiadó Publishers

ISSN: 0238-1249 (Print) 1588-2691 (Online).