FACTORS AFFECTING THE RESPONSE OF THRIPS TO AN OLFACTORY CUE

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy (Ph.D.)

at

Lincoln University

by

Mette-Cecilie Krause Nielsen

Bio-Protection Research Centre

Lincoln University

2013

Dedicated to the women who raised me

Marie Cecilie Krause Nielsen

&

Deborah Marie Risk

Abstract of a thesis submitted in partial fulfilment of the
requirements for the Degree of Ph.D.

Factors affecting the response of thrips to an olfactory cue

By
Mette-Cecilie Krause Nielsen

Some thrips (Order Thysanoptera) are key pests of many greenhouse and outdoor crops worldwide. Biological attributes such as polyphagy, rapid reproduction, cryptic behaviour and insecticide resistance make them particularly difficult to manage. Because a large number of insects utilize plant volatiles in host finding, there is growing interest in exploiting the olfactory cues that insects use to find their hosts, including the use of semiochemicals as attractants in pest management. However, our understanding of the mechanisms eliciting the response of thrips to semiochemicals is still only rudimentary and a greater understanding of their behavioural response to olfactory cues is needed to optimize the use of semiochemicals.

An ecological approach has been taken to investigate the reasons why the use of a known thrips attractant, methyl isonicotinate (MI), in combination with coloured traps works better in some situations than in others that appear very similar. The approach investigated the influence of selected intrinsic and extrinsic factors on the response of the worldwide polyphagous pest western flower thrips (*Frankliniella occidentalis* (Pergande)). Several studies undertaken previously using MI-based kairomone lures have shown large variations in trapping efficacy both within and between different trials. Most notable were results from southeast Spain where there appeared to be an overall lack of effect. Specific objectives include examination of the release kinetics and release rates of MI from passive dispensers, the effect of different release rates of MI on thrips response in greenhouse environments, the attraction of WFT to MI under controlled laboratory conditions, and further investigations of the lack of responsiveness of Spanish WFT to MI to determine whether there are intraspecific differences among WFT populations.

Two main results can be drawn from the research in this thesis. First, the results showed that intrinsic factors such as phenotype play a much larger role than previously expected in the responses of thrips to an olfactory cue. Y-tube olfactometer assays testing MI under controlled conditions using populations collected from different countries showed that WFT differ in their response to an olfactory cue depending on which region of the world they are from. The work in this thesis confirmed previous experiments that WFT collected from

southeast Spain did not respond to MI. Significantly more WFT collected from New Zealand (73%), The Netherlands (72%), Kenya (70%) and France (61%) preferred the MI laden arm in the Y-tube olfactometer over the clean arm. However, Spanish WFT populations did not show any significant preference for the MI laden arm. Additional Y-tube olfactometer studies undertaken to further explore this non-response, using different doses and concentrations, and an alternative thrips kairomone lure, showed the same result: no positive behavioural response was found for the WFT population collected in Spain. This suggests the development of a different behavioural phenotype of WFT in Spain has occurred. Additional experiments using electroantennogram (EAG) technique showed that Spanish WFT can detect MI at least at the peripheral olfactory reception level, and suggest that the non-response is due to failure in the olfactory signal pathway of the central nervous system. While the work undertaken in this study suggests that underlying genetic variation between populations is the basis of the difference, the reason for this non-response is still to be determined.

Second, the study identified that several extrinsic factors influence the level of response in populations of WFT that showed a significant response to MI. While the dispensers were used for releasing MI at a constant rate (zero-order kinetics), temperature was shown to influence the release rate strongly. Increasing the temperature from 15 to 25^{o}C, resulted in a 2.5-fold increase in the release rate of MI for a commercially available sachet. Airflow was also shown to affect the release rate. Considering the critical role release rates of kairomones play in insect trap responses, it is reasonable to infer that differences in release rates as a result of choice of passive dispenser and extrinsic factors, such as temperature and air flow, could account for some of the variation previously experienced in field and greenhouse trials using MI to trap thrips. This was confirmed in a greenhouse study showing the response thrips to MI was at least partially positively dose related. The greenhouse trial also highlighted other areas believed to play a role on the responsiveness of thrips such as odour saturation of the air surrounding traps used in the greenhouse.

The fundamental information on the intrinsic and extrinsic factors affecting the behavioural response of thrips to odours obtained from this research can be used to design better tools for monitoring and trapping thrips pests using semiochemicals in New Zealand and worldwide.

Keywords: Thysanoptera, *Frankliniella occidentalis*, western flower thrips, semiochemicals, allelochemicals**,** kairomone, attractant, methyl isonicotinate, intrinsic factors, extrinsic factors, olfactometer, trapping variation, phenotype, genotype, dispenser, dose.

Table of contents

List of tables

.

List of figures

List of appendices

Chapter 1

General introduction

Thrips are key pests of many greenhouse and outdoor crops worldwide (Parker et al. 1995, Lewis 1997a) because of their ability to damage plants directly through feeding and oviposition and indirectly through transmission of plant viruses. Biological attributes such as polyphagy, vagility, rapid reproduction, cryptic behaviour and insecticide resistance make them particularly difficult to manage (Mound and Teulon 1995, Morse and Hoddle 2006). Consequently, there has been considerable interest in developing a range of new and alternative methods for thrips pest management. Because a large number of insect pests utilize plant volatiles in host finding (Metcalf and Metcalf 1992), there is growing interest in utilising the cues that insects use to find their hosts in applied pest management, including the use of semiochemicals as attractants (Koschier 2008). Untangling the mechanisms of these insect-plant interactions is essential for developing and optimising tools used for pest management, in addition to improving our understanding from an ecological and evolutionary perspective.

The search for kairomone (an interspecific semiochemical that provides an adaptive advantage to the receiver) lures that attract thrips has been carried out for almost a century (Howlett 1914). By adding a kairomone lure to traps, more effective thrips traps could improve thrips monitoring at low population densities (i.e. early infestations) and potentially reduce thrips numbers by mass-trapping. It has been demonstrated many times that thrips are attracted to colours and odours (Moffit 1964, Kirk 1985, Kirk 1987, Teulon and Ramakers 1990, Teulon and Penman 1992, Frey et al. 1994, Mateus and Mexia 1995, Pow et al. 1998, de Kogel et al. 1999, Teulon et al. 1999, Koschier et al. 2000, Murai et al. 2000, Smits et al. 2000, Chermenskaya et al. 2001, Imai et al. 2001). Many of the kairomones tested are odours found in flowers such as *p*-anisaldehyde (Brødsgaard 1990, Teulon and Ramakers 1990, Teulon et al. 1993a, Hollister et al. 1995), geraniol, eugenol, myrcene (Kirk 1985, Frey et al. 1994) and benzaldehyde (Teulon et al. 1993a). Some kairomones not reported from flowers have also been shown to be attractive to flower thrips species, such as ethyl nicotinate (Koschier et al. 2000, Koschier 2006), increasing trap capture of *Thrips obscuratus* (Penman et al. 1982). In the search for attractants to manage one of the most important cosmopolitan thrips pest species, the western flower thrips (WFT), *Frankliniella occidentalis* Pergande, methyl isonicotinate, a 4-pyridyl carbonyl compound, has been recognised (Davidson et al.

2005, Davidson et al. 2007, Davidson et al. 2008). Methyl isonicotinate (MI) is also an effective kairomone lure to a range of other polyphagous pest thrips species such as *Frankliniella schultzei* (S. Sevgan, unpubl. data), *Thrips imaginis* (Broughton and Harrison 2012), *Thrips major* (Teulon et al. 2008b) and *Thrips tabaci* (Teulon et al. 2007b, Davidson et al. 2009) and MI is the current active ingredient of the commercially available thrips lure LUREM-TR (Teulon et al. 2011).

There are a growing number of examples of the successful use of attractants for thrips and other insects, but for most the underlying mechanisms are not fully understood. To optimize the use of traps in thrips management a better understanding of the interaction that occurs between the insects, the host plants, the environment and the trapping systems is needed. A combination of both intrinsic and extrinsic factors will likely affect the way insects perceive and respond to visual and olfactory cues when they are in the close vicinity of a host (Figure 1.1). To manipulate the behavioural response of thrips in their host-finding using a kairomone as an olfactory cue, in combination with colours, we need to understand how intrinsic and extrinsic factors may influence the way thrips respond to the visual and olfactory cues under different conditions.

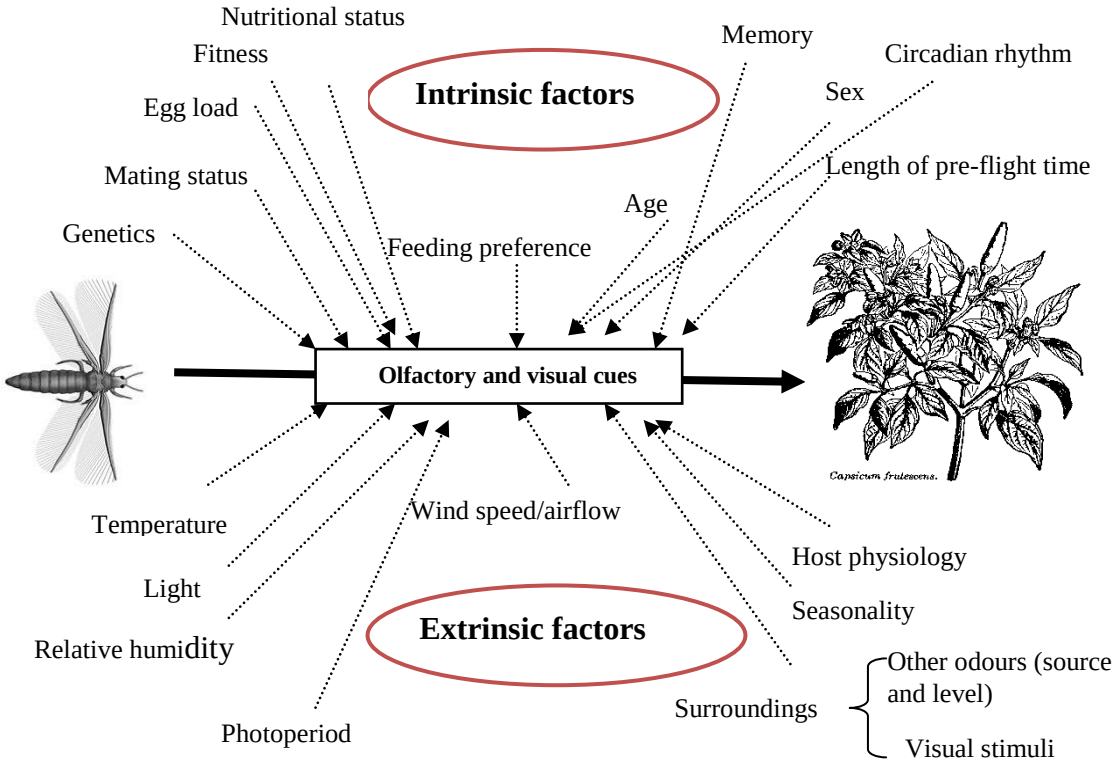


Figure 1.1 *Intrinsic and extrinsic factors that may influence thrips using olfactory and visual cues to locate potential host plants.*

Smits et al. (2000) suggested that the local situation and other stimuli determine the type of trap most attractive to a thrips species and that simply adding a kairomone lure to a trap leads to very variable and sometimes contradicting results because our understanding of thrips behaviour in regards to host finding is still insufficient. Several studies undertaken over the last few years using similar thrips kairomone lures support this implication as large variations in trapping efficacy of thrips on traps baited with a kairomone lure have been reported. Table 1.1 shows records of WFT trapping using LUREM-TR in combination with colour traps in different cropping systems, locations and times of year. The records clearly show that large variations in trapping efficacy (i.e. within trial areas, between seasons and between locations) have been recorded with respect to trapping of WFT when using LUREM-TR. Of particular note are the greenhouse experiments undertaken in southeast Spain which have failed to achieve any significant increases in the number of WFT caught on traps baited with either LUREM-TR or the active ingredient MI (M-C Nielsen, unpubl. data).

The overall aim of this study was to investigate how selected intrinsic and extrinsic factors affect the response of WFT to olfactory cues that are used in host finding and determine if these factors explain the variation observed in trapping efficacy. **Specific objectives** include:

1. Examination of the release kinetics and measure the release rates of MI over time from passive dispensers under constant laboratory conditions to establish the effect dispenser type and abiotic factors have on the release rate; and
2. Investigate release rates under greenhouse conditions and determine the effect of different release rates of MI on thrips response in greenhouse environments.
3. Investigate the attraction of WFT to MI under controlled laboratory conditions to determine the influence that feeding history, virus status, genetic variation and geographic origin have on the response; and
4. Further investigate the lack of responsiveness of Spanish WFT to MI to determine whether there are intraspecific differences among WFT populations.

Table 1.1 *Records of western flower thrips trapping using LUREM-TR in combination with colour traps.*

Crop	Location	Cropping system	Trapping system	Season	Trapping results*	Reference
Beans	Kenya (Nairobi)	Greenhouse	Blue sticky traps	Summer (January–February)	1.1–4.2x mean increase (Average 2.5x increase)	S. Sevgan, unpubl. data
Eggplant	Spain (Almeria)	Greenhouse	Blue sticky traps	Summer (June)	0–2.7x mean increase (Average 1.1x increase)	J.E. Belda, unpubl. data
Capsicum	Argentina (Currients)	Greenhouse	Blue sticky traps	Winter (June–July)	2.2–7.8x mean increase (Average 4.45x increase)	S. Caceres, unpubl. data
	Tunisia (Moknine)	Greenhouse	Blue sticky traps	Spring (April–May)	2.6–9.0 x mean increase (Average 7.0x increase)	M. Elimem, unpubl. data
	Netherlands (Wageningen)	Greenhouse	Blue sticky traps	- -	1.6–1.8x mean increase (Average 1.7x increase)	Ramakers and van Wensveen (2008)
	Australia (Perth)	Greenhouse	Blue sticky traps	Summer (February)	1.2–2.0x mean increase (Average 1.5x)	S. Broughton, unpubl. data
	Spain (Almeria)	Greenhouse	Blue sticky traps	Winter (January–February)	0–1.1x mean increase (Average 1.0x increase)	M-C Nielsen, unpubl. data
Cyclamen	Germany (Straele)	Greenhouse	Blue sticky traps	Summer/Autumn (July–September)	7.0–22.0x mean increase (Average 12x)	Ruisinger (2008)
Fallow field	USA (Riverside)	Field	Yellow sticky traps	Summer (August)	2.8–3.4x mean increase (Average 3.1x)	M-C Nielsen, unpubl. data
Gerberas	Australia (Perth)	Greenhouse	Blue sticky traps	Summer (February)	3.0–3.6.x mean increase (Average 3.2x increase)	S. Broughton, unpubl. data
Nectarine	Australia (Perth)	Orchard	Blue sticky traps	Spring (October–November)	2.3–5.3x mean increase (Average 3.1x increase)	S. Broughton, unpubl. data
Roses	Australia (Perth)	Greenhouse	Blue sticky traps	Summer (February)	1.9–4.0x mean increase (Average 2.8x increase)	S. Broughton, unpubl. data
Vegetable nursery	Australia (Melbourne)	Greenhouse	Blue sticky traps	Spring (October)	- (Average 2.6x increase)	Till et al. (2009)

** Increased capture over control traps. Decreased trap capture (western flower thrips numbers caught on LUREM-TR baited traps lower than western flower thrips numbers caught on control traps) was denoted as zero.*

Structure of the thesis. The thesis is divided into two parts. The first part is a review of the literature (**Chapter 1**) aimed at presenting and discussing topics that are important for understanding the basis and perspective of the experimental work of the thesis. This part deals with topics such as thrips as pests in general and WFT in particular, the biology and ecology of WFT, and host finding mechanisms. Special focus is on semiochemicals, the role kairomones play in host finding behaviour and how intrinsic and extrinsic factors influence these interactions. The second part of the thesis presents the experimental work of the study.

The experimental work is covered in five chapters (Chapter 2 to 6). Each chapter consists of an introduction, methods and materials, results and discussion section. The objective of **Chapter 2** and part of **Chapter 3** was to use gravimetric analysis to determine the release kinetics of MI from selected passive dispensers used for thrips lures and establish their release rate under a range of environmental conditions. Work in **Chapter 2** was used to establish release rates under several controlled laboratory conditions, whereas **Chapter 3** examined the performance of the dispensers under greenhouse conditions. The main objectives of **Chapter 3** were to investigate the relationship between the MI dose released and how extrinsic factors such as temperature influenced the dose released and the flying response of thrips. In **Chapter 4** olfactometer studies under laboratory conditions were used to validate the robustness of the response of WFT to MI and explore if populations of WFT responded consistently to MI or if intrinsic factors such as previous feeding history and genotype influence the response. Given the apparent non-responsiveness of some Spanish WFT populations to MI, in contrast to WFT from other countries, the objective of **Chapter 5** was to investigate these differences and to identify whether intrinsic factors were involved, and whether these differ among a range of WFT populations. Based on the results obtained in Chapter 5 further work on the lack of responsiveness of Spanish WFT to MI and other semiochemicals under controlled conditions were examined in more detail in **Chapter 6.**

Chapter 7 includes a general discussion and a conclusion based on the results obtained in the experimental chapters and the literature presented in the first part of the thesis in regards to the overall aim of the thesis identified in the general introduction. This chapter also reflects on shortcomings of the research and on avenues for future research.

Literature review

This chapter contains a selected review of the literature on thrips, especially western flower thrips (*Frankliniella occidentalis*), their interactions with host plants, the cues thrips use to locate host plants and how intrinsic and extrinsic factors influence these interactions. Special emphasis is on the role kairomones play in the host-finding process. A brief summary of insect orientation mechanisms is also included. Extended reviews on thrips as pest insects and their biology and ecology can be found in Lewis (1973), Parker et al. (1995) and Lewis (1997b). A review on host plant selection by phytophagous insects is covered in Bernays and Chapman (1994) and Metcalf and Metcalf (1992) cover the role of plant kairomones in insect ecology.

1.1 Thrips

Thrips are tiny, slender insects with fringed wings belonging to the order Thysanoptera, of which there are presently over 5500 described species (Mound 2002). The order is divided into two suborders, the Tubulifera and Terebrantia. Approximately 60% of the species are in the Tubulifera, which contains a single family, the Phlaeothripidae. There are eight recognised, extant families in the Terebrantia: Uzelothripidae, Merothripidae, Aeolothripidae, Adiheterothripidae, Melanthripidae, Fauriellidae, Heterothripidae, and Thripidae (Mound and Morris 2007). Thrips occur worldwide with a preponderance of tropical species, many temperate ones and a few inhabiting cool regions (Lewis 1997a). While most thrips species are not considered pests, some show all the features (e.g. fast development, extensive host range, rapid development of resistance against pesticides) that predispose them to be major pest species by causing direct feeding damage and by spreading viral diseases to food, fibre and ornamental crops (Brunner and Frey 2010). Thrips that breed on only a single host plant are rarely serious pests (Mound 2005a). Thrips that are serious pests are usually highly adaptable and polyphagous species (Stavisky et al. 2002). To control pest thrips numerous treatments by insecticides are often used. This tactic has resulted in increasing resistance to the particular pesticides used (Brødsgaard 1994, Espinosa et al. 2002, Bielza et al. 2008, Cloyd 2009, Diaz-Montano et al. 2011) and in addition, creates problems for the implementation and improvement of some integrated pest management (IPM) programmes (Morse and Buhler 1997). A search for alternative management techniques has stimulated intensive investigations of a chemical nature in plant–insect relationships. One thrips species

that is a polyphagous pest worldwide in agricultural and horticulture crops, and that is also a vector of plant-damaging tospoviruses, is the western flower thrips (*Frankliniella occidentalis*) (Pergande) (Mound 1996, Lewis 1997a, Cloyd 2009, Reitz et al. 2011).

1.1.1 Western flower thrips

Western flower thrips (WFT) belongs to the suborder Terebrantia (Moritz et al. 2001, Mound 2005a). The genus *Frankliniella* is grouped within the subfamily Thripinae (family Thripidae) that contains 1400 species worldwide (Mound and Teulon 1995). Most of the species of economic importance such as *Thrips tabaci, Thrips obscuratus* and *Frankliniella intonsa* belong to the subfamily Thripinae (Mound and Walker 1982, Mound and Teulon 1995). WFT was first described in California, USA, in 1895 by Theodore Pergande (Pergande 1895). For many years the species was known to be restricted to the western part of the North American continent, both on outdoor and indoor crops. It was not considered a pest and could easily be controlled by insecticides if outbreaks of complexes of pestiferous thrips occurred that included WFT. However, location restrictions and low pest status changed in the 1970s (Robb et al. 1995) and in 1976 the first outbreak of WFT on the east coast (Pennsylvania) was recorded on chrysanthemums (Kirk and Terry 2003). By the 1980s it had spread to glasshouses throughout the USA. A likely cause for the spread and extensive establishment in glasshouses was the use of, and increased tolerance to, insecticides (Brødsgaard 1994, Robb et al. 1995) in conjunction with the trade of plant material (Morse and Hoddle 2006). WFT is highly polyphagous and can be found feeding on more than 240 plant species in 62 different plant families (Brunner and Frey 2010).

In 1983 pesticide resistant WFT were discovered in a greenhouse in Holland and by the late 1980s this species was found in the rest of Europe (Kirk 2001, Kirk and Terry 2003). In only three decades this species has spread throughout the world and today it is one of the most economically important thrips species in greenhouse production. Since 1980 WFT has been the most intensively studied species of Thysanoptera, accounting for over one third of the publications on the order (Reitz 2009). A search on Web of Knowledge for publications with the topic "*Frankliniella occidentalis*" or "western flower thrips" showed that 468 articles were published over a short period from 2010 to April 2013.

1.1.2 Morphology of WFT

Adult WFT are approximately 0.5–2 mm in length and pale white, yellow, yellow-orange, brown or dark brown. Temperature during development is the most likely factor affecting body colour in thrips. Darker forms are found when development occurs during colder temperatures, while light forms are more common when development occurs during higher temperatures (Lewis 1997a, Mound 2005b). However, Bryan and Smith (1956) demonstrated that the colour variations among WFT in California are to some extent also genetically determined. Male WFT are pale yellow and much smaller than females (Mound and Walker 1982, Ananthakrishnan 1984). The variation in colour can make distinguishing WFT from other *Frankliniella* species, and some *Thrips* species, difficult. Three characteristics for visual identification of WFT under the microscope (x20–100) are: 1) long pair of setae under the compound eyes, 2) four pairs of elongated setae on the pronotum, and, 3) a complete row of setae on both the first and second veins of the forewings.

1.1.3 The WFT complex

In New Zealand, a putatively monophagous population of WFT can be found in non-cropping situations, which is largely restricted to the flowers of yellow tree lupins, *Lupinus arboreus* (Mound and Walker 1982). These WFT (in previous literature referred to as the "WFT lupin strain") are not considered to be a pest of crop plants (greenhouse or field) and are morphologically identical to WFT collected from greenhouse sand field crops (Mound and Walker 1982, Mound 2005a), highly susceptible to pesticides (Martin and Workman 1994) and exhibit significant differences in some life history parameters (Nielsen et al. 2010). These factors and recent studies on the molecular range of variation within WFT (Brunner and Frey 2010, Rugman-Jones et al. 2010) has indicated that what is commonly considered a different strain of WFT is, in fact, closer to being a different species (see below). This has also been suggested for other thrips species such as *Scirtothrips dorsalis* (Rugman-Jones et al. 2006) and *T. tabaci* (Brunner et al. 2004).

Research by Rugman-Jones et al. (2010) revealed the existence of two separate cryptic species of WFT. The WFT populations in that study were represented by 20 different haplotypes forming two distinct clusters separated by 17 nucleotide changes (see Appendix I) for the global distribution of WFT mtDNA haplotypes. The two clusters identified by Rugman-Jones et al. (2010) are also reflected well in WFT from New Zealand. The so-called "glasshouse pest strain" of WFT collected from greenhouses fell in Cluster 1, and non-pest

WFT collected from outdoor lupins fell into Cluster 2 (Rugman-Jones et al. 2010). The different haplotypes of WFT represented in Cluster 1 is the worldwide pest WFT referred to in the majority of published literature. A recent study using genetic barcoding for the identification of WFT populations found in The Netherlands revealed that all the Dutch WFT populations studied belonged to the "glasshouse pest strain" (Mirnezhad et al. 2012).

1.2 Host plants

More than 95% of the species within the Terebrantia suborder are associated with green plants (Mound 2005a). However, many plants associated with thrips are probably not actual host plants according to the strict definition of a host; in other words, a breeding site that can successfully maintain a population. To limit the definition of a host plant to "true" host plants only is nevertheless inappropriate with respect to crop protection since adult thrips can transmit tospoviruses to another previously uninfected plant simply by feeding on it (Mound 2005a). Within the Terebrantia sub-order many of the species are only associated with grasses whereas others are associated only with dicotyledonous plants, some in flowers but others only on leaves (Mound 2002). On dicotyledonous plants most Terebrantia suck out the cell contents from leaves, flowers, fruits and young shoots, and many flower-dwelling species suck out the contents of pollen grains (Lewis 1973). On green plants many thrips species live in narrow crevices, for example, in tightly packed flower buds where they are protected and where it is moist enough for them to live. This creates a major problem for the control of thrips since it is very difficult to reach them with control agents when they live deep inside flowers and other narrow crevices. When a favoured site has been located the thrips will reduce its walking activity on the plant (Lewis 1973).

1.3 Semiochemicals

Semiochemicals are chemicals mediating interactions between organisms either within the same species (pheromones) or between different species (allelochemicals) (Nordlund et al. 1981, Pickett and Glinwood 2007). Allelochemicals include allomones, kairomones and synomones (Fig. 1.2). A single chemical signal may, however, act as both as pheromone and allelochemical (Heuskin et al. 2011).

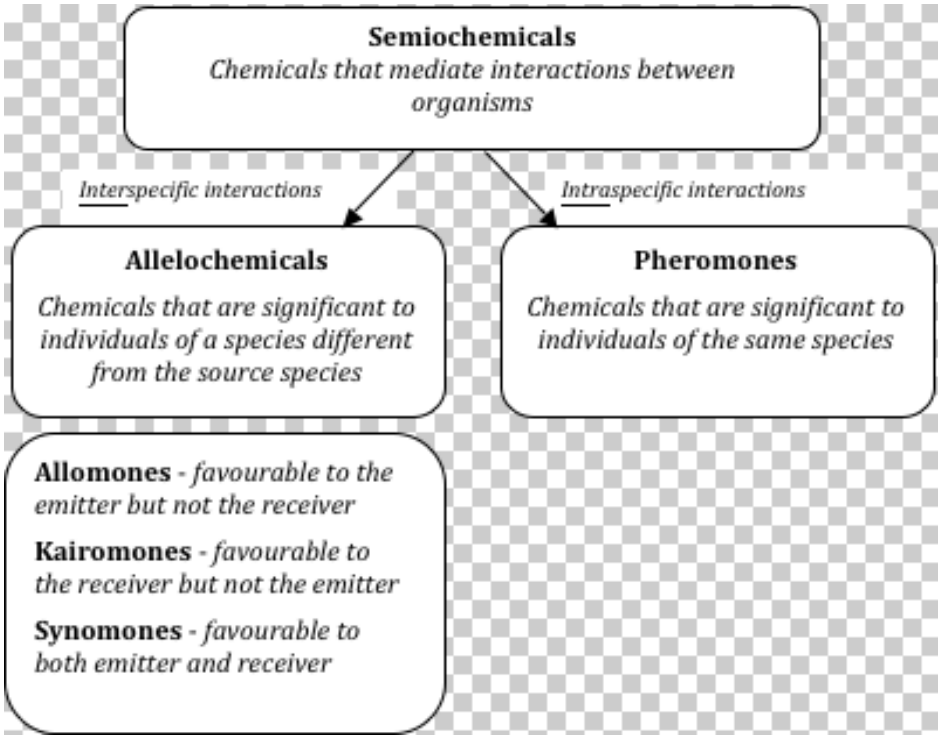


Figure 1.2 *Classification of semiochemicals (modified from Heuskins et al. 2011)*

A semiochemical may influence interactions involving a number of organisms from several trophic levels. Bark beetles, for example, aggregate on trees using semiochemicals produced by conspecific beetles, the attraction of which is synergised by volatiles released from the tree itself (Byers 1995). Semiochemicals offer enormous potential for managing many of the world's most devastating pests when used in integrated pest management schemes, and so for almost a century researchers have searched for kairomone compounds that attract thrips (Howlett 1914).

1.3.1 Kairomones

Kairomones synthesised by plants are used by insects as cues for host plant selection as attractants, arrestants and feeding and oviposition stimuli (Metcalf and Metcalf 1992). Although kairomones can be used as repellents for thrips, such as *Origanum majorana* L. (Lamiaceae) for *Thrips tabaci* (van Tol et al. 2007), the main focus on kairomones in relation to thrips research has been on their role as attractants. Today a range of kairomone attractant chemicals derived from host plants and related compounds have been found for thrips (Koschier 2006, 2008).

1.3.2 Response assays for semiochemicals

To test the response of insects, including several thrips species, to semiochemicals several assays have been developed depending on what level of response is of interest. The sense of smell in insects and the overall response of the olfactory receptors located on an insect's antennae can be studied using electroantennography (EAG) (Schneider 1957). This technique is widely applied for screening insect pheromones by examining the responses to fractions of a compound mixture separated by gas chromatography (Gothilf et al. 1978, Dunkelblum et al. 1980, Baker et al. 1991, Plettner and Gries 2010), but also to test the response towards allelochemicals (Chermenskaya et al. 2001, Light et al. 2001, Han and Chen 2002, Qiu et al. 2004). The odour response at the olfactory sensory level can be further examined in detail by sensilla recording (Nagai 1983, Van der Pers and Minks 1993, Kurtovic et al. 2007).

To test walking and/or hopping responses of insects to semiochemicals several laboratory assays have been developed and used to date: the Petterson four-arm olfactometer or variations thereof (Pettersson 1970, Vet et al. 1983, Campbell et al. 1993, Quiroz and Niemeyer 1998, Birkett et al. 2000, Glinwood and Pettersson 2000, Francis et al. 2004, Turlings et al. 2004, Tapia et al. 2005, Saïd et al. 2006, Baoyu and Baohong 2007, Karunaratne et al. 2009), V-shaped olfactometer (Gerin et al. 1994), T-shaped olfactometer (Gruber et al. 2009) and Y-tube olfactometer (Pivnick et al. 1994, Shimoda et al. 1997, Venzon et al. 1999, Koschier et al. 2000, Smits et al. 2000, Davidson et al. 2006, Mochizuki and Yano 2007, Stenberg and Ericson 2007, Davidson et al. 2008, Tatemoto and Shimoda 2008, Wenninger et al. 2009).

For behaviours involving take-off and flight in response to olfactory and/or visual cues several experiments have been conducted on leaf /petal disc assays (van Tol et al. 2012), in flight chambers (Blackmer and Phelan 1992, Foster and Howard 1998, Cunningham et al. 2006) and in wind tunnels (Visser and Taanman 1987, Blackmer and Phelan 1992, Rojas and Wyatt 1999, Teulon et al. 1999, Birkett et al. 2000, Smits et al. 2000, Berry et al. 2006, Davidson et al. 2006, Brevault and Quilici 2010). The response of small flying insects to attractants and colours has also successfully been tested in greenhouses, semi-field and field experiments by using different trapping systems (e.g. pan-traps, delta-traps, sticky-traps) (Chambers 1990, Duan and Prokopy 1994, Birkett et al. 2000, Bhasin et al. 2001, Schmera et al. 2004, James 2005, Laubertie et al. 2006, Njiru et al. 2006, El-Sayed et al. 2008). For thrips, different coloured sticky traps and water traps have been used extensively to screen

and test colours and kairomones and other ecological aspects (Moffit 1964, Penman et al. 1982, Burger and Munro 1986, Czencz 1987, Kirk 1987, Teulon and Ramakers 1990, Teulon et al. 1993a, Teulon et al. 1993b, Frey et al. 1994, Hollister et al. 1995, Mateus and Mexia 1995, Antignus et al. 1996, Teulon et al. 1999, Koschier et al. 2000, Smits et al. 2000, de Kogel and Koschier 2001, Imai et al. 2001, Rhainds and Shipp 2004, Davidson et al. 2006, Davidson et al. 2007, Teulon et al. 2007a, Teulon et al. 2007c, El-Sayed et al. 2009, Vassiliou 2010, Wogin et al. 2010). The culmination of this research has resulted in the combination of various traps and lures for pest management purposes.

Informational overview of chemical ecology and thrips in particular can be found in the following articles; Blum (1991), Smits et al. (2000) and Koschier et al. (2008).

1.3.3 Use of kairomones

Commercial thrips lures are used in combination with coloured sticky traps. So far they are mainly employed in greenhouses and for monitoring purposes. However, results from field settings have showed promise with respect to kairomone lures being use in pest management strategies outdoors. The aim of current research and application of kairomone lures is to create traps that are more effective at thrips trapping, providing useful tools for monitoring thrips at low population densities, such as in early infestations or during eradication attempts, and to reduce thrips numbers via mass-trapping. In a study by Teulon et al. (1993b) in nectarine orchards, *T. obscuratus* males were detected 2 weeks earlier using odour baited traps than using un-baited traps. Other applications of attractants not yet fully tested in thrips pest management are “attract and kill”, “attract and infest” and “push-pull” strategies. To optimize the use of traps in thrips management a better understanding of the interactions that take place between the insects, the host plants, the environment and the various trapping systems are needed. A better understanding of the host-finding behaviour will play an important part in developing more effective means of monitoring and controlling thrips populations.

1.4 Host finding by phytophagous insects

The host finding behaviour of insects represents their search for a suitable host to support the development of their offspring and to fulfil its nutritional requirements. Host finding cues are received through peripheral receptors located primarily in the antennae and compound eyes and then processed in the brain. Host selection of thrips generally follows several sequential

or simultaneous “decision” processes that lead to detection and landing on the host (Terry 1997). Like other insects, thrips locate hosts using colour, shape, size and volatiles associated with them. Because of their small size, it has been widely assumed that once “air borne” assisted by winds exceeding the flight speed of the thrips, the individuals have minimal control over their flight path and where they land (Lewis 1997a). However, there is now abundant evidence that thrips have sufficient control to choose to alight on a host plant, at least near vegetation level in light wind conditions. Without the ability to control their flight path and where they land, the very different response of small thrips species to various colours and UV-reflecting traps (Moffit 1964, Kirk 1984, Czencz 1987, Matteson and Terry 1992, Teulon and Penman 1992, Teulon et al. 1999, Smits et al. 2000) would not occur. While shape and size of a host plant show variability within species and depend on growth stage, and even colour to an extent, visually mediated responses can be relatively unspecific. However, in the context of a specific odour cue visually cues play a key role in host location (Bernays and Chapman 1994). Finch and Collier (2000) developed the hypothesis that once phytophagous insects have located the habitat by use of plant volatile chemical information, visual stimuli then govern the next phase of host plant location.

1.5 Olfactory and visual cues in host finding

1.5.1 Olfactory volatiles

Flowers of a single plant species can vary markedly in the composition and concentration of volatile components (Loughrin et al. 1992, Shaver et al. 1997, Kolosova et al. 2001, Pichersky and Gersherzon 2002). While different flower species often share many volatile components (Dudareva and Pichersky 2000, Bruce et al. 2005), their combination and concentration is unique to each species, forming a particular ‘odour code’. The perception of these chemical messages supports two vital functions for insects, namely their reproduction and feeding (Visser et al. 1990).

The search for kairomone compounds that attract thrips has been going on for more than a century. The first reported experiment was undertaken in 1912 testing thrips responses to several aldehydes (Howlett 1914). Morgan and Crumb (1928) listed several chemicals that caught more thrips when tested in combination (baited) with traps compared with unbaited traps. In 1973 the first patent was applied to the use of anisaldehyde and/or cinnamaldehyde in traps for thrips (Utchida 1973). Most of the pure compounds that are currently known to

attract thrips have been screened either because they were already known to attract other insects, or were related to those compounds, or were extracted from thrips host plants (Koschier 2008). There are many examples of insects being attracted to the odour of their host plants, both flying and by walking or crawling (Bernays and Chapman 1994). For thrips in particular, olfactory attraction has been recorded both for host derived kairomones but also from non-plant derived kairomones (Kirk 1985, Teulon et al. 1993a, Teulon et al. 1999, Koschier et al. 2000, Berry et al. 2006, Teulon et al. 2007c, van Tol et al. 2007, Davidson et al. 2008). Since fragrant flowers seem to attract more flower-dwelling thrips than non-scented flowers, it is likely that flower thrips use floral volatiles as kairomones to locate their host plant (Annand 1926).

In this project a non-floral kairomone lure, methyl isonicotinate (MI), was used. The decision to use this kairomone was based on: 1) the vast amount of data gathered from laboratory, greenhouse and field studies testing the response of WFT to MI both in New Zealand and overseas, 2) the results of these trials (large variations in efficacy reported), 3) its potential in thrips pest management, 4) its wide use and versatility with regard to its proven attractiveness to several different species, and 5) its current use as the active ingredient in the commercial thrips lure LUREM-TR (Teulon et al. 2011).

1.5.2 Visual cues

It is well documented that insects respond to visual stimuli, in particular certain colours (Lewis 1973, Kirk 1984, Yudin et al. 1987, Jermy et al. 1988, Mateus and Mexia 1995, Leong and Thorp 1999, Teulon et al. 1999, Smits et al. 2000). Visual attraction in phytophagous insects is thought to result not only from responding to the colour but also the form of the host plant. But because these characteristics vary so greatly within a plant species, visual responses often only occur with an appropriate olfactory stimulus (Bernays and Chapman 1994). It is clear, however, that vision, particularly response to leaf colour, will often be of critical importance in the final stages of host finding (Bernays and Chapman 1994). Leaves are various shades of green and their spectral reflectance patterns tend to be similar. Nevertheless, differences in the shade of colour and the interaction between thrips and their host plants may account for differences in colour preference for a given species in different experiments. For example, Brødsgaard (1989) showed that trap catches of WFT varied significantly with three different shades of blue. Many kinds of traps, such as sticky traps and water pan traps of different structure and colour, have already been designed for

capturing thrips (Lewis 1997a). Polyphagous thrips that feed on a variety of plant tissues including flowers have been shown to have a general response to white, yellow and blue colours, whereas thrips with specific floral host requirements are often attracted to their host plant flower colour (Kirk 1984, Czencz 1987). Colour does not influence catches of grass-inhabiting thrips species to the same degree (Kirk 1984, Czencz 1987).

1.6 Olfactory versus visual cues in host finding

The relative importance between olfactory and visual cues in relation to insect host finding behaviour and searching varies between species (Schoonhoven et al. 2005) and between several abiotic and biotic factors affecting both the individual insect and the host plant. While visual cues are important, olfaction is often considered the most important search cue in the host finding process of insects (Bernays and Chapman 1994, Schoonhoven et al. 2005). In some previous literature, it has been assumed that optical cues cannot be used to recognize host plants for the reason that "all plants are green". However, the intensity of the reflected light from the foliage and flowers from different species may differ significantly because of different leaf surfaces and because of biotic and abiotic factors such as heat stress and water avalibility (Hofmann et al. 2003, Dobrowski et al. 2005) and insect pest attack (Riedell and Blackmer 1999, Davies 2004). Also, it is likely that the attraction of an insect to a colour may vary with both age and sex (Southwood 1978) so that the efficiency of a coloured trap may not be constant.

The importance of colour in host finding has also been demonstrated for insects such as the monophagous leaf beetle *Altica engstroemi* (Stenberg and Ericson 2007) and some thrips species where previous studies have indicated that colour is a dominant factor for their orientation (Smits et al. 2000, de Kogel and Koschier 2001). It has been demonstrated many times that thrips are attracted to colours and odours (Moffit 1964, Kirk 1985, Kirk 1987, Teulon and Ramakers 1990, Teulon and Penman 1992, Teulon et al. 1993a, Frey et al. 1994, Mateus and Mexia 1995, Pow et al. 1998, de Kogel et al. 1999, Teulon et al. 1999, Koschier et al. 2000, Murai et al. 2000, Smits et al. 2000, Chermenskaya et al. 2001, Imai et al. 2001) and the results of the studies could indicate that presence of a kairomonel lure is the reason for the significant increase in capture of thrips on coloured traps. The significant influence of adding a kairomone lures to colour traps was supported in a study by Teulon et al. (1999) where they found a significant interaction between trap colour and the presence of anisaldehyde. The presence of the odour increased the numbers of WFT (females and males)

trapped proportionately more in black traps (no visual cue) than in yellow traps (visual cue) in a capsicum glasshouse crop. However, before that study, Brødsgaard (1990) found that clear window traps (no visual cue) did not catch more WFT with an odour source added to the traps indicating that WFT does not orientate directly towards the olfactory cue but that the visual cue needs to be present as well to successfully find the trap. Such results were also found in strawberry fields in Australia for *Thrips imaginis* and WFT caught in black water traps. The black traps baited with a thrips lure did not increase the capture of the two species over the unbaited black water traps as it did when using white traps (M-C Nielsen, unpubl. data). Mainali and Lim (2011) found that higher numbers of WFT responded to a combination of an olfactory cue (*p*-anisaldehyde) and a visual artificial flower cue in a Y-tube olfactometer study compared with the olfactory or the visual cue used alone. Preference studies have shown that the response to colour by thrips also depends not only upon the thrips species but also more generally to the feeding preference of the thrips (flower feeder versus a grass and foliage feeder) (Kirk 1984, Czencz 1987). In field experiments Kirk (1987) also showed that the catch of *T. imaginis* on white sticky traps was dependent on the size of the increased according to a constant power of trap size.

Based on current literature, the variability that results from the influence of intrinsic and extrinsic factors on both visual and olfactory cues of a plant, it seems plausible to give weight to both cues as the correct combination of sensory inputs at a particular stage of the insect species is required to recognise a host plant. Host finding of individual insects will most likely be strongly influenced by the particular need of the insect at the time for oviposition, mating, feeding, shelter or a combination of these.

1.7 Orientation mechanisms of insects

The sequence of behavioural steps that an insect species carries out during searching differs among species and their development stages and can vary from random search to highly directed search pattern (Schoonhoven et al. 2005).

Orientation can be viewed in the classical way as undirected (kineses) and directed reactions (taxis) (Visser 1988). Both involve response to a stimulus. Several different types of kinesis and taxis exist but only some relate to orientation in insects. Types of kinesis and taxes have been identified as orthokinesis (change in linear speed of movement), klinokinesis (change in rate and frequency of turning), anemotaxis (wind stimuli), barotaxis (pressure stimuli),

chemotaxis (chemicals stimuli), hydrotaxis (moisture stimuli), phototaxis (light stimuli) thermotaxis (temperature changes stimuli). Anemotactic behaviour, influenced by plant odour, is seen in a number of herbivorous insects under laboratory conditions (Schoonhoven et al. 2005). Positive odour-conditioned optomotor anemotaxis and olfactory-induced visual orientation are currently considered as the main mechanisms used during host plant searching in large herbivorous insects, both in specialized and polyphagous species (Schoonhoven et al. 2005).

1.7.1 Orientation mechanisms for thrips

Several mechanisms have been suggested in the literature to explain the behaviour of thrips in the presence of volatile compounds. Behavioural response of insects to odour sources can be directly induced (i.e. chemokinesis and chemotaxis) or indirectly induced (i.e. odour induced anemotaxis and odour-induced visual response) (Kennedy 1977). For larger insects, responses in flight to the scent of host plants and flowers are often indirect. If thrips respond to odours in flight are the same as for larger insects, they could only be successful using a scent in a very narrow range of wind speed as their host finding activities is likely to be limited to calm air conditions (Teulon et al. 1993b). Anemotaxis therefore seems unlikely because thrips are weak flyers. It seems probable that thrips could use a scent cue more efficiently as an arrestant, or to stimulate a visual response, than for anemotaxis, because the cue could then also be used when the air is completely still (Kirk 1985). It seems most likely that searching behaviour and host finding in insects represents the confluence of three kinds of factors: 1) biological characteristics and abilities of the insect including the locomotory patterns and perception of sensory information, 2) external environmental factors, and 3) internal factors, such as food deprivation or sexual receptivity that determine individual needs at a particular time.

1.8 Factors affecting host finding of thrips

The host finding behaviour and the way both visual and olfactory cues are used and interpreted by thrips and other insects to recognize host plants at a distance are meditated by several intrinsic and extrinsic factors.

When considering extrinsic factors affecting host finding in thrips, optical plant characteristics are fairly constant with respect to their distribution and largely independent of temperature and wind speed, but clearly optical characteristics depend on solar radiation

(Schoonhoven et al. 2005). Odours emanating from the plants, however, have a highly variable spatial distribution and concentration, depending on wind speed and temperature and to some extent also on solar radiation (Schoonhoven et al. 2005) along with plant age and physiological state (e.g. stress).

Another aspect to consider with respect to host finding is the affect that different extrinsic factors may have on a given behaviour depending on the locomotory state, such as walking and hopping, migration and dispersal flight and take-off. For example, relative humidity is suggested to restrict the duration of the flight of thrips (Lewis 1997a) and depending on species may affect their take-off behaviour (Lewis 1963).

1.8.1 Intrinsic factors

The intrinsic factors influencing host finding of thrips and other small flying insects can be divided into 1) genetic factors, 2) acquired factors such as learned behaviour and 3) physiological factors.

Genetic factors affect the characteristics of the insects and perception of environmental information. Host preferences of phytophagous insects often vary genetically within or between populations and have been documented in a variety of insects (Futuyma and Peterson 1985, Via 1990, Jaenike and Holt 1991, Chiu and Messina 1994, Nylin et al. 2005). However, in most studies that report on genetic variation in host selection, the variation is detected by bioassays of oviposition and feeding preference and not by examining the actual response of the insect to different cues. Genetic variation may presumably affect any of the many discrimination mechanisms associated with finding a host plant. For example, searching behaviour may be altered by genetic changes that affect sensitivity to visual or odour stimuli stemming from a host plant or an insect trap with a lure. For example, Frey et al. (1992) demonstrated a significant difference between the apple and the hawthorn races of *Rhagoletis mendax* and the closely related *R. pomonella* to several fruit odour components using antennograms. Wieczorek (1976) showed that the response of genetically different strains of the noctuid caterpillar, *Mamestra brassicae*, responded significantly differently to 12 secondary metabolites due to differences in their chemoreceptors. Lower rates of flight take-off and mobility have been reported in different strains (insecticide-resistance and susceptible strains) of weevils (Guedes et al. 2009). Furthermore, (Loxdale and Lushai 1999) showed that for the highly insecticide resistant peach aphid *Myzus persicae*, some mutations

that confer pesticide resistance affect the physiology and behaviour of this species directly by influencing the take-off response from deteriorating leaves. Unfortunately, there are few experiments that demonstrate which specific components of the host-finding behaviours are influenced by genetic variations.

Acquired factors include the experience gained by the insect throughout its life. In the past insect behaviour was generally thought to be determined largely by closed genetic programmes leaving little room for learning in behavioural development. This perception has changed over recent decades with more evidence for the importance of acquired factors in host finding. What appears to be associative learning to host odour and olfactory cues has been shown in several different insects (Cheah and Coaker 1992, Chiu and Messina 1994, Landolt and Molina 1996, Cardé and Willis 2008, Desouhant et al. 2010). For example, female cabbage looper moths that were caged with cotton, celery, or soybean foliage were attracted significantly more than inexperienced moths to the odour of the same species of plant the following night (Landolt and Molina 1996). Similarly, learning influences the visual ability of the female apple maggot fly, *Rhagoletis pomonella* to detect host fruit (Prokopy et al. 1994). Experience with the host may then modify the ranking and specificity of host location stimuli, so that a more specific search image and perhaps olfactory cues are used subsequently (Vet et al. 1990, Wackers and Lewis 1994, Landolt and Molina 1996).

Host location and searching behaviour of insects are influenced by the physiological state of the insect and this interaction has to be superimposed upon the already very complex system of host finding (Finch and Collier 2002). Sex, age, egg load, hunger/food deprivation, circadian rhythmicity and mating status of the individuals are among the parameters known to influence host selection behaviour. Differences between males and females in response to host plant odours have been observed amongst several insect species (Jones 1996, Pompanon et al. 1999, Davidson et al. 2007, Wenninger et al. 2009, Brevault and Quilici 2010). Response to plant volatiles may not be as markedly different between the sexes as the response to pheromones, but differences in host plant use are expected based on the roles plants play in insect reproduction. The activities of male thrips are likely to be driven by the search for mates, whereas host finding stimulates female activities such as oviposition. Thus, females may have a stronger response to plant volatiles than males (Curtis and Clark 1978, Hern and Dorn 2004) and are likely to be affected by plant volatiles, such as those induced by herbivory, when searching for oviposition sites (de Moraes et al. 2001). For various species,

responses to visual and olfactory stimuli from host plants have been shown to be influenced by age and reproductive status. In the apple maggot (*R. pomonella*) the age and sexual maturity of females significantly affect the probability and time to discover fruit models (Duan and Prokopy 1994). It is likely that the attraction of an insect to a colour may vary with both age and sex (Southwood 1978). Age is known to affect the trapping efficiency of *Thrips palmi* adults on coloured sticky traps in greenhouses and may also influence the response of thrips to volatile chemicals (Kawai and Kitamura 1987). Food-related stimulants may have a reduced chance of being detected when feeding is 'irrelevant", in other words, when the insects are satiated. Davidson et al. (2006) found that more thrips walked up the odour-laden arm of a Y-tube when starved for at least 4 h than satiated thrips. In wind tunnel experiments, the percentage of thrips that fly or land on a sticky trap increased between satiated thrips and those starved for 4 h but then decreased again when thrips were starved for 48 h or longer (Davidson et al. 2006). The suggestion of an input being "irrelevant" is also likely to relate to other responses than just food stimuli. The amount of sensory information obtained by insects within a certain time window is potentially huge and, as a result, insects must be able to select among the incoming items of information and rank them in terms of immediate relevance. Mating status of both females and males and egg-load carried by the females will also affect the relevance of host plant cues and their relevance.

Another aspect to take into account when dealing with thrips and host finding are physiological factors and flight ability. Dispersal and flight are essential for thrips to survive. The structure of the minute, fringe wings of thrips and other small insects such as Mymaridae and Ptiliidae suggest that they function differently from the larger, sparsely fringed or completely membranous wings of other insects. Thrips are generally not considered to be good flyers, although their fringed wings enable them to remain airborne long enough to travel short and long distances (Lewis 1997a). Long-distance dispersal has been recorded for a few species of thrips (Mound and Marullo 1996). The thrips fauna of Central America studied in Costa Rica by Mound and Marullo (1996) included species that originated far to the north and south of the Costa Rica region. This is possible only under specific circumstances (e.g. temperature and wind conditions) as small organisms dehydrate rapidly and must drink plant fluids often to survive (Funderburk and Stavisky 2004). Most flower thrips disperse over a series of short flights. The frequency and duration of flight varies with the species and gender, and is influenced by the weather, by the suitability of food and possibly by crowding.

1.8.2 Extrinsic factors

External environmental factors can alter searching behaviour directly, by acting upon environmentally sensitive physiological processes (Bell 1990). It is well documented that temperature, relative humidity and day length exert a strong influence on insect behaviour. For thrips, the important environmental factors affecting behaviour include the leading stimuli from the host plant as well as abiotic factors, mainly climatic conditions. In particular, temperature, humidity, wind speed/airflow, UV light and the phenological stage of the host plant all affect thrips host finding behaviour. The influence of wind is particularly complex. For example Teulon et al. (1993b) showed that thrips responded to volatile chemicals in both windy open fields and relatively calm conditions in the greenhouse, so clearly behaviour for thrips host finding must be effective in both situations. Dispersion of odour in wind is dominated by the forces of turbulent diffusion that stretches and stirs the odour filaments as they are released from the odour source, simultaneously creating pockets of odour-free air within the plume as it expands and is transported downwind (Murlis et al. 1992). The effect of wind speed on small- and large-scale turbulence is complex. As wind velocity increases, generally the directional flow of the plume is more consistent in that it is straighter (Cardé and Willis 2008). The slow rate of molecular diffusion means that the distribution of the odour in the plume is due mainly to turbulence rather than molecular diffusion (Cardé and Willis 2008).

Flight is strongly influenced by temperature in insects. For example, preliminary field trials in strawberries in Australia undertaken in early spring and late summer indicated that the attractiveness of volatiles are lower at higher temperatures with a lesser response recorded in late summer trials than early spring trials at cooler temperatures (M-C Nielsen, unpubl. data). Activity outside the protected microclimate of the plant may be restricted to short flights in the middle of the day and early afternoon where temperatures and vapour pressure may be much higher than early morning and late afternoon. Thus, trap catches will not only depend on population density, but on temperature which determines activity and reproduction potential of the thrips (Lublinkhof and Foster 1977). Barometric pressure has been shown to influence flight activity in *Fopius arisanus* females, where low barometric pressure resulted in less flight activity (Rousse et al. 2009). Wellington (1946) suggested that sensitivity to barometric pressure variations may be associated with a higher risk of mortality in the insects, such that they may have evolved to undertake less flight activity at low baraometric pressure that signals rainy and windy conditions that cause high mortality in small species.

However, compared with temperature, atmospheric pressure is expected to have little effect on flight (Lewis 1973). However, it may have an effect on an insect species' response to an odour. For example, Leskey and Prokopy (2003) conducted laboratory experiments to elucidate the influence of barometric pressure on odour discrimination of adult female plum curculios, *Conotrachelus nenuphar*, and found that barometric pressure had a significant positive effect on the response to hexane-extracted volatiles made from apple fruiting tissue. Barometric pressure may also affect the release rate of the volatiles used.

Limited information is published on relative humidity and the effect that it has on insect activity and searching behaviour of small flying insects. From the work that has been done it seems that there is a negative correlation between humidity and flight activity in small insects. Rousse et al. (2009) found flight and parasitism activities of the egg-parasite *F. arisanus* decreased with relative humidity. A negative influence of humidity on flight activity has also been shown for some Apidae (Iwama 1977, Sihag and Abrol 1986) and Megachilidae (Abrol 1987). However, because temperature and relative humidity are negatively correlated there is a potential confounding effect, but the level of thrips activity is affected to a much larger extent by variation in temperature than humidity (Shipp and Zhang 1999) and direct effects of humidity variation on thrips are buffered within the boundary layer of live plant tissue (Shipp and Gillespie 1993).

The climatic conditions in greenhouses are usually well controlled. Additionally UV levels can be regulated for optimal plant growth. Polyethylene plastic films, used in most greenhouses, contain UV-light absorbing components to prolong the life of the materials while maintaining an appropriate level of photosynthetically active radiation to optimize plant growth and development. This manipulation of the UV-light is presumed to interfere with the visual cues used by insects and/or resulting insect behavioural responses (Antignus et al. 2001, Costa et al. 2002, Chyzik et al. 2003). Ultraviolet-absorbing plastic films covering greenhouses appear to interfere with thrips orientation behaviour, but their applied use to alter the behaviour of thrips is still under evaluation (Costa and Robb 1999, Antignus et al. 2001, Costa et al. 2002, Nguyen et al. 2009, Kigathi and Poehling 2012).

When using traps to catch insects in cropping situations the relative attractiveness of the trap is an important parameter influencing how successful the trap is. The attractiveness of the trap is relative to the attractiveness of other environmental cues; in other words, the more

attractive the environment the fewer pests caught on the traps (Smith 1976, Prokopy and Owens 1983, Berlinger et al. 1993). According to Finch et al. (2003) host-finding by the cabbage root fly and the onion fly was disrupted by surrounding plants due to the size of the plants with respect to their weight, leaf area and height, and not from their odours when tested in field-cage experiments. With respect to the efficiency of coloured traps in crops, Czencz (1987) found that the colour of the crop was not to be neglected with traps efficacy potentially weakened or strengthened by the background colour. Another aspect of how the general environment can affect trap efficiency is how the geometry of the crop canopy and the heterogeneity in the crop environment will influence insect movements. Results obtained from trials using intercropping indicates that under field conditions a non-host odour can interfere with the orientation and host utilisation by the bean flower thrips, *Megalurothrips sjostedti* (Kyamanywa et al. 1993) towards the host plants.

Density dependence is another aspect that can influence dispersal of insects (Rhainds and Shipp 2003). In most insects, crowding on the host plant is correlated with a high incidence of dispersal, an adaptive response stimulating members of the population to colonise new plants (Herzig 1995, Rhainds et al. 1997, Albrectsen and Nachman 2001). Optimal foraging behaviour of individuals that maximise their fitness by dispersing from crowded resources has important implications for population dynamics such that density-dependent dispersal is thought to regulate local populations and generate a relatively uniform spatial distribution of the population (Herzig 1995, Albrectsen and Nachman 2001). Several parameters associated with crowding with respect to food resources have been shown to enhance the rate of dispersal by adult flower thrips. Besides high population density (Gopinathan et al. 1981) and depletion of the food resources (Forbes and Beck 1954), alarm pheromone released by larvae (MacDonald et al. 2002), or heavy incidence of damage on host plants (Rhainds and Shipp 2003) can result in dispersal. In comparison with males, the rate of dispersal by female WFT is more strongly impacted by the crowding of chrysanthemum inflorescences (Rhainds and Shipp 2003), which may reflect sex-specific constraints affecting the dispersal behaviour of males and females.

1.9 Conclusion

In conclusion, research on utilizing the host-finding behaviour of thrips has been undertaken for a century and, although great advances have been made, research on thrips kairomone lures is still at the elementary stage. There are a growing number of examples of the

successful use of kairomone lures for thrips and other insects, but for most the underlying mechanisms are not fully understood. To optimize the use of traps in thrips management a better understanding of the interaction that occurs between the insects, the host plants, the environment and the trapping systems is needed. A greater understanding of the behavioural response of thrips to a kairomone, including the intrinsic and extrinsic factors that may affect these responses will be essential if kairomone-based approaches are to be integrated into thrips management programmes.

Chapter 2

Evaluation of different passive dispenser systems for the controlled release of volatile insect lures

2.1 Introduction

A large number of insect pests utilise plant volatiles for host finding (Metcalf and Metcalf 1992), and there is growing interest in exploiting these cues in applied pest management. For example, adding a lure to a trap could provide an effective tool for: 1) monitoring insects at low population densities (i.e. early infestations or in eradication attempts); 2) reducing insect numbers via mass-trapping for control; 3) use in strategies such as attract-and-kill; and 4) disruption of host-finding behaviour (Rodriguez-Saona and Stelinski 2009). For thrips (Order Thysanoptera), attraction to odours has been recorded both for host-derived compounds (Koschier et al. 2000, Berry et al. 2006, van Tol et al. 2007) and from non-plant derived chemical compounds (Koschier et al. 2000, Davidson et al. 2007, Teulon et al. 2007b) .

The successful use of these kairomones in pest management is strongly dependent on the manner in which they are dispensed (Byers 1988, Heuskin et al. 2011). Semiochemicals are primarily volatile compounds which require an appropriate delivery and dispensing system to modulate their release in a manner that optimises insect attraction in the time period desired. The delivery systems used for semiochemicals in applied thrips research are often passive dispensers including open-ended glass vials (Teulon et al. 1993a, Hollister et al. 1995, Teulon et al. 2007b, Davidson et al. 2009, Wogin et al. 2010), impregnated rubber septa (Hamilton et al. 2005, Broughton and Harrison 2012), impregnated dental cotton rolls (Teulon et al. 2008b), semi-permeable polyethylene bags (M.M Davidson unpubl. data, M-C Nielsen unpubl. data, Skill et al. 2012) and semi-permeable barriers covering a reservoir containing the compound such as a sachet (Teulon et al. 2008a, Till et al. 2009, Broughton and Harrison 2012). Some kairomones have also been used successfully by spraying or painting the compounds directly onto traps, leaving it to diffuse freely (Teulon et al. 1993b, Davidson et al. 2007, van Tol et al. 2007). These different passive delivery systems all have their advantages and disadvantages when used in experimental situations, and the choice of dispenser has commonly been guided by technical requirements (e.g. trap design, cropping

system), the initial amount of kairomone needed, protection needed from environmental factors and duration of experiment, rather than knowledge of ecological aspects such as optimal release rate of the kairomone lure for the target species.

Several studies have shown that release rates of semiochemicals are critical to insect response (Miller and Borden 1990, Mathieu et al. 1997, Phillips 1997, Ross and Daterman 1998, Reddy et al. 2005, Sweeney et al. 2006). To fully understand the chemical ecology of an organism under experimental conditions it is important to first quantify the release rate of the chosen kairomone compound from the passive dispenser (Byers 1988). With respect to the recognised thrips kairomone, methyl isonicotinate (MI), no published data are available on its release rate from any of the different dispensers identified above. Differences in release rates and/or, the choice of a passive dispenser may account for some of the variation previously experienced in field and greenhouse trials using MI to attract thrips. Although release rate studies, *per se*, do not identify the biological efficiency of a semiochemical (i.e the response), the results from release rate studies are important so that estimates can be made of the actual release rate during field and greenhouse trials and thus assist in estimating the dose the thrips are exposed to (Kraan and Ebbers 1990).

While the release rate depends on the physical characteristics of the passive dispenser (Golub et al. 1983, Hofmeyr and Burger 1995) and the physical properties of the semiochemical itself (Heuskin et al. 2011), environmental factors like air temperature and air flow also dictate the release of the semiochemical molecules into the air. Torr et al. (1997) found that the release of tsetse fly attractant from polyethylene sachets (zero-order release kinetics) increased considerably as the temperature increased from 21^{o}C to 38^{o}C. The important effect of the increase in release rates with increasing temperatures has been found in several other studies (Kraan and Ebbers 1990, Bradley et al. 1995, Stipanovic et al. 2004, Shem et al. 2009). Air flow also has an important effect on release rate. Kraan and Ebbers (1990) found that an increase in the air speed increased the release rate of moth sex pheromones, but concluded that this factor was not as important as temperature on the release kinetics. However, other studies have reported increased air flow can, under constant temperature, increase the release rate considerably. Bierl-Leonhardt et al. (1979) also found the release rate of a moth sex pheromone increased exponentially with an increase in temperature, but in addition was found to be proportional to the rate of air flow. Under both greenhouse and open field situations environmental factors can be very different and vary greatly both between and

within a location depending on the site, time of year and duration of the trapping period. Even in greenhouses where abiotic factors can be manipulated to a certain degree, temperature may still vary (Glenn 1984, Yunis et al. 1990, Berenguel et al. 2003) as does the internal air flow (Baptista et al. 1999, Wang et al. 1999, Tanny et al. 2005, Sase 2006). Wang et al. (1999) found that within a naturally ventilated greenhouse (tomato crop) in France, airflow within the house varied between < 0.1 m/s and 0.6 m/s depending on location within the greenhouse and outside wind speed (0.1–1.2 m/s) and direction over an 18-h period. Temperatures in greenhouses are managed to encourage optimal growth of crops, but variations of more than 10^{o}C within a 24-h period can occur (M-C Nielsen, unpubl. data). To achieve reliable estimates of release rates under different field conditions it is therefore important to determine the relationship between release rates and environmental factors.

The aim of this chapter was to use gravimetric analysis to determine the release kinetics of MI from selected passive dispensers used for thrips kairomone work and establish its release rate under a range of environmental conditions. The passive dispensers of interest in this chapter were polyethylene bags, a commercially available sachet, a commercially available thrips lure LUREM-TR (Koppert Biological Systems, the Netherlands) and uncovered cotton dental rolls containing different amounts of MI (0.5, 1.0 or 2.5 ml), under different temperatures (15, 25 or 35^{o}C) and air flows (0.1–0.15 m/s or 0.25–0.3 m/s). The temperatures chosen were influenced by the range previously measured under greenhouse and field experiments for thrips kairomone work. The airflows were dictated by the limitations of the operational parameters of the wind tunnel (minimum and maximum airflow used). The hypotheses were that MI was released at a constant rate (zero-order kinetics) and that temperature and air flow are major determinants in the release rates.

2.2 Methods and Materials

The experiments were carried out in a low-speed laminar-flow wind tunnel located at Plant & Food Research, Lincoln, between November 2010 and August 2011. For full description of the wind tunnel see Berry et al. (2006) and Davidson et al (2012a). The airflow in the wind tunnel was measured using a TA5 thermal solid probe anemometer (Airflow Developments Ltd, High Wycombe, England) and HOBO data loggers (Onset, Scott Technical Instruments, New Zealand) were placed in the room and inside the wind tunnel to record temperature and relative humidity during the experiment.

Passive dispensers. Polyethylene bags (150 micron in thickness) were made from polyethylene layflat tubing (50 mm wide) (Accord Plastics Ltd, New Zealand). The tubing was cut into 75 mm long strips and heat sealed twice at one end using an industrial heat sealer (Accolade Packaging Ltd, New Zealand). A piece of white acrylic felt (Spotlight, New Zealand) measuring 40 x 12 mm (for 0.5 ml MI), 40 x 25mm (for 1.0 ml MI) or 40 x 40 mm (for 0 and 2.5 ml of MI) (Fig. 2.1 a) was placed inside the tubing/bag. Commercially available kairomone sachets (LUREM-TR lures emptied of the contents) (Koppert Biological Systems, the Netherlands) were prepared prior to the experiment by placing the sachets in a fume hood for 6 weeks at room temperature, followed by freeze-drying to exhaust the sachet of the original kairomone. In addition to these dispensers, the release rate of the commercially available thrips lure LUREM-TR (Koppert Biological Systems, the Netherlands) was tested. The commercial sachet and the LUREM-TR dispensers measured 105 x 44 x 6 mm with the centre part (50 x 34 x 5 mm) containing a white piece of absorbent material (40 x 25 x 3 mm) covered with a perforated membrane on one side (Fig. 2.1 a,b). The final dispenser type used comprised of uncovered cotton dental rolls (size 2) (Biomedics, New Zealand). The cotton dental rolls were not modified (Fig. 2.1 c).

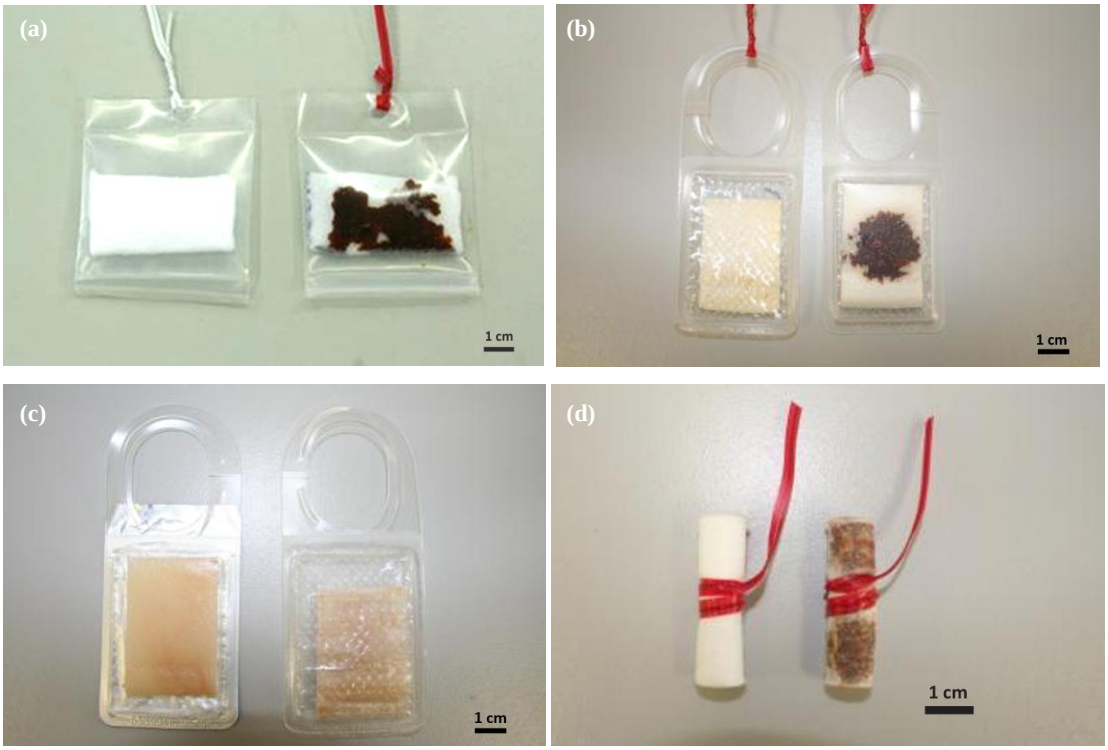


Figure 2.1 *Passive dispenser systems tested; (a) polyethylene bags (without and with methyl isonicotinate (MI)), (b) commercial sachet (without (permeable side shown) and with (non-permeable side shown) MI), (c) LUREM-TR with MI (permeable and non-permeable side shown) and (c) cotton dental rolls (with and without MI). (Photo: M-C Nielsen).*

Treatments. The release rates were tested at 15±1, 25±1 and 35±2°C and under 0.1–0.15 m/s air flow (air flow I) and 0.25–0.3 m/s air flow (air flow II). The experiment was carried out under ambient relative humidity that was recorded. The cotton dental rolls were tested using 0 (control), 0.5 and 1.0 ml MI. The emptied commercially available sachet and the 150 micron polyethylene bag were tested using 0 (control), 0.5, 1.0 and 2.5 ml MI. The commercially available thrips lure LUREM-TR contains approximately 2.69 ml MI (F. Griepink 2013, pers. comm. to M-C Nielsen, 22 June 2013). The emptied commercial sachet with 0 ml MI was also used as the control treatment for LUREM-TR as the dispensers are similar. The physical and chemical properties of MI can be found in Appendix II.

Assay and design layout. The release rate was determined gravimetrically by recording weight loss (g/day). Each of the dispensers were weighed before and after adding the liquid MI (±0.001 g) to establish the weight of the added volumes (0.5, 1.0 or 2.5 ml MI). The MI was added to the cotton dental rolls and the polyethylene bags using a 3ml disposable plastic pipette (Sarstedt, Global Science, New Zealand). The top of the polyethylene bags were immediately sealed after adding the MI. Adding the MI to the emptied commercial sachets was done by directly injecting the MI through one of the existing premade holes in the perforated membrane using a 1 ml insulin syringe (BD New Zealand Ltd), thereby not puncturing the sachet. A twist tie was added to each dispenser to hang them from a wire frame within the wind-tunnel. The experiment was a split-plot design, with each temperature by air flow combination (six runs) in randomised blocks. Each run contained three sets of 12 treatments (randomised split-split plot) with each set positioned randomly within a run thus comprising a split-plot 'pseudo' treatment to increase replication. The layout is illustrated in Fig 2.2 below.

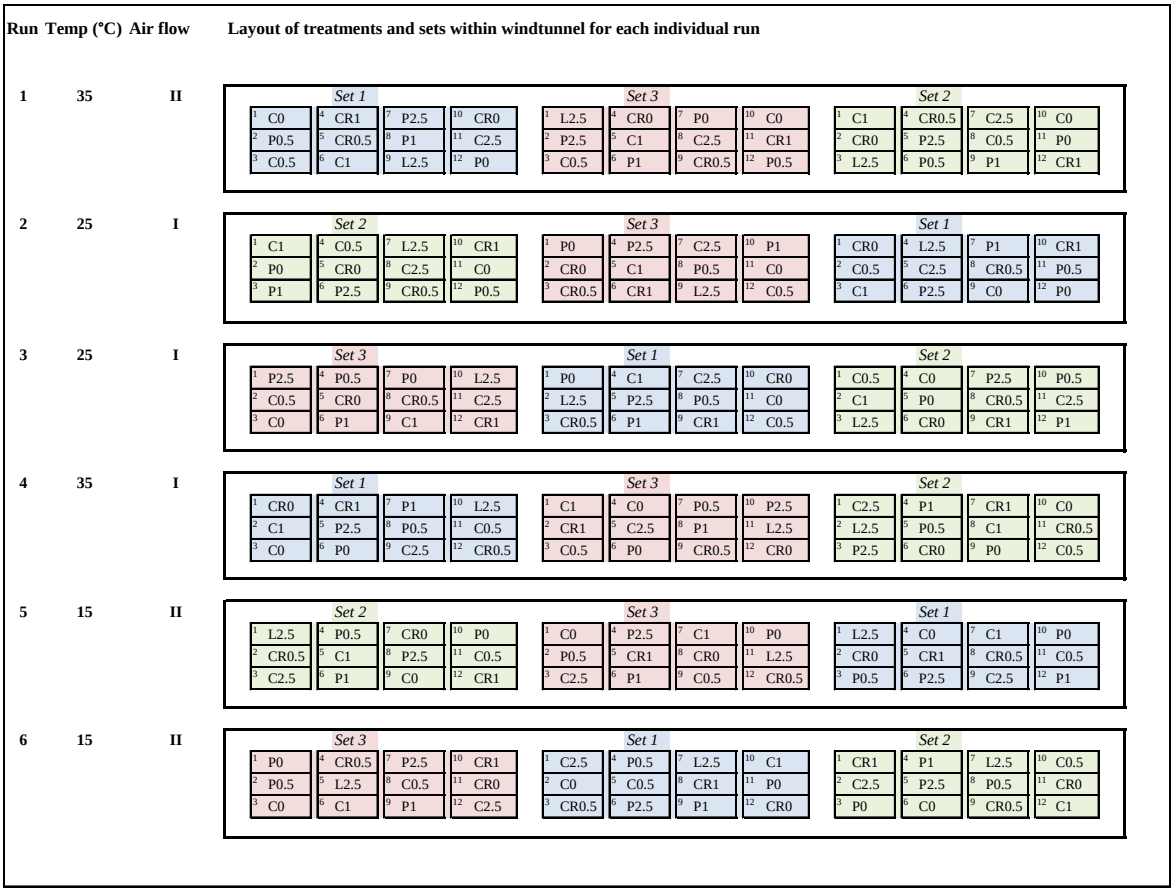


Figure 2.2 *Experimental design of MI release rate experiment. The experiment comprised six runs; each run was one temperature by one air flow combination (air flow I (0.1–0.15 m/s) or II (0.25–0.3 m/s). Each run included three sets, each set comprising 150 micron polyethylene bags containing 0 (P0), 0.5 (P0.5), 1 (P1.0) or 2.5(P2.5) ml MI, emptied commercial sachet containing 0 (C0), 0.5 (C0.5), 1 (C1.0) or 2.5 (C2.5) ml MI, cotton dental rolls (CR) containing 0 (CR0), 0.5 (CR0.5) or 1 (CR1.0) ml MI and LUREM-TR containing 2.67 (L2.5) ml MI.*

The dispensers were weighed daily (24±2 h) for all treatments run at 35°C. At 25 and 15°C the dispensers were weighed daily (24±2 h) for the first 3 days, then every 2 days (48±2 h). The observations for each dispenser were stopped at day 42 or earlier when the dispenser's weight was within 5% of its original weight.

2.2.1 Data analyses

Rates of release were calculated from the average daily loss in weight (g). The data were graphed to show trends of the release rates and to examine the hypotheses to be tested. Linear regression was used to fit straight lines to the linear part of each curve, with a separate analysis for each dispenser by temperature combination (including both air flows). The linear

part of the data was identified by including only data above 0.1 g of MI remaining. For each dispenser by temperature combination, separate constants (y-intercepts) were estimated for each initial amount (as the constant estimates the initial amount), but with a single slope estimated. The slope of these regressions gave the estimated average release rate for each dispenser by temperature combination. Because the treatments involved an element of pseudo-replication where all replicates were tested over time within the same wind tunnel, no other formal statistical analyses were applied to the data.

2.3 Results

The mass of MI lost from the different passive dispensers was constant, with the release rate being independent of amount of initial MI (Fig. 2.3). Evident fluctuation occurred in some data sets due to differences in days of weighing due to the split-plot layout within the run. The release rate of MI varied between the different dispensers, decreasing from the highest in the following order: cotton dental roll, LUREM-TR, commercial sachet and 150 micron polyethylene bag. The results showed that temperature is a major determinant of release rate. Increasing the temperature from 15^{o} to 35^{o}C produced an increase in release rates of MI with the recorded increases being similar for all the dispensers. Increased air flow also increased the release rates, with the effect increasing with temperature. Cotton dental rolls were depleted within 4 days for all treatments. LUREM-TR lures were depleted within 20 days at 25 and 35^{o}C but at 15^{o}C the depletion had not occurred at day 42. Using the average release rates sourced from fitting a linear regression model to the data (Table 2.1) depletion of LUREM-TR would take approximately 82 days at 15^{o}C. The commercial sachets were depleted within 20 days for all treatments, except when 2.5 ml MI was used at 15^{o}C. On average 40% of the MI was still left in the dispensers at day 42 at 15^{o}C. For the 150 micron thick polyethylene bags, depletion within 20 days only occurred for 0.5 and 1.0 ml MI at 25^{o}C and 35^{o}C and 2.5 ml MI at 35^{o}C. At 25^{o}C and 15^{o}C depletion of 2.5 ml MI took more than 55 and 250 days, respectively.

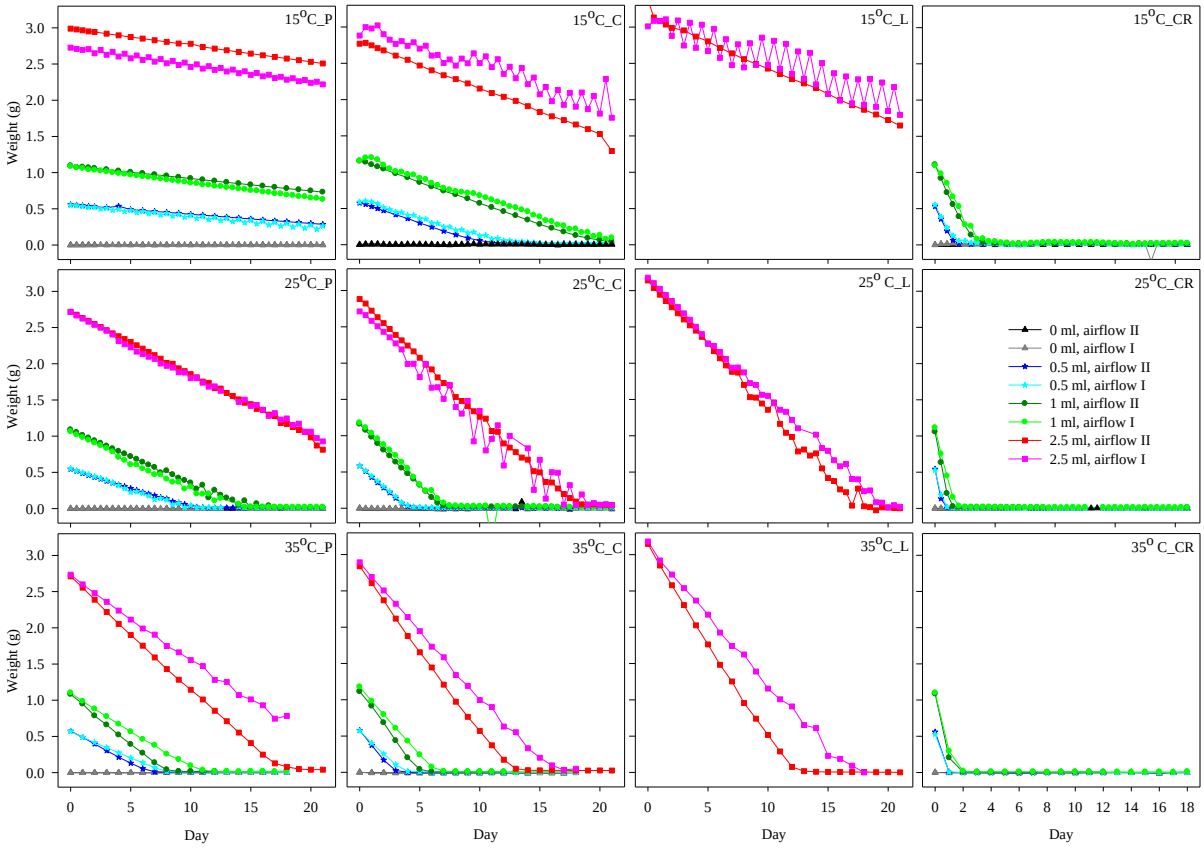


Figure 2.3 *The release of MI (g) from passive dispensers (polyethylene bag 150 micron (P), commercial sachet (C), LUREM-TR (L) and cotton dental rolls (CR)) under different temperatures (15, 25 or 35°C), air flows (0.1–0.15 m/s (I) and 0.25–0.3 m/s (II)) and initial amount MI (0, 0.5, 1.0 or 2.5 ml).*

The linear regression model gave excellent fit to the linear part of the response curves and precise estimates of release rates from the different dispensers (see Table 2.1). Based on the results described above an average air flow was used for the linear regression model. Some lack of fit described by the sum of squares occured mainly due to fluctuation in data sets (see comments above). Increasing the temperature produced an exponential increase in the release rate of MI for all dispenser types; however, this relationship was established from only three data points (15, 25 and 35°C).

Table 2.1 *Release rates of MI (mg/day) from different passive dispensers (polyethylene bag 150 micron, commercial sachet, LUREM-TR and cotton dental rolls) under different temperatures (15, 25 or 35°C). The release rates are estimated from fitting a linear regression model with separate constants (y-intercepts) and a common slope.*

Dispenser	Temperature (°C)	n	Mean Release rate (s.e.) (mg/day)	R^2 * (%)
Polyethylene bag (150 micron)	15± 1	9	9.4 (0.5)	97.8
	25± 1	9	40.4 (0.3)	99.4
	35± 2	9	129.3 (2.9)	95.8
Emptied commercial sachet	15± 1	9	28.7 (0.6)	98.0
	25± 1	9	76.0 (1.0)	97.2
	35± 2	9	194.8 (4.0)	96.6
LUREM-TR	15± 1	9	32.6 (0.9)	88.6
	25± 1	9	83.4 (1.1)	97.6
	35± 2	9	207.0 (8.0)	89.9
Cotton dental roll	15± 1	9	152.7 (5.5)	95.7
	25± 1	9	320.9 (20.1)	93.2
	35± 2	9	845.0 (53.9)	94.4

** Percentage of sums of squares accounted for by the regression*

2.4 Discussion

The results of the experiment supported the hypothesis that the MI would be released at a constant rate (zero-order kinetics) and that temperature is a major determinant of release rate. Increased air flow also increased the release rates, with the effect increasing with temperature; however, this factor was less important than temperature on the release kinetics for the parameters examined. For all the dispenser types the release rate data indicated an exponential increase with temperature from 15°C to 35°C. The importance of temperature on the release kinetics of a target chemical through a permeable membrane is in agreement with other studies (Kraan and Ebbers 1990, Bradley et al. 1995, Stipanovic et al. 2004, Shem et al. 2009). Although a zero-order kinetics release rate was indicated for the cotton dental rolls it should be noted that the frequency of the weighing (every 24 h) for this dispenser type was relatively insufficient in comparison to the total time for MI to be release. Hammack and Petroski (2004) tested the release rate of several volatile lures for the corn rootworm beetles (*Diabrotica barberi* and *D. virgifera virgifera*) with uncovered cotton rolls and found that the amount loaded onto the rolls influenced the release rate indicating first-order release kinetics. This could mean that the release rate of MI from the cotton dental rolls are time and concentration dependent and will vary depending on initial amount of MI added and the amount present at any given time displaying a curvilinear relationship, however further experiments are needed to validate the release kinetics of the cotton dental rolls.

The cotton dental rolls released MI at the highest rate and depletion occurred within 1–5 days depending on temperature and air flow. The 150 micron thick polyethylene bag had the lowest release rates and therefore released the chemical over the longest time period. The commercial sachet and LUREM-TR had equivalent release rates. This was to be expected since the commercial sachet was prepared from an emptied LUREM-TR sachet and MI is the current active ingredient of LUREM-TR (Teulon et al. 2011). At constant temperatures above 25^{o}C the regression results estimated that LUREM-TR would have depleted within 32 days. LUREM-TR is marketed to have a duration of 42 days when used in protected crops (Koppert Biological Systems) and to be most effective in summer. As daytime temperatures in greenhouses during summer reach temperatures well above 25^{o}C this could mean the lure may be depleted before the intended trapping period is completed. This could produce biased results when comparing trap catches between sticky traps alone and sticky traps used in combination with LUREM-TR lures. Though greenhouses in temperate zones like the Netherlands experience daytime summer temperatures well above 25^{o}C, the night temperatures are much lower, influencing the total release. A greenhouse experiment with LUREM-TR carried out during warm summer weather in the Netherlands in 2012 (see Chapter 3) recorded an average of 23^{o}C in the greenhouse during the experiment, with a recorded minimum of 16^{o}C and a maximum of 33^{o}C (M-C Nielsen, unpubl. data). Because roof vents were fully open during the daytime, moderate to high air movement occurred (M-C Nielsen, personal observ.). Under these conditions LUREM-TR lures lost on average of 87 mg/day. With 2.67 ml of active ingredient in LUREM-TR this would mean that the lure would be depleted within 30 days. The experiment in Chapter 3 was only carried out for 6 days; however, if the experiment was carried out over 42 days the lures would be depleted prematurely. Under the subtropical conditions of southeast Spain this problem would worsen. Greenhouse temperatures during summer (June–August) in the Almeria area of southeast Spain were recorded at an average of 33^{o}C, with a minimum of 20^{o}C and a maximum up to 41^{o}C (Montero et al. 1985). Under these conditions depletion of the dispensers would occur well within 20 days, under less than half the expected depletion time. In studies testing the biological efficiency of a semiochemical it is important to know when the quantity released daily declines due to depletion of the compound from the dispenser (Heuskin et al. 2011) and either end the experiment or replace the dispenser before depletion occurs. For example, the cotton dental rolls released MI in a matter of days, potentially leading to ‘failure’ of enhanced trapping after a few days. The results showed that MI can be delivered at a constant rate from the polyethylene bags and the LUREM-TR sachets. In addition, experiments undertaken in

Chapter 3 in this study have shown that polyethylene bags allow for easy manipulation of release rates by changing the thickness of the polyethylene used. By using a thinner polyethylene bag (50 micron in thickness) the results showed a three-fold increase in mean release rate of MI (tested with 1 ml at 25°C) compared with the 150 micron polyethylene bag used in this chapter.

Under field or greenhouse conditions several factors will play important roles in the actual release kinetics of a semiochemical from a passive dispenser. While constant and predictable release rates are reproducible in a laboratory setting they would not likely be replicated in field or greenhouse conditions. As shown in this chapter, temperatures and air flow affected the release rate. Thrips pests such as WFT and onion thrips (*Thrips tabaci* Lindeman) are worldwide pests throughout the year in many different indoor and outdoor cropping systems. Therefore the release rate of a kairomone lure like LUREM-TR needs to be calibrated under different temperature and air flow regimes for optimum and effective use in pest management. Even in greenhouses where these abiotic factors can be "manipulated" to a certain degree, temperature (Glenn 1984, Yunis et al. 1990, Berenguel et al. 2003) and air flow (Baptista et al. 1999, Wang et al. 1999, Tanny et al. 2005, Sase 2006) still vary greatly due to influences from the outside environment. The maximum airflow tested here, from 0.2 to 0.3 m/s, should be considered a weak airflow in a greenhouse setting, but was based on the range reproducible in the wind tunnel. Airflow in an experimental greenhouse (400 m^2) with continuous open roof vents was recorded from 0 to 10 m/s in a greenhouse in France (Boulard and Draoui 1995). However, according to studies by Bartzanas et al. (2004), Kittas et al. (1996), Liu et al. (2005), Teitel & Tanny (1999) and Teitel et al. (2008) airflows between 2 and 5 m/s seem more common. Optimisation of minimum, average, and maximum temperatures in greenhouse settings vary depending on crop, variety and growth stage, but during summer, temperatures inside vegetable greenhouses often range from 15^{o}C during the night to 35^{o}C during the daytime (M-C Nielsen, unpubl. data). Torr et al. (1997) found that the release rate of tsetse fly (Diptera: Glossinidae) attractants sealed in polyethylene bags varied 100-fold according to temperature differences relating to the time of day when used in the field. Another important factor reported to increase the release rate of kairomones from dispensers is the exposure to direct sunlight (Torr et al. 1997, Shem et al. 2009). Torr et al. (1997) found the cumulative loss of a tsetse fly attractant over 1 year from a dispenser in full sun was 17.4 g compared with 4.8 g from a sachet in the shade. In the field and greenhouses, thrips semiochemicals are often used in combination with coloured traps positioned above the

crop canopy in greenhouses, or at various crop heights in fields. Such positioning exposes the dispenser to potentially direct sunlight, and therefore will likely affect release rates. The variation in release rate when dispensers are exposed to sunlight is likely due to: 1) increased volatility as the chemical heats up (Heuskin et al. 2011), 2) prolonged exposure degrading the polyethylene material and thereby changing the characteristics of the dispenser (Torr et al. 1997) and/or 3) subtle chemical changes in the composition of the chemical used (Brown et al. 1992). Therefore, any data obtained under controlled and stable conditions such as in the laboratory is best used to determine the release kinetics and to indicate the release rates under specific controlled conditions. To optimise trapping with a kairomone like MI under variable field conditions it would seem appropriate to produce lures that are specially designed for different conditions, such as summer and winter trapping periods, temperate and subtropical trapping locations.

Although this chapter has demonstrated the release rate characteristics of MI for dispensers used for thrips trapping, it does not reveal any information about the response of thrips to MI at the various release rates. Dose-response studies of several thrips kairomone lures, including MI, have been performed on WFT in Y-tube olfactometer experiments (Koschier et al. 2000, Davidson et al. 2008), and will be discussed further in Chapter 3. These studies showed that female WFT walking response to kairomone lures was mostly dose-dependent. The results in Davidson et al. (2008) of female WFT attraction to MI showed a dose-response curve that was very broad/flat-topped indicating that once an appropriate minimum level was reached, the response obtained remained consistent over a wide dose range. However, whether a release rate of 40, 83 or 320 mg MI/day released by the 150 micron polyethylene bag, LUREM-TR or the cotton dental roll, respectively, at 25^{o}C reaches that minimum and how this level is perceived by flying thrips in a field situation is unclear. Considering the critical role the release rates of a semiochemicals play in insect trap responses (Miller and Borden 1990, Mathieu et al. 1997, Phillips 1997, Ross and Daterman 1998, Reddy et al. 2005, Sweeney et al. 2006), it is reasonable to infer that differences in release rates as a result of choice of passive dispenser and abiotic factors, such as temperature and air flow, could account for some of the variation previously experienced in field and greenhouse trials using MI to trap thrips. To explore this variation further, field validation of the release rates and the response of thrips to different release rates are examined in Chapter 3.

Chapter 3

Dose response of thrips to a kairomone lure in a greenhouse environment

3.1 Introduction

One of the key objectives for successful application of a semiochemical for insect pest manipulation and management is to achieve a controlled release rate that elicits an appropriate level of response from the target insect (Byers 1988, Heuskin et al. 2011). To establish the release kinetics over time for the passive dispensers previously used in thrips semiochemical work, laboratory assays were undertaken (see Chapter 2). The results showed that all dispenser types tested release the thrips kairomone lure methyl isonicotinate (MI) in a linear and constant fashion, independent of amount added (zero-order kinetic). This is desirable and allows for manipulation of the release rate in greenhouse and field applications depending on choice of dispenser. However, the release rate data does not specify the biological efficiency (i.e. thrips response when exposed to different dosage) of the semiochemical (Heuskin et al. 2011).

Variation in number of thrips caught on kairomone baited traps in field and greenhouse experiments may, to some extent, be due to differences in the amount of the kairomone released from the passive dispensers used and ultimately in differences in molecular concentration of the kairomone near the trap. Understanding the actual release of odour under greenhouse or field conditions and subsequent estimation of the exposure of thrips to the odours may help to elucidate such variation.

The importance of dose in regards to optimizing traps for insect trapping when applying semiochemicals has been shown for different insects such as the striped cucumber beetle, *Acalymma vittatum* (Lewis et al. 1990), oriental fruit moth, *Grapholita molesta* (Najar-Rodriguez et al. 2010) and the oriental fruit fly, *Bactrocera dorsali* (Zhang et al. 2007a). Dose-response studies to several thrips kairomones, including MI, have been performed on WFT in Y-tube olfactometer experiments (Koschier et al. 2000, Davidson et al. 2008). These show that female WFT walking responses to kairomones were mostly dose-dependent (i.e.

the response changed when the dose changed). These findings correspond to similar studies conducted on other thrips species (e.g. *Thrips obscuratus*) (Davidson et al. 2008, El-Sayed et al. 2009). Davidson et al. (2008) showed female WFT attraction to MI was dose-dependent and that once an appropriate minimum level was reached (activation threshold), the walking response obtained remained consistent over a large range of dosages (10^{-6} to 1 µL). In addition to the dose-dependent walking response found in Y-tube olfactometer experiments to MI, van Tol et al. (2012) showed in a leaf disc laboratory experiment that MI stimulated take-off behaviour for adult female WFT was dose-dependent. However, whether dose-dependent behaviour is found in flying thrips under field and greenhouse conditions is unknown.

The aim of this study was to investigate the relationship between the MI dose released and the behavioural responses of thrips in greenhouse environments. The objective was to use different passive dispensers releasing different rates of MI in combination with blue sticky traps in greenhouse experiments to link release of MI with number of thrips caught. The hypothesis was that flying responsive thrips will show a positive dose-dependent response to MI with more thrips caught on traps with high release of MI compare to thrips caught on traps with a lower release of MI. The results are discussed with respect to the appropriate release level of kairomone needed in a greenhouse environment to achieve optimum insect response (i.e. attract the greatest number of thrips to a trap) and relationship between laboratory and greenhouse results in regards to release rates of kairomone and response of thrips.

3.2 Materials and Methods

Two separate greenhouse experiments were carried out: one in July 2011 (Experiment 1) and one in August 2012 (Experiment 2) in capsicum crops (*Capsicum annuum* L.) in the Netherlands. The 2012 experiment was intended to be repeated in the same location as the 2011 experiment but the lack of WFT in the greenhouse required a change in location. The passive dispensers used to release the MI were evaluated in Chapter 2 and the release rates were therefore known under selected temperature and air flow parameters. In both years, blue Horiver sticky traps (25 x 10 cm) (Koppert Biological Systems, The Netherlands) were used. At the end of each sample period, the individual sticky traps were wrapped in super clear sheet protectors (Staples Inc., The Netherlands) and transported back to the laboratory. The numbers of female and male thrips caught on each trap were counted. For all traps a

maximum of 100 individuals per trap were randomly selected and identified to species (WFT) or genus (all other thrips) based on morphological characteristics described in Moritz et al. (2004) under a stereomicroscope (> x100) directly on the sticky traps. Appendices III–IV show the morphological criteria used to identify WFT (female and male) directly on the sticky traps. If more than 100 individuals were caught, the total number of thrips of a given species or genus were estimated using the proportions found in the 100 identified. The kairomone dispensers used in combination with the blue sticky traps (see below) were weighed prior to and after the experiment ($\pm$0.001 g) (Mettler AE163, Mettler Toledo). All the dispensers were sealed in foil pouches (Accord Plastics Ltd, New Zealand) until they were ready to be used in the greenhouse to avoid any release of the kairomone prior to the start of the experiment. When the dispensers were collected after the experiments they were put into an insulated box with freezer blocks and weighed immediately upon returning to the laboratory (i.e. approx. within 2 h). Data loggers (Onset Computer Corporation, USA) were used to record temperature and relative humidity in the greenhouse during each experiment. Daily averages were calculated for temperature and relative humidity recorded during the photophase (6 a.m. to 10 p.m.). Light intensity is usually the factor that affects the time of flight in insects (Lewis and Taylor 1965) and previous studies on flight activity of WFT have shown that no flight occurred during scotophase (Pearsall 2002, Liang et al. 2010). The sticky traps from the experiments were stored at -5^{o}C in the entomology laboratory at Plant Research International, The Netherlands, or Plant & Food Research, Lincoln, New Zealand, for future reference and record keeping.

3.2.1 Experiment 1

The first greenhouse experiment was conducted in a commercial organic capsicum crop (*C. annuum* var. Ciroco (compartment B) and Spider (compartment D)) at Groenland Biologische Groentekwekerij B.V., Schalkwijk, The Netherlands. The experiment was carried out between Monday 18 July and Monday 25 July 2011 (i.e. 7 days). The house was a naturally ventilated glass greenhouse with 21,000 m^2 of growing space, supplying markets in Europe and the US. The experiment was split between two compartments (compartment B (5000 m^2) and D (6000 m^2)) in the greenhouse. Biological control agents (mainly *Amblyseius cucumeris* (Oudemans) and *Orius laevigatus* (Fieber)) were used for ongoing thrips control and no insecticides were used at anytime in the growing season. Tomatoes were grown in the adjacent compartments (compartment A and C). All the compartments in the greenhouse were linked via a central pathway and separated by glass walls and sliding doors. In addition

to the data loggers used to record temperature and relative humidity, wind flow and wind direction were measured at crop height in compartment D using a WindLog datalogger (Rainwise Inc., USA).

The kairomone dispensers used for the experiment were: an emptied commercial sachet (Commercial sachet), 150 micron thick polyethylene bags (P150), and 50 micron thick polyethylene bags (P50). To each of the dispensers 2.5 ml of MI (Sigma-Aldrich, New Zealand) was added. The release rate was determined gravimetrically by recording weight loss (g/day). Each of the dispensers were weighed before and after adding the liquid MI ($\pm$0.001 g) to establish the weight of the added volumes (0.5, 1.0 or 2.5 ml MI). For the control traps (Control) a 150 micron thick polyethylene bag with no MI added was used. The methods and materials used to make the polyethylene bags and to prepare the commercial sachet are described in Chapter 2, Section 2.2.

Twelve replicates of the four treatments (Control, P150, P50 and Commercial sachet) were used, laid out as four (3 rep. x 4 treatments) incomplete Latin squares, with two squares in each compartment, one either side of the path (Fig. 3.1). The blue sticky traps were positioned 10–20 cm above the crop canopy (plants 2.0–2.5 m in height), attached to the plants' climber strings with green twist ties spaced with 10 m between all traps. Traps could freely rotate vertically around the plants' climber strings in accordance to the wind direction. This allowed a minimum of 10 m from the edges of the greenhouse and the foot path to the nearest trap. All the sticky traps were hung in position in the greenhouse before the dispensers were applied. The dispensers were attached to the plants' climber strings with green twist ties at the top of the blue sticky traps. The dispensers were applied and collected by replication (rep. 1 to 12) and in the following order: Control, Commercial, P150 and P50. The dispensers were applied in the chosen order to minimize any cross contamination among treatments and especially to avoid any contamination of the control dispensers and control traps.

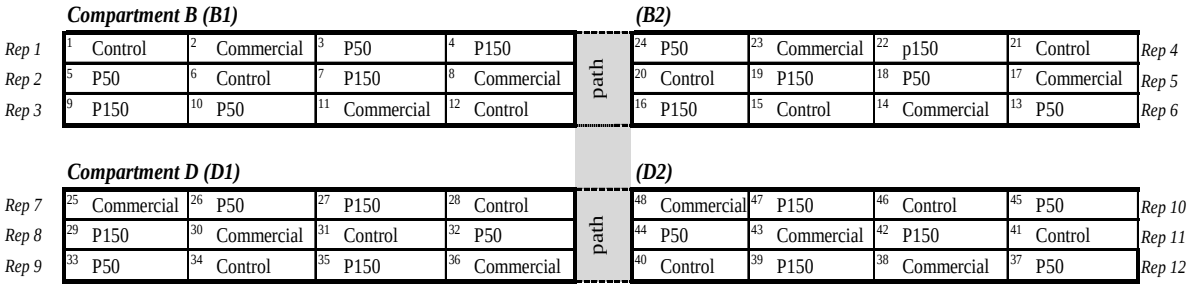


Figure 3.1 *Layout of blue sticky traps and treatments in July, 2011 greenhouse experiment. Small numbers in top left corner indicate trap number in experiment. The dispensers were: an emptied commercial sachet (Commercial), 150 micron thick polyethylene bags (P150), and 50 micron thick polyethylene bags (P50) all with 2.5 ml MI added. For control traps (Control) a 150 micron thick polyethylene bag with no MI added was used.*

In one section of compartment D (traps 25–36) additional flower sampling and daily trap capture counts were undertaken as part of a pilot study to record daily thrips capture and potential increase/decrease in flower-dwelling thrips near the traps. Every 24 h (between 1 and 1.30 p.m.) five flowers within a 1 m^2 area of each of the traps were surveyed for the number of adult thrips. Only fully open flowers found on the top third of the plants were surveyed. Adult thrips were observed and numbers recorded in the flowers by gently blowing onto the flower causing any resident thrips to move around. Numbers of thrips caught on traps 25–36 every 24 h (between 1 and 1.30 p.m.) were recorded daily in the greenhouse by using a hand lens (x10). On the last day when the traps were collected the number of thrips caught per trap were recorded under a stereomicroscope (<100 x) in the laboratory.

3.2.2 Experiment 2

The second greenhouse experiment was conducted in a commercial capsicum crop (*C. annuum* var. Inzell Rz) at SdG Paprika's BV, Harmelen, The Netherlands, between Friday 10 August and Thursday 16 August 2012 (i.e. 6 days). The day prior to starting the experiment (Thursday 9 August 2012, day 0) a base-line sampling was undertaken in the greenhouse (see below for details). The greenhouse was a naturally ventilated glass greenhouse with 51,750 m^2 of growing space. The company supplies markets in England, France and Germany. Biological control agents (*A. cucumeris* and *Orius vicinus* (Ribaut)) were used for ongoing thrips control and no insecticides were applied immediately before or during the week of the experiment.

As in Experiment 1, 150 and 50 micron polyethylene bags (P150 and P50) with 2.5 ml MI added were used. In addition, two dispensers made by ChemTica International (Costa Rica) and LUREM-TR commercial thrips lures were added to the experimental design (Fig. 3.2). The ChemTica dispensers were made from white semi-permeable plastic (fully sealed) covering a medium containing 2.5 ml MI and designed to release MI at 8.9 mg/d at 20^{o}C (CmLow) and 60.9 mg/d at 20^{o}C (CmHigh) (C. Oehlschlager, ChemTica, pers. comm. to DAJ Teulon, 29 June 2012). Each of the dispensers were weighed before and after adding the liquid MI ($\pm$0.001 g) to establish the weight of the added volumes (0.5, 1.0 or 2.5 ml MI). The reservoir containing MI measured 50 x 35 x 2 mm in CmLow dispensers and 50 x 60 x 2 mm in CmHigh dispensers. LUREM-TR lures contained approximately 2.69 ml MI (F. Griepink, Pherobank, pers. comm. to M-C Nielsen, 22 June 2013). All dispensers were used in combination with blue Horiver sticky traps. For the control traps a 150 micron polyethylene bag without any kairomone was used. The polyethylene bags were made as described in Chapter 2, Section 2.2.

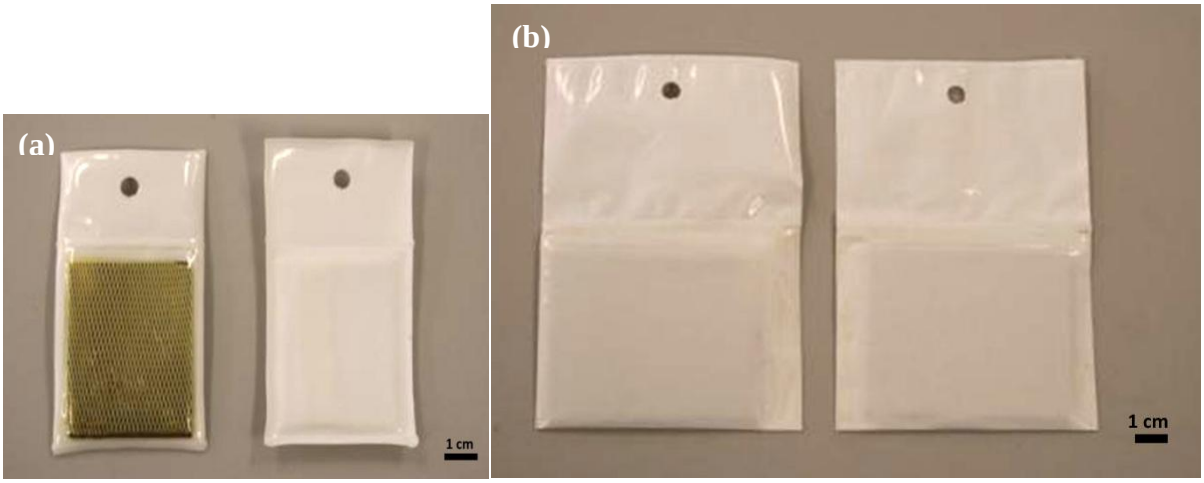


Figure 3.2 *ChemTica lures used in August, 2012 greenhouse experiment; (a) CmLow and (b) CmHigh (Photo: M-C Nielsen).*

Six replicates of the six treatments (Control, P50, P150, CmLow, CmHigh and LUREM-TR) were laid out in a complete 6x6 Latin square (Fig. 3.3). The blue sticky traps were positioned 10–20 cm above the crop canopy (plants 3.0–3.5 m in height), attached to the plants' climber strings with green twist ties. Traps could freely rotate vertically around the plants' climber strings in accordance with the wind direction. Traps were spaced out with 20 m between all traps and 20 m between any trap and the crop edge, walls, doors and pathways in the greenhouse. All the sticky traps were hung in position in the greenhouse before the dispensers were put out. The different treatments were applied and collected by replication (rep. 1 to 6)

and in the following order: control, P150, P50, CmLow, CmHigh, LUREM-TR. The dispensers were applied in the chosen order to minimize any cross contamination between treatments and especially to avoid any contamination of the control dispensers and control traps. The dispensers were attached to the plants' climber strings with green twist ties at the top of the blue sticky traps. The traps were left out for 24 h, and then replaced with new traps (between 12 noon and 2 p.m.). Trap replacement was repeated for six continuous days. The dispensers were not replaced throughout the experiment. Average daily release of MI was calculated by dividing the measured total loss of MI from the dispensers with number of days of the experiment. To identify and adjust for any spatial distribution of thrips at the different trap positions a base-line sampling was done before the experiment started (day 0). This was achieved by placing blue sticky traps only in all of the 36 trap positions in the 6x6 Latin square layout for 24 h before adding any treatments (day 1 to day 6) and counting the number of thrips caught in each position. The data collected provided information on the spatial distribution of thrips in the trapping area prior to adding any treatments. The trap count was used to identify trap positions that had either noticeable higher (e.g. "hotspots") or lower number of thrips caught/ trap position than the average lower number of thrips caught/ trap position over a 24-h period. The results of the trapping (total thrips/trap) can be found in Appendix V.

In addition to trap capture, flower scouting was undertaken on day 1, 4 and 6 to assess the potential increase/decrease in flower-dwelling thrips near the traps. Five flowers within a 1 m^2 area of each of the traps were surveyed for number of adult thrips (between 11 a.m. and 12 noon). As in Experiment 1, only fully open flowers found on the top third of the plants were surveyed, in the same manner as Experiment 1.

Rep 6	31 P150	32 Control	33 P50	34 CmHigh	35 LUREM -TR	36 CmLow
Rep 5	25 Control	26 P50	27 CmHigh	28 LUREM -TR	29 CmLow	30 P150
Rep 4	19 P50	20 CmHigh	21 LUREM -TR	22 CmLow	23 P150	24 Control
Rep 3	13 CmHigh	14 LUREM-TR	15 CmLow	16 P150	17 Control	18 P50
Rep 2	7 LUREM -TR	8 CmLow	9 P150	10 Control	11 P50	12 CmHigh
Rep 1	1 CmLow	2 P150	3 Control	4 P50	5 CmHigh	6 LUREM -TR

Figure 3.3 *Layout of blue sticky traps and treatments in August, 2012 greenhouse experiment. Small numbers in left side indicate trap number in experiment. The dispensers used were: 150 micron thick polyethylene bags (P150), 50 micron thick polyethylene bags (P50), ChemTica low release (CmLow), ChemTica high release (CmHigh) each with 2.5 ml of MI added and LUREM-TR. For control traps (Control) a 150 micron thick polyethylene bag with no MI added was used.*

3.2.3 Statistical analysis

Experiment 1. The number of thrips counted on sticky boards with different lures were analysed with a Poisson-gamma hierarchical generalised linear modelling approach (Lee et al. 2006) to allow for various random spatial effects (e.g. row and column effect). This included log (thrips identified/total on trap) as an offset, to adjust numbers for the proportion of thrips identified. Various random effects (glasshouses, Latin square within house, position within Latin square) were assessed with change of deviance tests, as implemented in GenStat's HGRTEST procedure (GenStat Committee 2011). Only some variations in mean trap count between the Latin squares were found, and in the final analysis adjustments were made only for the position of the four Latin squares. Treatments were included as a fixed effect within the analysis, and contrasts between treatments assessed with a change of deviance test, similar to the random effects, using HGFTEST (GenStat Committee 2011). Scatterplots were used to evaluate the relationships between daily trap capture (trap 25–36) and MI released over time and also between daily trap capture and climatic factors. The strength of any linear relationship was measured using the Least Squares method to estimate the Pearson correlation coefficient. All analyses were carried out with GenStat (GenStat Committee 2011), and graphics were produced using SigmaPlot v.10. No statistical analysis was carried out on the assessment of thrips in the flowers due to low numbers.

Experiment 2. The number of thrips counted on sticky boards with different lures were analysed with a Poisson-Gamma hierarchical generalised linear modelling approach (Lee et al. 2006). The analyses of male and female WFT were adjusted for the sub-sampling done for species identification, by including log (thrips identified/total on trap) as an offset (McCullagh and Nelder 1989). Patterns in trap catches across the greenhouse were examined by exploring the residuals graphically (where the residuals used were deviance residuals). Differences between the treatments were assessed using F-tests of contrasts included within the analysis of deviance done as part of the analysis. In a second set of analyses, spatial patterns in the counts were explored and adjusted for. These were carried out using a Poisson-Gamma hierarchical generalized linear model (Lee et al. 2006), which extends the Poisson GLM by allowing for the inclusion of random effects, in this case with a Gamma distribution and a log.link. The analyses included the offset to adjust for the sampling for identification, as for the GLM. Spatial patterns associated with the rows and columns were assessed, including one or both as random effects. The importance of these was assessed using a X^2 test of the change in deviance, as implemented in GenStat's HGRTEST procedure (GenStat Committee 2012). Counts of adult thrips found on flowers within the vicinity of sticky traps with different lures were analysed using a Poisson generalized linear model (GLM) with a log.link (McCullagh and Nelder 1989), with log(number of flowers) as an offset to adjust for the cases when fewer than five flowers were sampled. As above, differences among the treatments were assessed using F-tests of contrasts included within the analysis of deviance done as part of the analysis. Predicted ('mean') counts per five flowers were obtained, along with 95% confidence limits. Scatterplots were used to evaluate the relationships between daily trap capture and MI released and also for daily trap capture and climatic parameters measured. The strength of any linear relationship was measured using the Least Squares method to estimate the Pearson Correlation Coefficient. All analyses were carried out with GenStat (GenStat Committee 2012), and graphics were produced using SigmaPlot v.10.

3.3 Results

Experiment 1. The total amounts (mg) of MI released from the different passive dispensers over the 7-day trapping period are shown in Fig. 3.4. The difference in weight of dispensers before and after the trapping period (i.e. amount of MI released) was on average 132, 281 and 290 mg for 150 micron polybags, 50 micron polybags and Commercial sachets, respectively. The temperatures recorded throughout the experiment ranged from 19.1 to 23.9^{o}C. The relative humidity ranged from 59.2 to 76.7%. Prevailing wind direction and wind speed

recordings made throughout the experiment did not yield any data due to wind being below the instruments recording threshold of 0.9 m/sec. The Commercial sachet showed the largest variation in total amount of MI released from the individual sachet (231–346 mg/sachet) as displayed visually in Fig. 3.4. All dispensers used still contained MI at the end of the experiment. Traps baited with a Commercial sachet caught on average the most thrips (62.4 thrips/trap) (Table 3.1). On average, P150, P50 and Control baited traps caught 47.6, 51.9 and 19.6 thrips/trap, respectively.

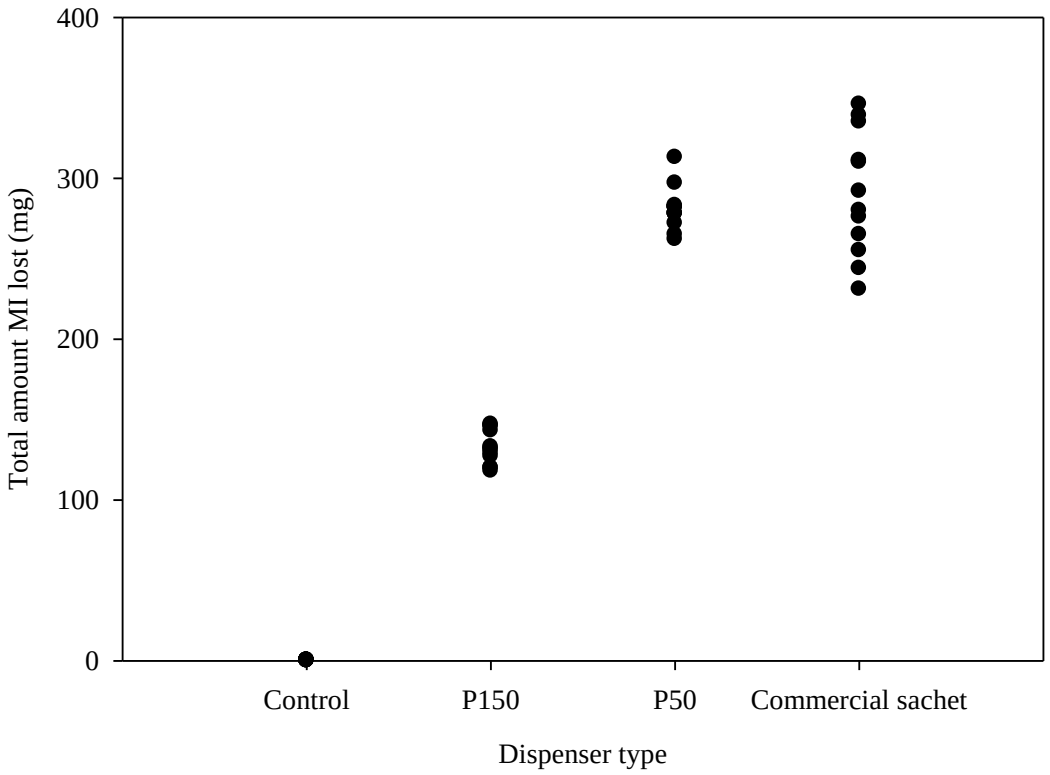


Figure 3.4 *Amount of MI (mg) lost from passive dispensers (n=12/dispenser type) after seven days when used in greenhouse experiment in July, 2011. The dispensers were: an emptied commercial sachet (Commercial sachet), 150 micron thick polyethylene bags (P150), and 50 micron thick polyethylene bags (P50) each with 2.5 ml of MI added. For control traps (Control) a 150 micron thick polyethylene bag with no MI added was used.*

Only a total of 32 WFT (female and male) were caught in the 2011 experiment. The majority of the thrips caught were female *Thrips* spp. (total of 1961). The total number of male *Thrips* spp. caught was 81. A total of 18 thrips were caught and grouped into "others". Tentative identification of the female *Thrips* spp. suggested that the majority of these were *Thrips tabaci* Lindeman. No tentative identification was carried out on the male *Thrips* spp. Formal analyses were carried out on total *Thrips* spp. (female and male), *Thrips* spp. (female), *Thrips* spp. (male) and total WFT (female and male) (Table 3.1). Trap catches of total WFT did not

vary significantly with treatment ($p = 0.386$), possibly due to the very low numbers caught. *Thrips* spp. were significantly lower on the controls than for any of the treatments ($p < 0.001$, $p < 0.001$, $p = 0.002$ for total *Thrips* spp., female *Thrips* spp. and male *Thrips* spp., respectively). There was an increase in trap capture with increased amount of MI released (Table 3.1) with catches of P150 traps being lower than commercial sachet and P50 traps for total and female *Thrips* spp. but the difference was not significant ($p = 0.065$ and $p = 0.060$, respectively). Only a total of 12 unidentified adult thrips were recorded from the 480 flowers scouted during the experiment near traps 25–36. No pattern was found and no statistical analysis was carried out on the data.

Table 3.1 *Average number of Thrips spp. (total, female (f) and male (m)) and western flower thrips (WFT)(total) caught on traps baited with MI released from different passive dispensers (n=12/treatment) (95% confidence limits) over whole trapping period. The traps and dispensers were left out for 7 days in greenhouse experiment in July, 2011. The dispensers were: an emptied commercial sachet (Commercial sachet), 150 micron thick polyethylene bags (P150), and 50 micron thick polyethylene bags (P50) each with 2.5 ml of MI added. For control traps (Control) a 150 micron thick polyethylene bag with no MI added was used.*

Treatment/dispenser	**Total *Thrips* spp.**	***Thrips* spp. (f)**	***Thrips* spp. (m)**	**Total WFT**
Control	19.6 (8.6,44.6)	18.6 (8.4,41.3)	0.8 (0.3,2.4)	0.8 (0.4,1.8)
P150	47.6 (21.3,106.4)	45.8 (20.8,100.8)	1.5 (0.5,4.2)	0.5 (0.2,1.3)
P50	51.9 (23.2,115.7)	49.0 (22.3,107.9)	2.5 (0.9,6.7)	0.9 (0.4,2.0)
Commercial sachet	62.4 (27.9,139.2)	59.7 (27.1,131.4)	2.3 (0.8,6.2)	0.4 (0.2,1.2)

Fig. 3.5 illustrates the result of the daily trap counts of thrips caught on traps 25–36 (see Fig. 3.1 for layout and trap position) and Fig. 3.6 shows the average daily temperature recorded throughout the experiment in the greenhouse (compartment B and D) and the average daily relative humidity recorded in compartment D. Due to a technical issue, relative humidity in compartment B was not recorded correctly. All MI baited traps showed a noticeable increase in thrips caught per day occurred over the first 3 days of sampling with a distinct peak occurring on day 3 followed by a marked drop in total and relative numbers.

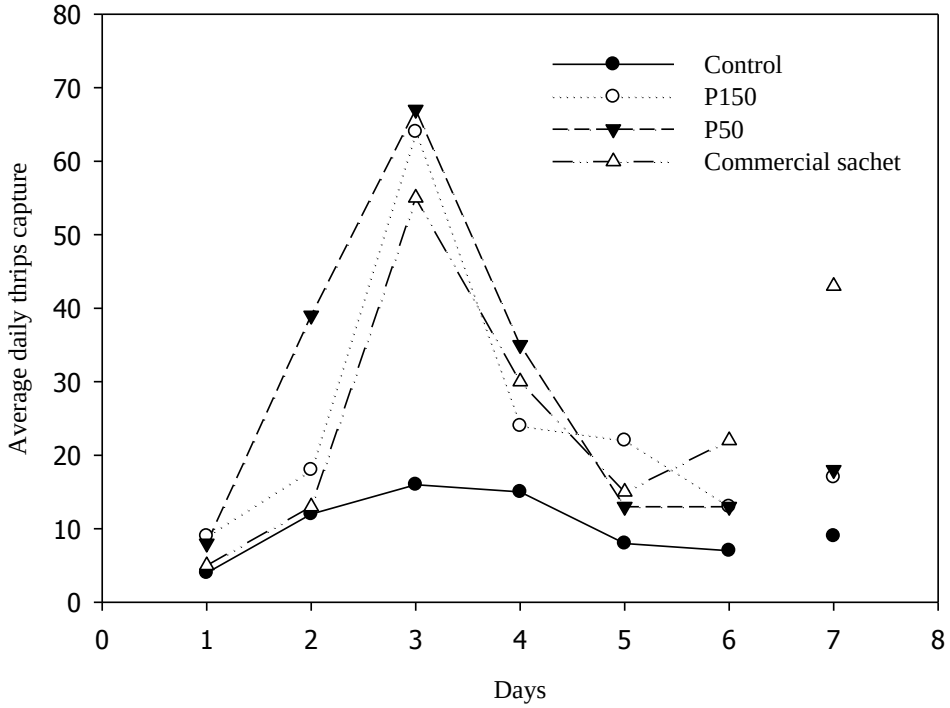


Figure 3.5 *Average daily thrips capture on blue sticky traps 25–36 (n=3/treatment) in the July, 2011 greenhouse experiment. Thrips were counted directly on the traps using a hand lens (x 10) between 1.00 and 1.30 p.m. The dispensers were: an emptied commercial sachet (Commercial sachet), 150 micron thick polyethylene bags (P150), and 50 micron thick polyethylene bags (P50), all containing 2.5 ml MI. Control traps (Control) were 150 micron thick polyethylene bags with no MI added. Data collected on day 7 was believed to be flawed due to methodology applied and therefore not included in the trend shown from day 1 to day 6.*

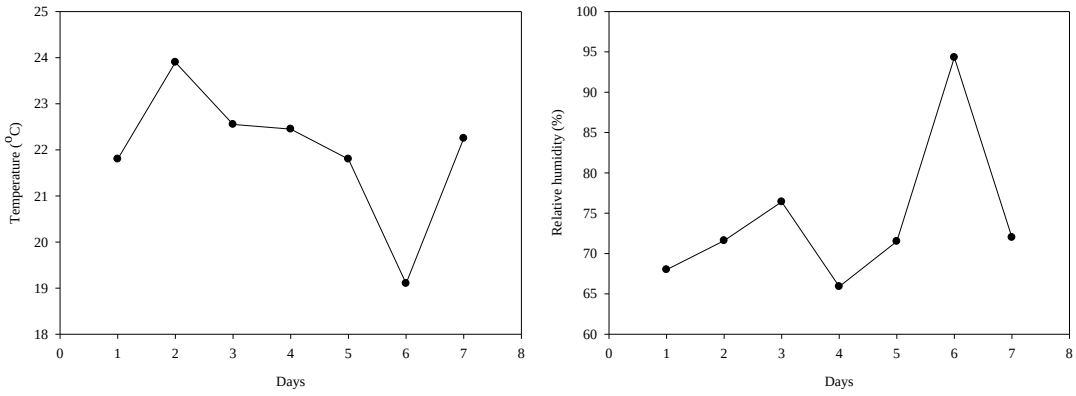


Figure 3.6 *Average daily temperature (a) and average daily relative humidity (b) recorded for the 24-hour period (excluding the hours between 10 p.m. to 6 a.m.) before daily thrips capture were recorded (ca. 1 p.m. to 1.30 p.m.) (n=32). The temperature and relative humidity recordings were taken every 30 min with a HOBO data logger hung adjacent to the trapping area in the greenhouse experiment in July 2011.*

Another increase in daily thrips capture occurred for all treatments on day 7 which was especially noticeable for traps baited with the Commercial sachet. This increase is possibly an

artefact of the methods used to assess the daily thrips capture. From day 1 to 6 the numbers of thrips caught on the traps over the previous 24 h were recorded using a hand lens (x10) while hanging in the greenhouse. While the hand lens allowed detection of female *Frankliniella* spp, female *Thrips* spp and male *Frankliniella* spp caught on the traps, male *Thrips* spp would be difficult to detect and easily missed using the hand lens thereby contributing to an underestimate of thrips caught on a trap. Traps were collected on day 7 and thrips caught over the previous 24 h for this day were recorded using a stereo microscope (>x100). This allowed for more accurate recording and any males overlooked previously using the hand lens only would be mistakenly included in the trap capture that had occurred over the last 24 h overestimating the number of thrips caught from day 6 to day 7 compared with previous data. The increase that occurred on day 7 on traps baited with a Commercial sachet was greater than the relative difference in increase seen between the other treatments. However, for traps 25–36, for which daily thrips numbers were recorded, no overall relationship was found for daily trap capture of thrips on traps baited with the commercial sachet and daily average temperature and relative humidity ($r < 0.1$, $p > 0.05$).

In regards to the relationship between daily trap capture on traps 25–36 and daily average temperature and relative humidity no strong relationships were found. A weak positive relationship ($r = 0.27$, $p = 0.02$) was found between the total number of thrips caught/trap/day and the mean daily temperature recorded 24 h prior (excluding the hours between 10 p.m. to 6 a.m.) to the daily recording of thrips caught (ca. 1 p.m. to 1.30 p.m.). For the individual treatments, a moderate positive relationship was found for traps baited with P50 dispensers ($r = 0.41$, $p = 0.05$) whereas no relationship was found for traps baited with the commercial sachet ($r < 0.1$, $p > 0.05$). P50 and commercial sachet baited traps caught on average similar number of thrips in the experiment (51.9 and 62.4 thrips/trap, respectively) and the dispensers released a similar mean amount of MI (281 and 290 mg/trap, respectively). A weak positive relationship was also found between the mean daily temperature and thrips caught on the control traps ($r = 0.31$), but the relationship was not significant ($p > 0.05$). No relationship was found between the total number of thrips caught/trap/day and the relative humidity ($r < 0.1$, $p = 0.01$).

A scatterplot showed a moderate positive relationship between the total number of thrips caught/trap and the amount of released MI/trap ($r = 0.44$, $p = 0.002$) (Fig. 3.7).

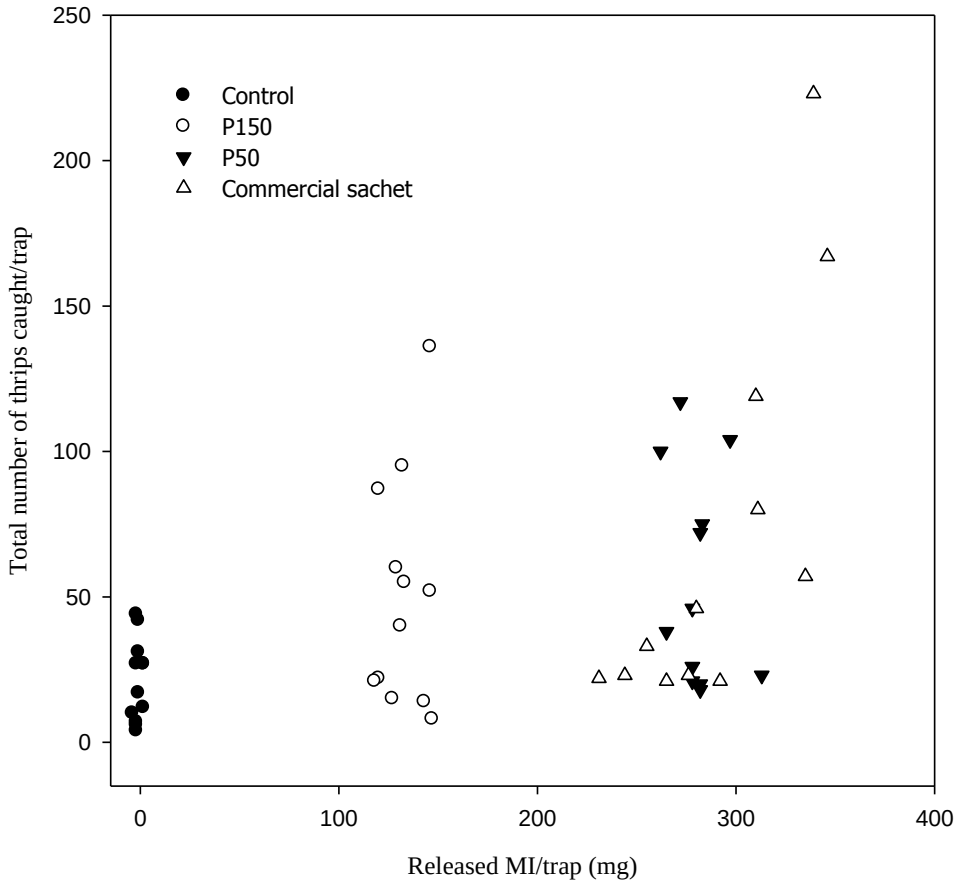


Figure 3.7 *Relationship between the total number of thrips caught/trap and the amount of released MI/trap (r = 0.44) using different passive dispensers in greenhouse experiment in July, 2011 (n=12/treatment). The dispensers were: an emptied commercial sachet (Commercial sachet), 150 micron thick polyethylene bags (P150), and 50 micron thick polyethylene bags (P50) each with 2.5 ml of MI added. Control traps (Control) were a 150 micron thick polyethylene bag with no MI added.*

Experiment 2. The total amounts (mg) of MI released from the different passive dispensers over the 6-day period are shown in Fig. 3.8. The average total amount of MI released from a dispenser ranged from 167 mg MI (P150) to 1192 mg MI (CmHigh). P50 dispensers released on average 395 mg MI and CmLow released 195 mg MI. LUREM-TR displayed the largest variation in total amount of MI released from the individual dispensers (399–687 mg) with a mean average of 521 mg MI. All dispensers used still contained MI at the end of the experiment. Mean daily releases of MI were calculated based on total amount lost at day 6 for all treatments to allow for comparison with daily trap capture.

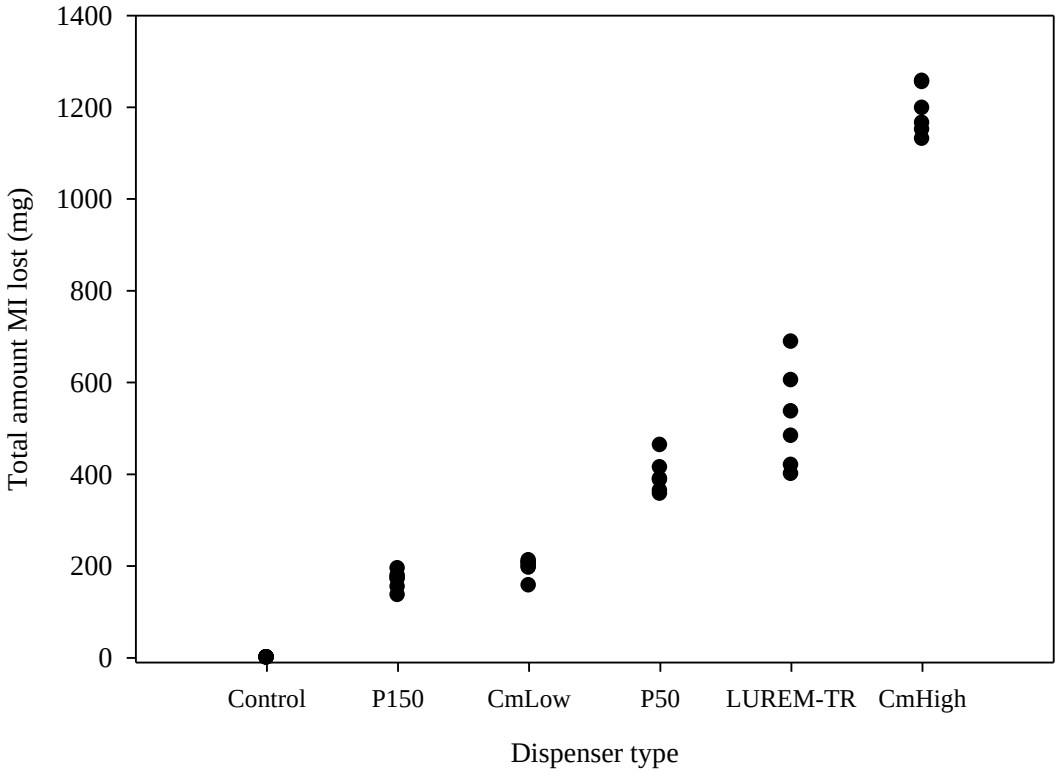


Figure 3.8 *Total amount of MI (mg) lost from different passive dispensers (n=6/dispenser type) after 6 days when used in greenhouse experiment in August 2012. The dispensers were: 150 micron thick polyethylene bags (P150), 50 micron thick polyethylene bags (P50), ChemTica low release (CmLow), ChemTica high release (CmHigh) each with 2.5 ml of MI added and LUREM-TR. For control traps (Control) 150 micron thick polyethylene bags with no MI added were used.*

The vast majority (>95%) of thrips caught on the traps were WFT. Formal analyses were only carried out for WFT (total, female and male). The results of the average catches of WFT total, female and male on the traps are presented in Appendix VI. The base-line trapping undertaken in each position on day 0 showed a trend in the spatial distribution, with a tendency for the average trap count to increase from replicate 1 to replicate 6 (column effects) (see Fig.3.3 for layout details). However, the overall effect was weak ($p > 0.1$), both in regards to column and row effect, and could generally be ignored. For the analyses, adjustments were made for row to row patterns only. The mean number of thrips caught on traps increased in the following order: P50, Control, CmHigh, CmLow, LUREM-TR. Large variations between trap captures were found for all treatments with the largest variation in number of thrips caught per trap recorded between traps baited with CmLow dispensers and the smallest found for P150 baited traps. LUREM-TR and CmLow caught similar mean numbers of thrips (825.1 and 791.8 thrips, respectively), whereas amount of MI released from the LUREM-TR and CmLow dispensers varied greatly between the two treatments (521 and

195 mg, respectively). For total WFT, there were some significant differences between treatments on all days except day 1 ($p = 0.206$ for day 1, and $p < 0.03$ for all other days); however, the patterns were inconsistent and varied from day to day. There was little difference between CmLow and CmHigh for any day ($p = 0.035$ on day 2 and $p > 0.05$ for all other days for this comparison). There were more differences between P50 and P150, with significant differences on days 3, 4 and 6 ($p < 0.05$), but differences on the other days were not significant (all $p > 0.05$). The inconsistent pattern found in the results for total WFT was also exhibited in the results for female and male WFT. For female WFT, there were some significant differences between treatments on some of the days (2, 3, 5, 6, with $0.0001 < p < 0.05$ for the overall treatment effect), but not on the remaining days ($0.07 < p < 0.33$). For male WFT, there were some significant differences between treatments on days 0, 2, 4 and 6 ($0.0001 < p < 0.03$ for the overall treatment effect), but not on the remaining days ($0.1 < p < 0.35$).

Fig. 3.9 illustrates the result of the daily trap capture of thrips. For traps baited with LUREM-TR and CmLow dispensers a noticeable peak in thrips caught per day occurred on day 2 compared with the other days (Fig 3.9). Although not as noticeable, this pattern also occurred on traps baited with CmHigh and P50 dispensers (day 3). Following the peaks on day 2 and 3 the number of thrips caught decreased as well as the relative differences between treatments. For traps baited with P150 dispensers the numbers of thrips caught decreased from day 1 to 6.

Only very few unidentified adult thrips were recorded from the daily visual scouting of flowers (Table 3.2). The results showed no pattern and no significant variation between the treatments at any of the dates ($p = 0.171, 0.278, 0.125$ for day 1, 4 and 6 respectively). The flower sampling did not support the trend observed in the spatial distribution, where the average trap count tended to increase from replicate 1 to replicate 6 (column effects). Further analyses (e.g. spatially adjusted) were not carried out because of the low numbers of thrips found.

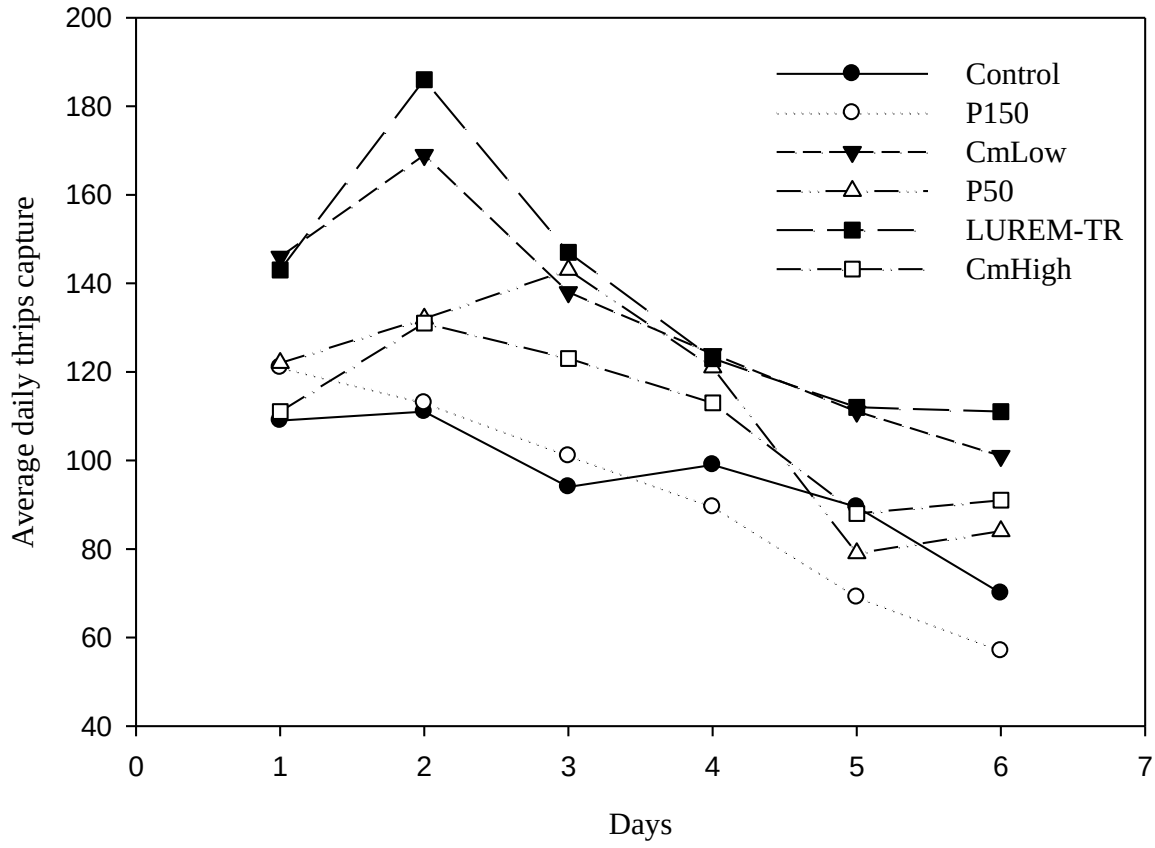


Figure 3.9 *Average daily thrips capture on blue sticky traps (n=6/treatment) in August 2012 greenhouse experiment (n=6/treatment). The dispensers were: 150 micron thick polyethylene bags (P150), 50 micron thick polyethylene bags (P50), ChemTica low release (CmLow), ChemTica high release (CmHigh) each with 2.5 ml of MI added and LUREM-TR. For control traps (Control) 150 micron thick polyethylene bags with no MI added were used.*

Table 3.2 *Mean number of adult thrips recorded from five open capsicums flowers in August 2012 greenhouse experiment. The flowers were in the vicinity (1 m^2) of blue sticky traps, paired with different passive dispensers containing MI. Recording were done on three separate days and the recording was done by visual assessment. The dispensers were: 150 micron thick polyethylene bags (P150), 50 micron thick polyethylene bags (P50), ChemTica low release (CmLow), ChemTica high release (CmHigh) each with 2.5 ml of MI added and LUREM-TR. For control traps (Control) 150 micron thick polyethylene bags with no MI added were used.*

Treatment on adjacent trap	Day 1	Day 4	Day 6
Control	0.50 (0.15,1.66)	0.17 (0.02,1.57)	0.33 (0.08,1.46)
P150	0.18 (0.02,1.40)	0.50 (0.14,1.85)	0.50 (0.15,1.67)
CmLow	0.17 (0.02,1.34)	1.33 (0.60,2.98)	0.67 (0.23,1.89)
P50	1.00 (0.43,2.34)	0.50 (0.14,1.85)	0.00 (0.00,*)
LUREM-TR	0.17 (0.02,1.30)	1.00 (0.40,2.53)	1.00 (0.43,2.34)
CmHigh	0.89 (0.35,2.26)	1.00 (0.40,2.53)	0.83 (0.33,2.12)

A scatterplot showed no relationship ($r < 0.1$, $p > 0.05$) between the total number of thrips caught/trap and the amount of released MI/trap (Fig. 3.10). There was a weak positive relationship ($r < 0.23$) between total females caught/trap and released MI/trap; however, the relationship was not significant ($p > 0.05$). The scatterplot revealed two clear outliers in the dataset: both traps (trap 32 and 36) were located in Rep. 6 (see comments above in regards to baseline sampling and spatial distribution of thrips in the trapping area). These traps may have caught more thrips due to being in thrips hotspots (i.e. area with a larger thrips population than mean density of thrips across the trapping area in the greenhouse). This is supported by the results from the baseline trapping, showing that traps located in Rep. 6 (traps 30–36) caught more thrips during a 24-h period on unbiased traps (see Appendix V). The outliers identified in Fig. 3.10 for Control (trap 32) and CmLow (trap 36) trap captures would have a strong influence on the results in regards to the relationship between the total number of thrips caught/trap and the amount of released MI/trap. This is also valid for the CmHigh trap data. As the trap capture of thrips on these two traps may not correctly reflect the treatment effect but may be due to an unusually high density of thrips in the trap location, it can be argued that the outliers could be ignored. If the outliers were excluded from the data a strong positive relationship ($r = 0.75$, $p < 0.001$) between the number of thrips caught per trap and MI released for Control, P150, CmLow, P50 and LUREM-TR, respectively, was found. This is supported by correlating the average trap capture and released MI for these treatments. The scatterplot presented in Fig. 3.10 suggests a peak in trap capture occurring between the LUREM-TR and CmHigh data points. Fitting a curvilinear regression analysis to the data (excluding the above outliers) showed a strong relationship between trap capture and MI released ($r = 0.71$, $p < 0.001$). However, this has to be further investigated testing release rates covering the range between LUREM-TR and CmHigh treatment release rates.

The average temperature and relative humidity recorded for the 24-hour period (excluding the hours between 10 p.m. to 6 a.m.) before traps were collected (between 12 noon and 2 p.m.) in the greenhouse are shown in Fig. 3.11. The temperatures recorded were very consistent between the days throughout the experiment, ranging from 23.5 to 25.6^{o}C. The relative humidity ranged from 59.2 to 76.7% during the experiment.

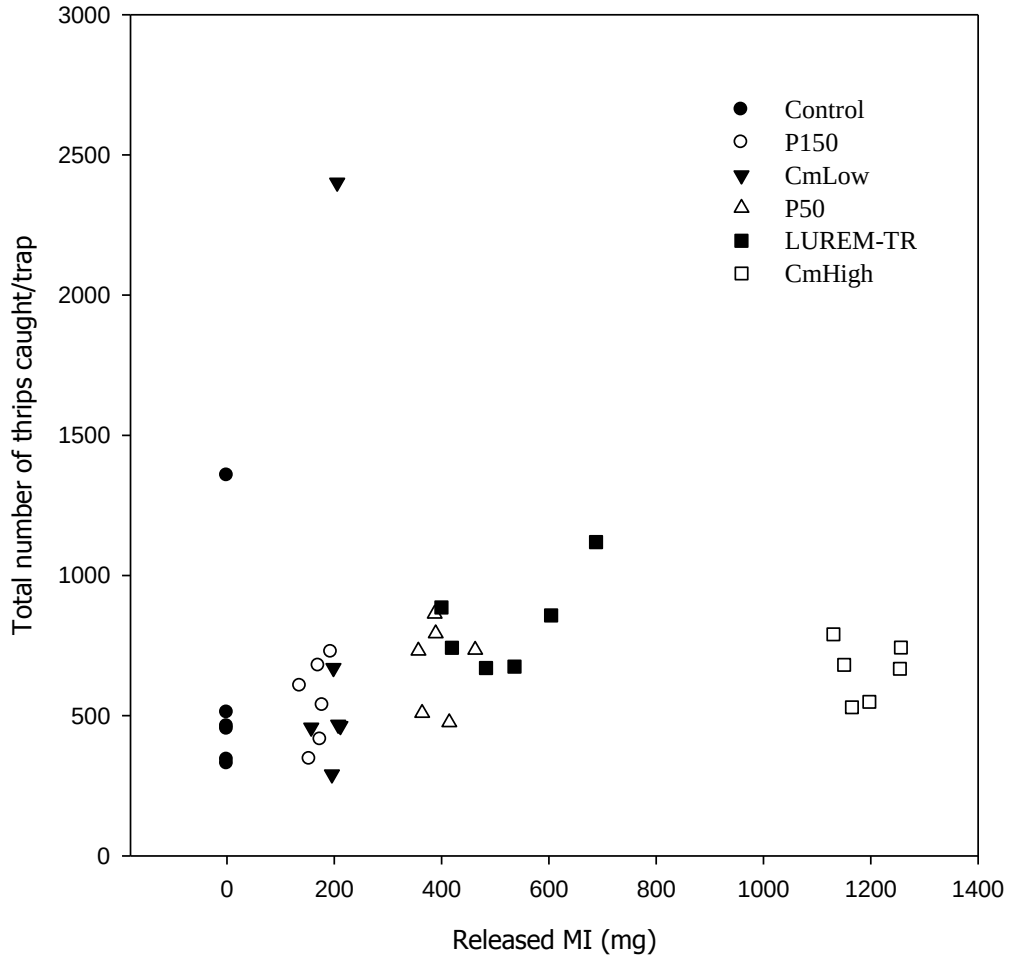


Figure 3.10 *Relationship between the total numbers of thrips caught/trap and released MI/trap during a 6-day greenhouse experiment August 2012 (average of n=6/treatment). The MI releasing dispensers were: 150 micron thick polyethylene bags (P150), 50 micron thick polyethylene bags (P50), ChemTica low release (CmLow), ChemTica high release (CmHigh) each with 2.5 ml of MI added and LUREM-TR. For control traps (Control) 150 micron thick polyethylene bags with no MI added were used.*

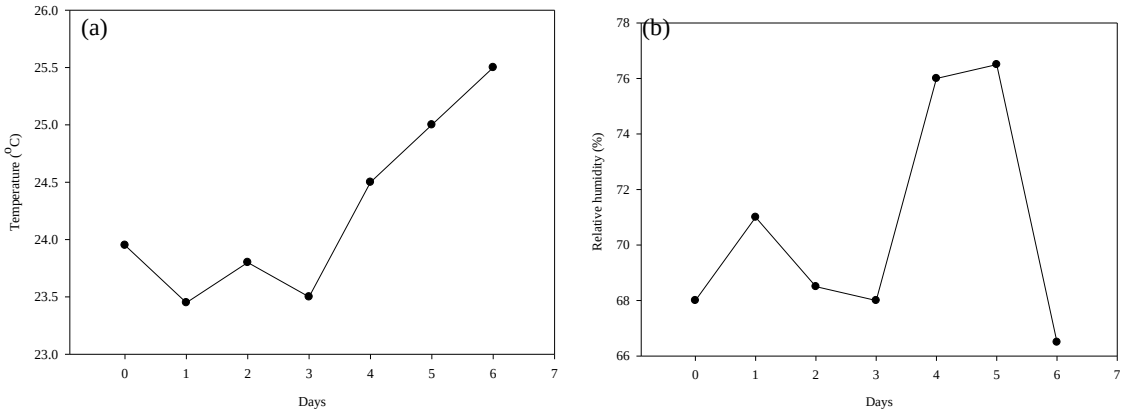


Figure 3.11 *Average temperature (a) and relative humidity (b) for the 24-hour period (excluding the hours between 10 p.m. to 6 a.m.) before traps were collected (ca. 12 noon to 2 p.m.)(n=32). The recordings were taken every 30 min with a HOBO data logger hung adjacent to the trapping area in the greenhouse experiment in August 2012.*

A strong negative relationship (r = 0.9, p = 0.01) was found between overall total number of thrips caught/trap/day and daily temperature recorded for the 24 h (excluding the hours between 10 p.m. to 6 a.m.) before traps were collected. Fig. 3.12 shows the relationship between the trap capture of the individual treatments and daily recorded temperature. For the individual treatments the negative relationship was strongest for P150 baited traps (r = 0.95, p = 0.003). Strong negative relationships were also found for CmHigh, CmLow and P50 baited traps ($0.80 < r < 0.88$, $p < 0.05$) but for LUREM-TR baited traps the relationship was weaker (r = 0.73, p = 0.09). A comparatively weaker negative relationship was found for the control traps (r = 0.61) and the relationship was not significant ($p > 0.05$). Trap capture of male thrips showed a stronger negative relationship between numbers caught and temperature (r = 0.87, p = 0.02) than female thrips (r = 0.73, p = 0.05). A weak negative relationship was found between the total number of thrips caught/trap/day and the relative humidity (r = 0.28), but the relationship was not significant ($p > 0.05$).

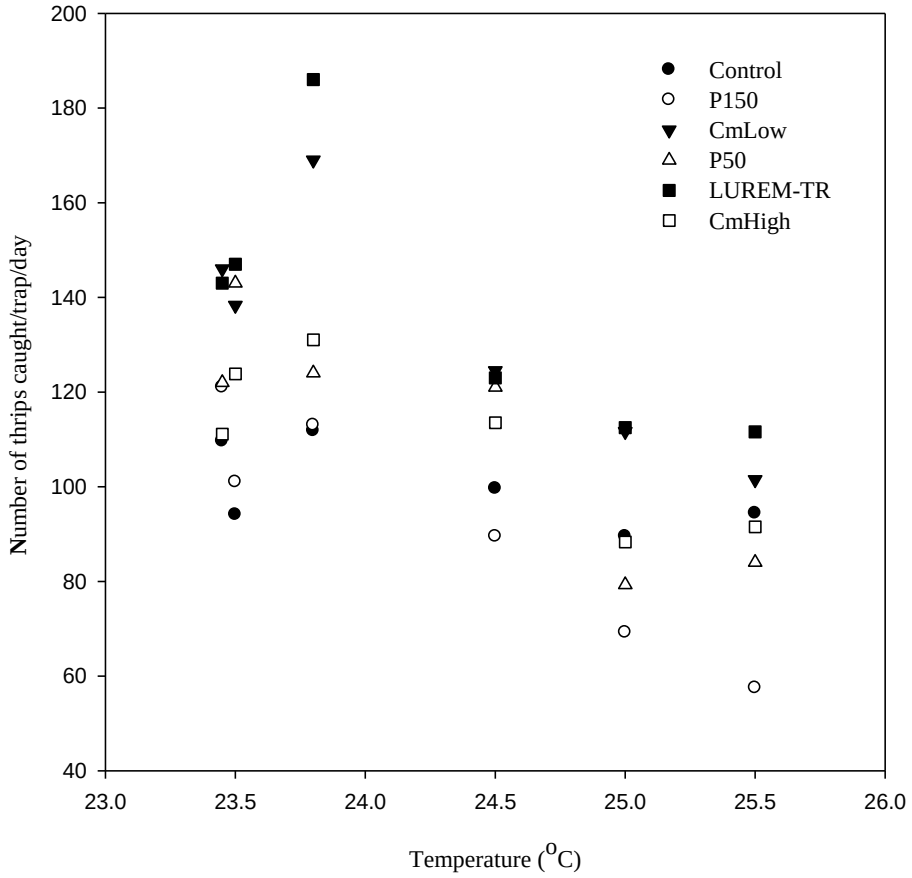


Figure 3.12 *Relationship between the number of thrips caught/trap/day and daily recorded temperature (n=6/treatment). The experiment was run over 6 days. The recordings were taken for the photophase only (6 a.m. to 10 p.m.) in the trapping period from 1 p.m. to 1 p.m. the following day. The recordings were taken every 30 min with a HOBO data logger hung adjacent to the trapping area in the greenhouse experiment in August 2012.*

3.4 Discussion

***Dose dependent response*.** The hypothesis that flying responsive thrips would show a dose-dependent response to MI in the greenhouse experiments was partially supported by the data. Results obtained for *Thrips* spp., the main genus caught in Experiment 1 (2011), showed a weak positive relationship between the total number of thrips caught/trap and the amount of released MI/trap ($r = 0.44$). For Experiment 2 (2012) the raw data showed no relationship ($r < 0.1$) between the total number of thrips caught/trap and the amount of released MI/trap. The majority of thrips caught in Experiment 2 were WFT. However, the results from Experiment 2 (Fig. 3.10) were further scrutinised because the number of thrips caught on one Control trap and one CmLow baited trap was considerably higher (2–3 fold and 3–5 fold, respectively) than the number of thrips caught on all other traps. The base-line trapping undertaken in each position on day 0 of the experiment identified that average trap count increased from replicate 1 to replicate 6, with trap capture in replicate 6 catching very high thrips numbers compared to trap capture in the other replicates. The two outlier traps were both located in replicate 6. It is plausible that these traps where located in thrips 'hotspots' within the greenhouse with increased numbers of thrips influencing the trap capture independent of the treatment. Thrips 'hotspots' can obscure underlying relationships as has been observed on a number of occasions in other thrips trapping experiments in greenhouses (DAJ Teulon, pers. comm.). The relationship between trap capture and MI released for Control, P150, CmLow, P50 and LUREM-TR excluding the outliers showed a positive relationship ($r = 0.75$, $p < 0.001$).

Secondly, the scatterplot presented in Fig. 3.10 suggests that the release rate produced by the LUREM-TR dispensers were near an optimum release rate of MI for WFT under the recorded conditions with the optimum release rate with regards to trap capture of WFT being between the LUREM-TR and CmHigh data points. Dose response curves for insects to kairomones tends to be bell-shaped, with the highest response found at a median release rate and low response at either very high or very low release rates (Pow et al. 1999, Knight and Light 2005, El-Sayed et al. 2009).

***Variable response over time*.** Differences between treatments were inconsistent and varied from day to day along with variation between trap capture within the treatments. While these results highlight the key issue addressed in this study (i.e. the variability of thrips response to kairomone lures) they complicate the interpretation of this chapters. Several issues such as uneven distribution of thrips within the greenhouse (i.e. hotspots) and the influence of

extrinsic (abiotic) factors may all have influenced the overall variation in response, potentially in combination with other extrinsic factors directly influencing flight activity and the behavioural response of WFT. These are considered below.

Effect of temperature. Rising temperatures in Experiment 2 may have had a negative influence on trap capture; the effect being strong for MI baited traps ($0.73 < r < 0.88$) and only moderate for control trap ($r = 0.61$). However, temperature tended to increase over time during the trial so that a temporal factor may have confounded these results. In contrast a weak positive relationship ($r = 0.27$, $p = 0.02$) was found between the total number of thrips caught/trap/day and the mean daily temperature in Experiment 1. Daily temperatures in Experiment 1 ranged from 19.1 to 23.9^{o}C whereas the daily temperature in Experiment 2 ranged from 23.5 to 25.6^{o}C. While it is difficult to make comparisons between the two experiments because of the different thrips species present there is some evidence that that the difference between baited traps and control traps is greater at lower temps (i.e. below 24^{o}C) than higher temperatures (i.e. above 24^{o}C). This might suggest that the effect of temperature is directly involved in the perception of odour. For WFT, studies carried out at different constant temperatures in Y-tube olfactometer to test the walking response to MI, showed a decrease in response with increasing temperature. At 25^{o}C significantly more WFT choose the MI laden arm versus the clean air at four difference doses of MI (at 0.1 µL), compared with WFT at 35^{o}C (M.M. Davidson, unpubl. data). Temperature has been shown to alter the response of insects to semiochemicals in other studies (Linn et al. 1988, Jonsson and Anderbrant 1993) highlighting the importance of temperature as a variable.

The effects of increasing temperature on total number of thrips/trap/day on baited traps between the two experiments are contradictory. Whereas a weak positive relationship was found for *Thrips* spp. in Experiment 1, a negative relationship was found for WFT in Experiment 2. However, this may be a function of temperature and other extrinsic factors directly effecting flight activity and dispersal of the thrips and not linked to MI release rate, since a similar patterns exists for control traps. Trapping data indicate that specific extrinsic factors affect dispersal of different thrips species differently (Morsello et al. 2008), and the effect observed may therefore not be linked to any treatment effect. Also, WFT have shown to be intolerant to high temperature in aspects of its life cycle (Robb and Parrell 1991, Gaum et al. 1994) and manipulation of temperature and humidity have even been investigated as a control measure for WFT in greenhouses (Shipp and Gillespie 1993). Therefore, the negative relationship observed for WFT in Experiment 2 may strictly be linked to decreased flight and

activity due to increasing temperatures although the average temperature range recorded (23.5–25.6^{o}C) corresponded with previously registered temperature range where flight occurred (Pearsall and Myers 2001, Liang et al. 2010). However, a combination of other factors such as wind flow and sunshine may have played an important role on the temperature experienced by the thrips at the crop canopy, as suggested on release rates (see discussion below).

Other extrinsic factors. Although the light intensity was not measured during the experiments meteorological data from local weather stations indicated that the duration of sunshine was very different between the experiments. During Experiment 2, the duration of sunshine ranged from 5.3 h to 13 h/day (average of 8.8 h/day). In contrast, Experiment 1 had 0 to 6.8 h/day (average of 3.2 h/day) of sunshine. Supplemental artificial lighting was not used during either of the experiments. While differences in species composition between trials confound comparisons between trials, analysis of relative trap capture within trials with respect to sunshine intensity might be worthwhile. Trap attractiveness and capture rates of thrips may vary due to differences in U.V. remittance and fluorescence (Vernon and Gillespie 1990) and degradation of the colours due to sunlight (Samways 1986, Grout and Richards 1990, Childers and Brecht 1996) decreasing the attractiveness of the trap to flying thrips. Previous studies have also shown that U.V. remittance and fluorescence can interfere with the flight orientation of insect (Raviv and Antignus 2004), and the large variation in number of thrips caught on traps within the treatments, and lack of difference between treatments, may have been influenced by this. Hours of sunshine during the experiment would also influence MI released from the dispensers (see discussion below) increasing the release rate with increasing sunshine.

Optimum release rates. The relatively small differences of WFT numbers on traps with lures and traps without lures and the weak dose dependent response could be that either a minimum odour concentration threshold to initiate thrips behaviour was not met (or it was too high and inhibited behaviour (e.g. repellency). As noted, dose response curves for insects tend to be bell-shaped, with the highest response found at a optimum release rate and a low response at either very high or very low release rates (Pow et al. 1999, Knight and Light 2005, El-Sayed et al. 2009). A few studies provide evidence that optimal dose is critical to achieve optimal attraction of flying thrips. Pow et al. (1999) found that significantly more WFT were caught on traps baited with 500 ng (*E*)-β-farnesene in vials, but not 100 or 1000

ng in a greenhouse experiment. In El-Sayed et al. (2009) field trapping experiments with six different *cis*-jasmone dosage ranging from 1 to 1000 mg showed an increase in *T. occidentalis* capture for all dosages except 1 mg and with highest catch being on traps baited with 500 mg *cis*-jasmone. Additionally, many semiochemicals that usually attract insects at one concentration may exert repellency at high concentrations (Visser 1986). For WFT this has been shown for *p*-anisaldehyde and 4-formyl pyridine in Y-tube olfactometer assays, both attractive at a low dose but repellent at higher dose (Koschier et al. 2000, Davidson et al. 2008). Regardless of this it seems very unlikely that the release rates tested here were too low or too high to elicit a behavioural response. In laboratory experiments (walking response) WFT has been attractive to MI across a wide range of doses (Davidson et al. 2008) and previous greenhouse and open field trapping experiments using LUREM-TR have shown an overall significant increase in the number of thrips caught compared with unbaited traps (Till et al. 2009, Broughton and Harrison 2012, Niassy et al. 2012). Nevertheless, CmHigh lures released on average over 40% more MI daily then the amount of MI released from LUREM-TR and caught fewer thrips suggesting that the higher rate of release may have in some way reduced the efficacy of the lure.

***Odour contamination*.** The level of MI within the greenhouses may have influenced the relative difference of WFT caught on traps with and without lures by saturating the air with MI around the trapping area (including control traps) over time. If this was to happen it could result in an increased trapping efficacy on control traps, essentially becoming an odour baited trap, obscuring any effect difference in trapping efficacy between odour baited traps and the control traps. Although no inhibiting threshold is believed to have been reached within either experiment the overall level of MI within the greenhouses may also have influenced the number of thrips caught. Previous studies in wind tunnel (Teulon et al. 1999, Berry et al. 2006) and Y-tube olfactometer (Koschier et al. 2000) bioassays show that activity of the thrips, and likely their flight response, can be inhibited by the presence of an odour, potentially leading to thrips becoming less active and/or attracted to the traps as the level of MI increases in the greenhouse over time.

The results of the daily trapping support this conjecture to some extent. After putting out the traps in the greenhouses the total number of thrips caught per trap peaked within day 2 (Experiment 2) or 3 (Experiment 1), followed by a decrease of thrips caught across all traps over the following days. Within the first days the MI released from the different dispensers

attached to the traps will elicit and stimulate movements of the thrips near the trap increasing the number of thrips caught on the trap significantly compared with the control traps. However, after that period the amounts released from the large number of dispensers saturate the air in the trapping area and decrease the differences between odour baited traps and control traps as all traps including the control traps have amounts of MI molecules nearby.

A greenhouse pilot study was undertaken in a capsicum crop in New Zealand in January 2012 to investigate the spatial dispersion of MI in the atmosphere within a greenhouse and to track and quantify levels of MI dispersed from one single release point. By using solid phase micro extraction (SPME) fibre sampling coupled with gas chromatography (GC) analysis, the results showed that MI dispensed from a P50 bag (similar to those used in this experiment) downwind from the point of release. After exposing the lure for just 1 h in the greenhouse MI could be detected 7.5 m downwind from the lure (M-C. Nielsen and C. Sansom, unpubl. data). This pilot study was carried out in a naturally ventilated greenhouse with the roof vents fully open during the trial period creating an updraft and temperatures under 21–23^{o}C. In hot summer conditions, over several days and with several dispensers continuously dispersing MI (such as in the experiments reported here), it is plausible that all traps including the controls have amounts of MI molecules nearby obscuring the effect of adding MI to the traps.
In Experiment 1 where a weak dose response was found for *Thrips* spp. both a potential difference in sensitivity towards MI between *Thrips* spp. and WFT (i.e. higher for *Thrips* spp. than WFT) and level of total MI released during the experiment could explain why a potential saturation did not seem to have same effect on trap catches as in Experiment 2. In Experiment 1 only 18 MI baited dispensers were hung in each compartment (1200 m^2) with an average release of 18.7–41.4 mg/day from any of the dispensers, compared with 30 dispensers in one area (10,000 m^2) in Experiment 2 with an average release of 28–198 mg/day. Even with the greenhouse used in Experiment 2 being more than twice the size of the greenhouse used in Experiment 1 the trapping area may still experience a saturation level of MI.

***Interspecific differences*.** A dose-related response was found for WFT in Experiment 2 **(**outliers excluded from the data) and for *Thrips* spp. in Experiment 1, showing a possible interspecific difference in regards to dose related response to semiochemicals. This has been found in other groups of insects such as beetles. In field trials Miller et al. (1995) showed that the effect of an anti-aggregation pheromone released at various rates on the attraction of three sympatric species of pine bark beetles (Coleoptera: Scolytidae) to pheromone-baited traps

differed between the different species. Although *Thrips* spp. (especially *Thrips tabaci*) and WFT are often found on the same host plant such as capsicum (Teulon and Nielsen 2005), lettuce (Workman et al. 2007), tomatoes (Salguero Navas et al. 1991) and roses (Till et al. 2009) profound differences in regards to response to kairomones have been reported. For example for WFT, carvacrol (essential oil extract) has been shown to be an anti-feedant whereas it was found to be a feeding stimulant for *T. tabaci* (Sedy and Koschier 2003). With regards to MI, previous field trials have shown that it is a strong attractant for *T. tabaci* (Teulon et al. 2007b). It is probable that the *Thrips* spp. caught mainly consist of *T. tabaci* and the tested dosage range may be more in line with what is optimum for this species. A previous trial in the same greenhouse showed that *T. tabaci* was the dominate species, followed by *T. major* (Teulon et al. 2008, DAJ Teulon, unpubl. data), a thrips species also attracted to MI (Teulon et al. 2008b). That *T. major* is present is supported by the male *Thrips* spp. caught on the traps as *T. tabaci* males are rare in many parts of the world except when populations are very high (Mound 1997). A number of studies provide strong evidence that selection of an optimal dose is critical to achieve attractiveness of thrips to a kairomone. In olfactometer studies dose dependent attractions of walking WFT towards different kairomones have been found (Pow et al. 1999, Koschier et al. 2000, Chermenskaya et al. 2001, Shamshev et al. 2003, Davidson et al. 2008).

Dispenser performance. Comparison of release rates obtained under laboratory conditions (Chapter 2) and the actual release rates obtained under greenhouse experiments are difficult as the changing temperature, variation in wind flow and sunlight direct influence on the dispensers all play a crucial role in the amount of kairomone released. Despite this the release rate established in Chapter 2 for MI from LUREM-TR dispensers under a constant $25\pm1^{o}C$ was on average 83.4 mg/day (Tabel 2.1), almost identical to the average daily release rate of 86.0 mg/day found for LUREM-TR dispenser in the 2012 greenhouse experiment. The average temperatures recorded in the greenhouse ranged from 23.5 to $25.6^{o}C$. The average temperatures in the 2011 greenhouse experiment were much lower (19.1 to $23.9^{o}C$) and so was the average daily amount of MI released from the commercial sachet (41.4 mg/day) compared with the release rate calculated in Chapter 2 under $25\pm1^{o}C$ (76.0 mg/day).

The results presented in Fig. 3.4 and 3.8 showed that for producing similar release rates in a greenhouse the closed system of the polyethylene bags is preferable. Much variation in the total amount of MI released between the individual dispensers was found for the commercial

sachet (LUREM-TR lures emptied of the contents) (231–346 mg) and LUREM-TR (399–687 mg); however, the traps baited with this dispenser outperformed all other dispensers used in regards to number of thrips caught on the traps, consistently catching more thrips than the control traps alone. Variation in released amount of MI is believed to be a problem created in part by problems with leakage of MI from the seals (W.J. de Kogel, pers. comm. to M-C Nielsen, 28 August 2013). Comparison of P150 and P50 dispensers in Experiment 1 and 2, showed that 60–70% more MI was released per day in Experiment 2. This is in part due to the increase in temperature (Section 3.2) and wind flow (M-C Nielsen, pers. observation 2012) in Experiment 2 but differences in sunshine are also believed to play an important role (see discussion above in regards to differences in daily sunshine between Experiment 1 and 2).

Thrips populations in flowers. Because of low numbers per flower recorded from daily flower scouting for adult thrips, no usable data in relation to the potential influence that kairomone baited traps might have on thrips numbers on adjacent plants was obtained. The low numbers of thrips found in flowers are possibly explained by sampling technique and sampling time choosen. Various methods are used to assess thrips density and results reported by Shipp and Zariffa (1991) and Hansen et al. (2003) showed that flower sampling gave the best correlation with regards to changes in WFT population in capsicum crops. Both Shipp and Zariffa (1991) and Hansen et al. (2003) removed the flowers and extracted any thrips residing in the flower. As the experiments in this chapter were conducted in commercial greenhouses removing or damaging the flowers was not an option and only a visual scouting of thrips numbers in the flowers *in situ* were done. This method would likely underestimate the number of thrips in the flowers. Also, WFT and other thrips species exhibit thigmotactic behaviour (Lewis 1973, Reitz 2009), protecting the thrips against unwelcome exposure and unfavourable conditions and hiding in tightly enclosed and concealed spaces of plants. Flowers were scouted just after midday in both experiments, and this may have resulted in thrips being hidden during peak daily temperatures. The sampling period was chosen based on the results of several studied recording peak densities of several thrips species in flowers during midday (Salguero Navas et al. 1991, Kiers et al. 2000, Kasina et al. 2006); however, all cited articles used a destructive flower sampling method to obtain the results.

Optimization of method. In addition to optimizing the flower scouting or using destructive flower sampling, several alterations to the methods used in this chapter would be beneficial to

obtaining more unambiguous results in regards to the dose response of thrips. Foremost, the actual amount of released MI during the period that the traps are set out for is needed instead of a calculated average based on total amount lost. In addition, the effect of direct sun on the temperature of the dispenser should be explored with, for example, an infrared thermometer. Second, to better understand the relationship between the MI dose released and the behavioural responses of thrips in greenhouse environments the influence of temperature on the results needs to be limited. This could be achieved by short trapping periods (e.g. 1–2 h). Thirdly, establishing the movement of the MI within the house and establishing levels of odour in the vicinity of traps will address the issue raised in the discussion above in regards to contamination. This issue can be addressed with the use of SPME fibres and has already been initiated (M-C. Nielsen and C. Sansom, unpubl. data).

Chapter 4

Olfactory responses of different western flower thrips (*Frankliniella occidentalis*) populations to a kairomone lure

4.1 Introduction

A substantial amount of research has been done on western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) since the early 1980s in relation to host preference, feeding mode, oviposition rate, development time, resistance to insecticide, spread and management (Terry and Schneider 1993, Bautista and Mau 1994, Gaum et al. 1994, Castane et al. 1996, Shipp et al. 1998, Kirk and Terry 2003, Chaisuekul and Riley 2005, Buitenhuis and Shipp 2006, Bielza et al. 2007, Zhang et al. 2007b, Bielza et al. 2008, Papadaki et al. 2008, Contreras et al. 2010, Nielsen et al. 2010, Herron and Langfield 2011, Zhang et al. 2011). Management of WFT is particularly difficult because of their small size, cryptic habits and their ability to develop resistance to insecticides (Lewis 1973, Lewis 1997a, Kirk 2001). A search for alternative management techniques has stimulated intensive investigations of the chemical basis of host plant–insect relationships, resulting in an increasing interest in the use of kairomones and other behaviour-modifying chemical kairomone in thrips pest management (Hamilton et al. 2005, Koschier 2006, 2008). By adding a kairomone lure, more effective thrips traps would be a useful tool for monitoring thrips at low population densities such as in early infestations or in eradication attempts, and to reduce thrips numbers via mass-trapping for control (Koschier 2006).

Methyl isonicotinate (MI) has been recognised as an effective lure of WFT (Davidson et al. 2007, Davidson et al. 2008). The response of WFT towards MI was initially evaluated in a Y-tube olfactometer using WFT females from a long established laboratory colony (Davidson et al. 2005), originating from an indoor capsicum crop. To date this has been the only population tested in laboratory bioassays under controlled conditions. Although increased trapping of WFT has been obtained in New Zealand greenhouses when using MI (Davidson et al. 2007), great variation in trap efficacy has been recorded (M.M. Davidson, unpubl. data;

M-C Nielsen, unpubl. data), and no validation of the Y-tube work has been attempted to confirm the initial results obtained by Davidson et al. (2005).

The Y-tube olfactometer method has been successfully used previously to discover and screen different kairomones in the search for effective lures for thrips (de Kogel et al. 1999, Koschier et al. 2000, Smits et al. 2000, de Kogel and Koschier 2001, Davidson et al. 2006). In addition to MI, *p*-anisaldehyde has also been tested against different thrips species as well as against different thrips populations of the same species, both in the field and in laboratory settings. The kairomone *p*-anisaldehyde is a common component of flower odours, and can significantly increase trap capture of many thrips species in greenhouse and open field situations (Kirk 1985, Teulon et al. 1993b) including WFT (Teulon et al. 1993a, Teulon et al. 1993b, Hollister et al. 1995, Roditakis and Lykouressis 1996). Y-tube olfactometer studies with *p*-anisaldehyde undertaken in different laboratories with different WFT populations show a consistent and robust result with 60–80% of the female WFT preferring the *p*-anisaldehyde laden arm compared to the clean arm (control) (Koschier et al. 2000, Smits et al. 2000, de Kogel and Koschier 2001, Davidson et al. 2006, Mainali and Lim 2011). The variations in response between the studies are believed to be mainly due to differences in the concentration and amount of *p*-anisaldehyde used, and in the choice of solvent. With the same concentration (10% w/v) and same dose (1 µl) of *p*-anisaldehyde, Koschier et al. (2000) and Davidson et al. (2006) recorded a positive response of 75% and 76%, respectively, for starved female WFT. However, despite the geographical differences between these two studies, both WFT populations were from long-term laboratory colonies, kept under similar rearing conditions and on a similar host plant (chrysanthemum). Differences in olfactory response may have occurred if these factors and/or other factors such as genotype, viruliferous or non-viruliferous status of the thrips populations or feeding history were different.

Between WFT populations, variation has been recorded with regard to genetic structure (Gillings et al. 1995, Brunner and Frey 2010, Rugman-Jones et al. 2010); morphology (Kirk 1997); reproduction (de Kogel et al. 1997); damage to plants and host adaptation (de Kogel et al. 1997); resistance to insecticides (Brødsgaard 1994); and their efficiency in transmitting tospoviruses (Sakimura 1962, van de Wetering et al. 1999b). In addition to these, a number of behaviour studies emphasise the occurrence of altered behaviour in mass-reared insects, specifically in relation to traits such as decreased dispersal (sedentary behaviour) (Boller

1972), decreased flight capacity (Remund et al. 1977) and decreased mate location and recognition (Dame et al. 1964, Fletcher et al. 1968, Gast 1968). Also pathogens such as viruses can have subtle but important effects on the behaviour and ecology of their hosts (Combes 1991).

In light of these intraspecific variations that can occur in populations of WFT from different locations, host plants and/or environmental conditions, variation in olfactory sensitivity and short distance behavioural responses to a kairomone lure such as MI may also differ depending on the population targeted. The aim of this chapter was to determine whether different WFT populations with different backgrounds, including feeding history, host plant and rearing conditions, have consistent responses to MI. In addition to those factors, the chapter explored whether differences in WFT genotypes have consistent responses to MI. Three of the populations tested belonged to the presumed 'glasshouse pest' genotype of WFT. This genotype of WFT is the worldwide pest WFT referred to in the literature above. However, to clearly distinguish the two genotypes studied in this chapter the "glasshouse pest" populations are referred to as WFT-G. In New Zealand, a putatively monophagous population of WFT can also be found outside restricted largely to the flowers of yellow tree lupins, *Lupinus arboreus* (Mound and Walker 1982). These WFT (in previous literature referred to as the "WFT lupin strain") are not considered to be a pest of crop plants either in the greenhouse or field , are considered to be morphologically identical to WFT-G (Mound and Walker 1982, Mound 2005a), are highly susceptible to pesticides (Martin and Workman 1994) and exhibit significant differences in some life history parameters (Nielsen et al. 2010). These factors and recent studies on the molecular range of variation within WFT (Brunner and Frey 2010, Rugman-Jones et al. 2010) strongly support that what is commonly considered a different strain of WFT is, in fact, closer to being a different species. Based on the paper of Rugman-Jones et al. (2010), WFT collected from lupin flowers for this experiment will be referred to as a separate cryptic non-pest species of WFT (WFT-L).

The objective of this chapter was to determine the response of three populations of WFT-G and one population of WFT-L with different backgrounds under controlled conditions to 1 µl of pure MI in a Y-tube olfactometer and validate the robustness of previous results obtained (Davidson et al. 2005, 2008). It was hypothesised that populations of WFT responded consistently to MI independently of their background and genotype and that the results would reflect previously published results on WFT response to MI in Y-tube olfactometer studies.

The results are discussed with respect to variability in olfactory perception and olfactory behaviour within a species and the influence of extrinsic and intrinsic factors.

4.2 Materials and Methods

4.2.1 Thrips populations

Table 4.1 describes the populations used in the experiments. Three of the populations belong to the presumed 'greenhouse pest' genotype of WFT (WFT-G) and one was the separate cryptic non-pest strain of WFT (WFT-L). The populations differed in respect to geographic origin, feeding history, rearing history, virus status and presumably genotype.

Table 4.1 *Classification and description of populations of western flower thrips used in the Y-tube olfactometer experiment.*

	Populations			
	Greenhouse WFT-G I	**Laboratory WFT-G II**	**Laboratory WFT-G III**	**WFT-L**
Collection location	Greenhouse, North Canterbury	Greenhouse, Auckland	Greenhouse, Auckland	Outside, Mid Canterbury
Original host plant	Capsicum, *Capsicum annuum*	Capsicum, *Capsicum annuum*	Capsicum, *Capsicum annuum*	Yellow tree lupin, *Lupinus arboreus*
Colony establishment date	NA	November 2001	November 2010	NA
Rearing methods	NA	Perspex cages 25±1°C 16:8 h light:dark	Environment cabinet 25±1°C 16:8 h light:dark	NA
Generations in the laboratory	0	> 222 generations	6 to 9 generations	0
Rearing host plant	NA	Chrysanthemum, *Dendranthema grandiflora* (cv. Onyx time yellow)	French Dwarf Bean, *Phaseolus vulgaris* (cv. Top Crop)	NA
Putative virus status of thrips populations	Unknown	Viruliferous	Non- viruliferous	Unknown
Putative genotype of thrips populations	Glasshouse pest genotype	Glasshouse pest genotype	Glasshouse pest genotype	Lupin non-pest genotype

'Greenhouse WFT-G I' was collected from a commercial indoor capsicum crop, *Capsicum annuum* (cv. 'Cupra') on the days the Y-tube bioassay were undertaken. The top part of several flowering plants was gently tapped over a white tray and female WFT were collected with an aspirator. The thrips were placed in a clean polyethylene tube for transport and taken to the laboratory where they were identified under a stereo microscope (> x100) using the keys of Mound and Walker (1982) to verify that they morphologically classified as WFT.

'Laboratory WFT-G II' were from a long established colony originally collected from a commercial greenhouse and maintained on potted flowering chrysanthemums, *Dendranthema grandiflora* (cv. 'Onyx time yellow') for over 10 years at The New Zealand Institute for Plant & Food Research Limited, Lincoln. The chrysanthemum plants and thrips have previously tested positive for tomato spotted wilt virus (TSWV). The plants used for the colony were held in two temperature-controlled perspex boxes (six plants per box) with the temperature maintained at 25 $\pm$ 1^{o}C under a 16:8 h light:dark cycle. A fresh chrysanthemum plant was placed in each box every 3 days and the oldest plant was removed at the same time.
'Laboratory WFT-G III', a non-viruliferus colony, was established from eggs laid by 'Laboratory WFT-G II' females and reared to adults on potted French dwarf beans, *Phaseolus vulgaris* (cv. 'Top Crop'). Only first instar thrips larvae acquire and transmit the tospovirus (van de Wetering et al. 1999a) and it is therefore assumed that a virus-free thrips colony could be established by rearing this colony from eggs despite the virus status of the female producing the egg. The eggs were obtained by allowing female Laboratory WFT-G II to deposit their eggs into an artificial parafilm membrane with sucrose water covering a rearing cage. The method was modified from that used by Murai and Ishii (1982) and Teulon (1992). The rearing cages were constructed from 30-ml polyethylene vials (Biolab Limited, New Zealand), from which both ends were removed (27 mm wide and 40 mm high) and subsequently covered by stretched parafilm (Pechiney Plastic Packaging, USA). At the top end of the cage, a second layer of stretched parafilm was added, and between the two layers, 0.05 ml of a 10% sucrose solution was deposited. The sucrose solution provided a nutrient medium for the adult thrips and an egg deposition site. Females were collected from the chrysanthemum colony using an aspirator and were placed in 10 individual rearing cages (10-20 individuals per cage) and allowed to oviposit into the nutrient solutions between the artificial membranes. The rearing cages were incubated at 25 $\pm$ 1^{o}C (60% RH, 16:8 h light:dark cycle) for a 24-h period in a controlled environment cabinet (Contherm Scientific Limited, New Zealand). After 24 h, the females were removed from the rearing cages, and the cages were placed inside a clear plastic box with a lid (Tellfresh, Australia). The eggs were immediately placed back in the controlled environment cabinet and observed every 24 $\pm$ 2 h. When the majority of the eggs reached the red-eye stage they were exposed, by removing the top membrane layer, to allow the larvae to hatch. Any excess sucrose solution on the membrane layer was absorbed with filter paper (Whatman™, UK) to prevent the larvae from drowning when they hatched. Newly emerged first-instar larvae were transferred onto the bean plants by moving the parafilm with the instars onto one of the plants. The process was

repeated every week for 5 weeks to ensure that a colony could establish. The bean plants used for the colony were grown in a controlled environment cabinet (Conviron E15, Controlled Environment Limited, Canada) (30–40 plants at various ages) with a temperature maintained at 25 $\pm$ 1^{o}C, RH 70% and a 16:8 h light:dark cycle. Fresh beans were planted weekly in the controlled environment cabinet to ensure a steady supply of healthy plants and old plants were removed when severely wilted. A tobacco plant was placed in the cabinet to detect the presence of tospoviruses in the thrips colony and extra care was given to not accidently transfer thrips from other colonies onto the beans.

'WFT-L' were collected from yellow tree lupins (*Lupinus arboreus*) growing in a vacant lot (43^{o} 33'S, 172^{o} 31'E) on the days the Y-tube bioassay were undertaken. The top part of several flowering plants was gently tapped over a white tray and female WFT-L were collected with an aspirator. The thrips were placed in a clean polyethylene tube for transport and taken to the laboratory where they were identified under a stereo microscope (>x100) using the key of Mound and Walker (1982) and Mortz et al. (2004) to verify that they morphologically classify as WFT.

4.2.2 Molecular tests to establish thrips genotypes

To determine the genetic variation, genotype and the alignment of all WFT-G populations and WFT-L used with respect to each other as well as other global WFT populations, total genomic DNA was extracted from individual thrips (approximately 5% of the total number of thrips used of each of the four populations in the assay and re-collected after the assay (see Chapter 4.2.4)) using a *prep*GEM insect kit (Zygem Ltd, New Zealand). It was impractical in terms of time and expense to test every individual thrips used in the experiment and therefore only a subset was tested. The manufacturer's protocol was followed for the DNA extraction of the thrips. In preparation for the DNA extraction, each thrips (whole individual) was crushed slightly with a clean pipette tip in PCR tube, as suggested by the manufacturer's protocol due to the small size of thrips. A region of the COI gene (Cytochrome oxidase sub unit 1) was amplified (433 bp amplicon) using mtD-7.2F and mtD-9.2R primers obtained from Brunner et al. (2002). Each PCR reaction contained 3.0 µl gDNA extracted for a individual thrips, 2.5 µl 10X Reddymix PCR buffer (Thermo Scientific, USA), 2.0 µl magnesium chloride 25 mM (Thermo Scientific, USA), 1.0 µl dNTP mix 5 µmol (Thermo Scientific, USA), 1.0 µl of each primer (5 µM concentration), 14.3 µl ddH_2O and 0.2 µl ThermoPrime *Taq* DNA polymerase 5 U/µl (Thermo Scientific, USA); the total reaction volume was 25 µl. Each PCR included appropriate negative (no template) controls. PCR

conditions were: one cycle at 96°C for 15 min, two cycles at 96°C for 30 s, 70°C for 30 s and 72° C for 1 min (repeated five times with the annealing temperature dropping 5°C with each repeat from 70°C to 40°C), 30 cycles at 96°C for 30 s, 40°C for 30 s and 72°C for 1 min, and one cycle at 72°C for 7 min. Each PCR was performed on a Corbett Research Palm-Cycler (CG1-96) and the PCR products were visualised on 1.5% SB buffer agarose gels. The PCR products were purified (Gel Extraction/PCR Clean-up Kit, Dnature Ltd, New Zealand) before being sent for DNA sequence analysis (Sequencing Facility, Lincoln University, New Zealand). Reference WFT specimens were deposited at Plant & Food Research, Lincoln, New Zealand.

4.2.3 RNA isolation and RT-PCR to confirm virus status of thrips

TSWV status of the Laboratory WFT-G II and Laboratory WFT-G III populations were determined using qRTPCR. RNA was extracted from individual thrips recollected after the Y-tube olfactometer assay (see Chapter 4.2.4) using the RNeasy Plus Mini Kit (Qiagen Inc., USA). Approximately 5% of the total number of thrips used for the assay of the putative virus-infected Laboratory WFT-G II (n = 8) and virus-free Laboratory WFT-G III colonies (n = 8) were tested. It was impractical in terms of time and expenses to test every individual thrips used in the experiment and therefore only a subset was tested. The manufacturer's protocol was followed for the RNA extraction, except the RNA was eluted into 20 µl of RNase-free water. RNA extracts were stored at -80^{o}C until examined. To design an internal control for amplification of thrips RNA, a fragment of the thrips elongation factor 1α (EF1α) gene was amplified and sequenced with the primers InsEF1F (TGGGTAAGGARAAGAYT-CAYATTAAC) and InsEF1R (CATGCAATGTGRGCNGTGTG) that were designed from conserved regions of an alignment of insect EF1α genes. A primer pair ThripsEF_1F/1R (ACGTGGTATCACCATCGACA/TTGTTCACACCAACAATCAGC) for qPCR was designed from the thrips using the Primer3 computer program. The primer pair for TSWV, TSW.1 and TSW.R (Roberts et al. 2000) were chosen. cDNA was synthesised using the qScript™ cDNA synthesis kit (Quanta Biosciences™, USA), with 5 µl RNA for thrips in a final volume of 10 µl, as per the manufacturer's instructions. cDNA was diluted 10-fold and 2 µl was used for each 20 µl reaction containing 10 µl iTaq SYBR Green Supermix with ROX (Bio-Rad Laboratories Inc., USA) with forward and reverse primers at a final concentration of 500 nM. The assay was carried out in a StepOnePlus™ Real-Time PCR System (Applied Biosystems, New Zealand), for 40 cycles at 95^{o}C for 15 s, 59^{o}C for 20 s, 72^{o}C for 20 s, with an initial activation step of 95^{o}C for 2 min 30 s. The ratios of virus (titer)

harbored in the individual thrips tested were calculated and visualized using StepOne Software v2.2.2 (Applied Biosystems).

4.2.4 Y-tube olfactometer assay

The response of walking female WFT to MI (98% purity) (Sigma Aldrich, New Zealand) was evaluated using a glass Y-tube olfactometer, following the method described by Koschier et al. (2000) and Davidson et al. (2006). The Y-tube was comprised of two branching arms at an angle of approximately 45^{o} leading from a single tube. Each section of the arms and the tube were 60 mm long and had an internal diameter of 5 mm. The 5-mm inner diameter of the Y-tube prevented thrips from flying within the tube. The arms at the top of the Y-tube were connected to glass adapters that attached to 4 ml glass vials each containing a 0.75 cm^2 piece of filter paper. The single stem (bottom of y-tube) was connected to tubing enabling the airflow (see below). The Y-tube was placed in a grey box (to prevent external visual stimuli influencing thrips' behaviour), located in a darkened, air-conditioned room under constant light intensity, temperature ($25 \pm 1^{o}C$) and relative ambient humidity (35–45%). The Y-tube was positioned at an inclining angle (25°) and illuminated from above by a halogen lamp (780 lux). One microlitre of MI was applied to the filter paper in one vial, while a control (1 µl of hexane) was applied to filter paper held in the second vial. Previous assays have shown that hexane does not elicit a response from female WFT in the Y-tube (Davidson et al. 2008). Air was drawn through activated charcoal using a suction pump producing airflow before entering the Y-tube. Airflow was 5 cm/s through each arm and 10 cm/s at the base of the Y-tube. Clean air was drawn through the Y-tube for 30 min before introducing the first thrips at the beginning of each bioassay to allow the hexane to fully evaporate. Female thrips of unknown age were collected for each of the four populations using an aspirator and held in perspex ring cages (8 cm diameter, 5 cm high) (Murai 1990) at $25 \pm 1^{o}C$ with access to water only for a minimum of 4 h prior to introducing them into the Y-tube olfactometer. Water was made available between two top layers of stretched parafilm covering the perspex ring cages. Six replicates each comprising 25 thrips making a choice (i.e. thrips that walked up the stem of the Y-tube and reached the end of either of the respective arms) of the four WFT populations were tested. All four populations were tested in a single day in four sequential sets and one replicate was run each day over six sequential days. A row-column design was used to randomize and balance the order of the thrips populations within a day using CycDesign (CycSoftware 2009). An individual female thrips was released into the base stem of the Y-tube using a small aspirator and the tubing reconnected. The thrips walked up the

stem of the Y-tube and when they reached the end of the respective arm their choice and the time it took was recorded. Thrips that failed to make a choice within 3 min were recorded as "no choice" and replaced with a new individual to ensure that a total of 25 thrips per replicate made a choice. Thrips that made a choice were re-captured and transferred to a clean 1.5 ml eppendorf tube and stored at -80^{o}C for future molecular analyses (see Chapters 4.2.2 and 4.2.3) or for reference collection. After every five thrips the Y-tube and apparatus were rotated 180^{o} to avoid position effects and the vial with MI was replaced to ensure a uniform concentration level of the volatiles for all thrips tested throughout the sets. After each run of 25 thrips, all parts of the Y-tube were cleaned with hexane and allowed to dry.

4.2.5 Data analysis

Molecular analysis. The DNA sequences were edited and aligned using Geneious Pro software (Drummond et al. 2010) and compared with a reference database constructed from sequences obtained from GenBank (www.ncbi.nlm.nih.gov) for WFT.

Y-tube olfactometer assay. The percentage of thrips choosing the MI laden arm was analysed using a Bernoulli generalized linear model (McCullagh and Nelder 1989) with a logit link function. The choice of each individual thrips was analysed. Comparisons were made between the thrips populations and the various other factors (i.e. variation in the starvation time due to sequential testing, odour side and odour position in the Y-tube and the interactions between these and thrips populations) within the analysis of deviance done as part of the analysis using Chi square tests. Percentages of thrips choosing the arm with MI and associated 95% confidence limits were obtained on the transformed (logit) scale, and back-transformed to percentages. The times taken to make a choice for each individual thrips were analysed using analysis of variance. All analyses were carried out with GenStat (GenStat Committee 2010) and graphics were produced using SigmaPlot v.10.

4.3 Results

Molecular analysis. Alignments of a fragment of the COI gene (445–496 bp) from the individual thrips tested revealed two distinct genotypes. The result confirmed that WFT-G I, II, and III all cluster into one group and aligned with other WFT 'glasshouse pest' populations from around the world. WFT-L individuals were discriminated by a 14-bp substitution separating them into a separate cryptic non-pest species of WFT (for sequence alignment example results see Appendix VII). For the thrips qPCR amplification with

primers of the EF1a gene confirmed that consistent amounts of amplifiable RNA were obtained from all thrips examined and qPCR from these samples showed that all putative virus-free thrips (Laboratory WFT-G III) were negative for TSWV. However, qPCR failed to show any detectable levels of TSWV in all putative virus-infected thrips (Laboratory WFT-G II) (n = 8), rendering them negative for TSWV. Re-testing a further 5% of the total number of Laboratory WFT-G II thrips used for the assay (n = 8) yielded the same negative result. Subsequent TSWV titer tests (Agdia ImmunoStrip® (Agdia, USA)) of leaf samples collected from the chrysanthemum host plants used to rear the putative virus-infected thrips showed only a very weak response to being infected with TSWV, indicating that TSWV was present in the plants but only at a very low level (see Appendix VIII).

***Y-tube olfactometer assay*.** No bias was found between the percentage of thrips choosing the MI laden arm and the order of the thrips populations tested during a repetition or position (left or right side) of the MI laden arm ($p > 0.1$ for both interactions) (Table 4.2). The average time taken for thrips to make a choice (Table 4.2) was significantly different among the thrips populations ($p < 0.001$). On average, the WFT-L population was faster to choose than the other populations ($p < 0.05$), followed by the Greenhouse WFT-G I population, which was quicker than the other two populations ($p < 0.05$), which took a similar time to choose ($p = 0.507$). There was no evidence that time taken influenced the final choice or was influenced by the order the thrips populations were tested in during a repetition ($p > 0.1$).

Table 4.2 *Mean percentage of western flower thrips (WFT) choosing the arm with 1 µl methyl isonicotinate (MI) vs. clean air in a Y-tube olfactometer (95% confidence limits) within 3 min. Values presented are for thrips responding with the MI laden arm on different sides of the Y-tube (right and left), mean time taken to choose, total number of thrips making a choice (< 3 min) and total number of thrips not choosing (> 3 min). Populations tested were Greenhouse WFT-G I, Laboratory WFT-G II, Laboratory WFT-G III and WFT-L.*

	% thrips choosing MI laden arm on the right (95% cl)	% thrips choosing MI laden arm on the left (95% cl)	Mean time (sec) to choose MI or control laden arm (se, df = 15)	Total number making a choice(n)	Total number not choosing(n)
Greenhouse WFT-G I	65.7 (54.0,75.8)	61.2 (50.2,71.2)	33.64 (2.14)	150	26
Laboratory WFT-G II	60.0 (48.6,70.4)	62.7 (51.3,72.8)	40.40 (2.14)	150	29
Laboratory WFT-GIII	58.6 (46.8,69.4)	73.6 (62.3,82.5)	42.49 (2.20)	142*	14
WFT-L	73.8 (61.9,83.1)	72.6 (61.3,81.6)	25.88 (2.23)	138*	1

* *Sample size (n total) was reduced to fewer than 150 thrips as several individuals escaped from the cages during replicate 1 (WFT-L) and replicate 6 (Laboratory WFT-G III).*

The total percentage of adult female thrips choosing the MI laden arm was similar for the Greenhouse WFT-G I and Laboratory WFT-G II populations, with slightly but not significantly more Laboratory WFT-G III thrips choosing MI ($p = 0.686$) (Fig. 4.1). However, more WFT-L chose MI (73%) than the Greenhouse WFT-G I ($p = 0.203$) and Laboratory WFT-G III ($p = 0.072$) but the difference was only significantly greater than the Laboratory WFT-G II thrips ($p = 0.033$).

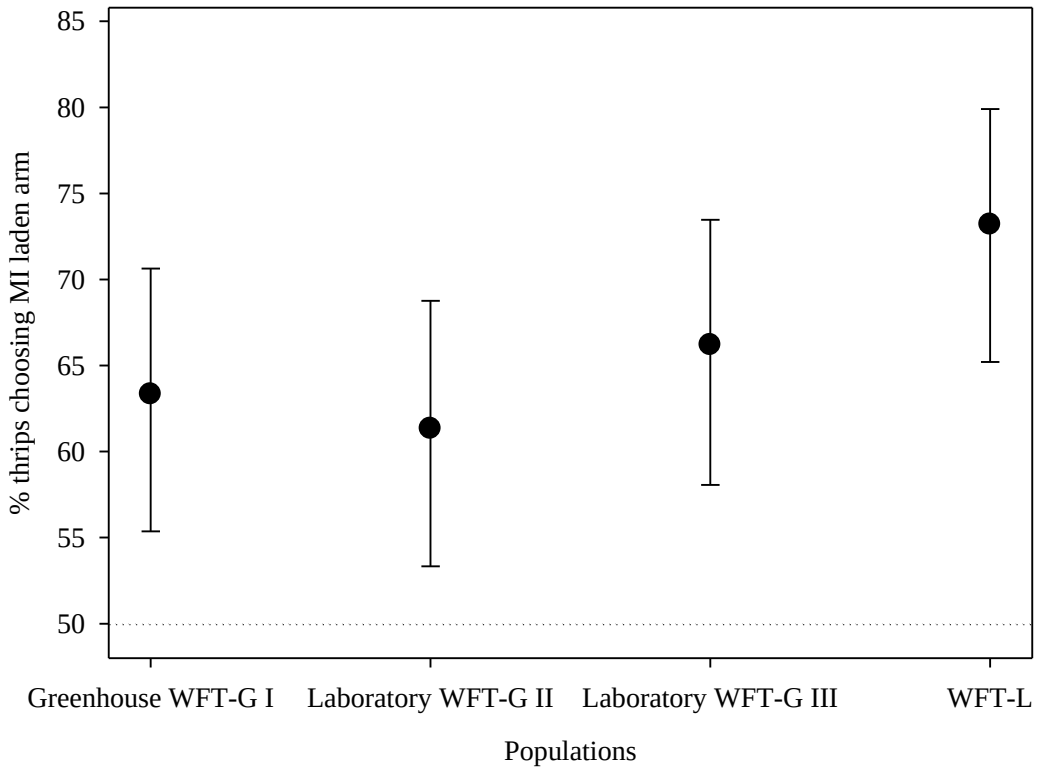


Figure 4.1 *Mean percentage of WFT choosing the arm with 1 µl MI vs. clean air in a Y-tube olfactometer (95% confidence limits). Populations tested were Greenhouse WFT-G I (n = 150), Laboratory WFT-G II (n = 150), Laboratory WFT-G III (n = 142) and WFT-L (n = 138).*

The average time taken for thrips to make a choice (Table 4.2) was significantly different among the thrips populations ($p < 0.001$). On average, the WFT-L population was faster to choose than the other populations ($p < 0.05$), followed by the Greenhouse WFT-G I population, which was quicker than the other two WFT-G populations ($p < 0.05$), which took a similar time to choose ($p = 0.507$). There was no evidence that time taken influenced the final choice or was influenced by the order the thrips populations were tested during a repetition ($p > 0.1$).

4.4 Discussion

The results of the experiment supported the hypothesis that WFT responded consistently to MI independently of their background and genotype. Significantly more female thrips from all populations, irrespective of their backgrounds, chose the MI laden arm. There were generally only non-significant differences between the different thrips populations. However, significant differences were found between the populations in regards to the average time taken for thrips to make a choice. WFT-G I, II and III populations responded in a consistent manner with 61.3–66.2% of thrips preferring the MI laden arm compared with the clean arm; the time to choose a particular arm was between 33 and 43 s. Although consistent, the percentage of thrips preferring the MI laden arm was lower than what was previously reported in Davidson et al. (2008) where over 70% of WFT tested preferred the MI laden arm over the clean arm. The lower percentage found in this experiment was believed to be due to a MI residue contamination in the clean arm. The lower percentage found in this experiment was believed to be the result of MI residue contamination in the clean arm. Contamination occurred when the Y-tube and apparatus were rotated incorrectly. Because the clean arm was potentially continuously contaminated with MI, the result was a consistent error throughout the bioassay experiment that would to some degree be obscuring the effect difference found between the MI laden arm and the clean air arm.

For populations of WFT from the Netherlands (Koschier et al. 2000, Smits et al. 2000, de Kogel and Koschier 2001), New Zealand (Davidson et al. 2006) and Korea (Mainali and Lim 2011) 60–80% of the female WFT preferred the *p*-anisaldehyde laden arm over the clean arm over a range of doses (1 µl (undiluted to 0.001% dilutions) and 10 µl undiluted *p*-anisaldehyde). Field trapping of WFT to MI may also reflect the consistent response of WFT to MI as found in the Y-tube olfactometer. Different WFT (glasshouse pest species) populations show similar responses to MI across a wide range of different cropping systems such as beans (Niassy et al. 2012), capsicum (Davidson et al. 2007, de Kogel and Teulon 2007), fallow field , fruit trees (Broughton and Harrison 2012) and roses (S. Broughton, unpubl. data). The variation in attractiveness that occurs both within and between these field and greenhouse trials is believed to be in part linked to extrinsic factors (this will be examined further in Chapter 5).

MI has so far not been found in host plants and therefore presumably has not been experienced previously by any of the populations tested, though MI is structurally similar to benzene carbonyl derivatives that are commonly found in flower scents (Davidson et al. 2008). However, a number of thrips kairomones are plant-derived (Koschier 2006). Using a plant-derived kairomone may alter the behaviour and responses as insect behaviours are modifiable through experience (Boller 1972). Wang et al. (2008) showed that experience can increase positive responses towards non-host plant extracts in diamondback moth (*Plutella xylostella*) females. Naive females showed aversion to the odours of the non-host plant whereas experienced females were no longer repelled or even became attracted by these non-host odours. Using plant-derived kairomone lures may change the influence that different feeding histories have on the response by different populations depending on previous experience with the kairomone.

Under laboratory rearing conditions, selection pressure, genetic drift and inbreeding may cause genetic changes in an insect population (Hopper et al. 1993). Also changes in the olfactory receptor neurons (ORNs) (Olsson et al. 2006) or a degradation of sensory sensitivity (de Weerdt and Kelling 2001) may be manifest in the population. These could cause variations in the olfactory responses to odours by laboratory reared populations and their 'wild' counterparts in greenhouses and fields. The results of this experiment did not support that any such changes had manifested in the laboratory populations. Although the thrips from the long-established laboratory colony reared on chrysanthemum for over 200 generations (WFT-G II) showed the lowest response to MI, this difference was not significantly different from the Greenhouse WFT-G I population. Maeda and Hinomoto (2006) demonstrated in a Y-tube olfactometer experiment that laboratory rearing over a 12-month period of the predatory mite *Neoseiulus womersleyi* did not induce genetic changes in the olfactory response of the mite to plant volatiles, and suggested that the selection pressure and limited time scale was too small to cause detectable changes. With some individuals of WFT (glasshouse pest species) occasionally added to the long established WFT-G II colonies over the last decade (both intentional from greenhouse populations and likely unintentional via the plant material) genetic decay or changes, if caused by long-term rearing, may not have manifested in the colony.

No detectable TSWV was present in the putative virus-infected thrips population (Laboratory WFT-G II) and therefore no information can be drawn directly from the results in this

experiment with regard to the influence TSVW may have on the olfactory response of vector thrips to an odour. However, Davidson et al. (2012b) found that the presence TSWV in WFT (greenhouse pest species) populations tested in the y-tube olfactometer against MI did not substantially affect the behavioural response. Nevertheless, numerous studies have shown differences in behaviour between virus-infected and non-infected insects (Eigenbrode et al. 2002, Fereres and Moreno 2009, Ingwell et al. 2012), and the potential influence a virus infection may have cannot be dismissed solely upon the results found in Davidson et al. (2012b). Changes in insect biology have been well documented in several studies with respect to the presence or absence of a plant pathogen within vector insects. Ingwell et al. (2012) provided the first experimental evidence that acquisition of a plant virus by an insect vector through *in vitro* feeding directly alters the host plant selection behaviour of the vector. They showed that the bird cherry oat aphid, *Rhopalosiphum padi*, after acquiring barley yellow dwarf virus (BYDV) during *in vitro* feeding preferred non-infected wheat plants, while non-infective aphids also fed *in vitro* preferred BYDV-infected plants. Studies have shown that differences in transmission of TSWV between WFT (glasshouse pest species) populations occur (van de Wetering et al. 1999a, Tavella et al. 2001) and Jacobsen et al. (2013) showed that transmission of TSWV by *Thrips tabaci* was positively correlated with the temperature at which the thrips were kept. These factors combined with potentially altered host selection behaviour as found in viruliferous bird cherry oat aphids could to some degree result in variation in attractiveness of any lure used in the field but this still requires experimental validation.

Compared with the WFT-G populations, WFT-L showed a stronger (73.2% choosing the MI laden arm compared to 58.6-65.7%) and faster (mean 25.88 s compared with 33.64-42.49 s) response, although these results were only significantly different between WFT-L and the WFT-G II population. This result indicates that genetic differences may be implicated in the olfactory sensitivity and behaviour of the insect. The result is similar to previous findings in studies on *D. melanogaster* that have thrown light on the genetic mechanism of olfaction. Work undertaken by Kikuchi (1973), Fuyama (1976) and Ayyub (1990) suggest that a considerable amount of genetic variation for an olfactory response exists in natural populations of *D. melanogaster*. In addition, Gailey et al. (1986) showed that olfactory deficient females of *D. melanogaster* changed their behaviour and became behaviourally sterile as they did not "participate" in the courtship with the males. Clearly, the difference in response between WFT-G II and WFT-L populations towards MI needs to be considered in

light of the differences in the basic biology of these populations (See Chapter 4.1). WFT (glasshouse pest species) populations from New Zealand, including the three populations used in the experiments, belong mainly to one of the largest haplotype groups (haplotype A) along with WFT (glasshouse pest species) from many other countries in northern Europe, Asia, North America, and Australia (Rugman-Jones et al. 2010). The influence different haplotypes of WFT may have on the variation in response to MI is further studied and discussed in Chapter 5.

Chapter 5

Variation in response of western flower thrips (*Frankliniella occidentalis*) populations from different geographic locations to the thrips lure, methyl isonicotinate

5.1 Introduction

Several thrips species worldwide have been shown to be attracted to a range of kairomones (Penman et al. 1982, Kirk 1987, Teulon et al. 1993b, Murai et al. 2000, Imai et al. 2001, Teulon et al. 2007a, Davidson et al. 2009, El-Sayed et al. 2009). Adding a kairomone lure to a coloured trap can make a trap more effective in attracting insects. Methyl isonicotinate (MI) has been recognised as an effective lure of western flower thrips (WFT), increasing the number of thrips caught on MI baited traps compared with unbaited coloured traps in greenhouse studies in New Zealand (Davidson et al. 2007), The Netherlands (de Kogel and Teulon 2007, R.W.H.M. van Tol, unpubl. data) and China (Liang et al. 2010) as well as in outdoor field studies in California (M-C. Nielsen, unpubl. data.), Australia (Broughton and Harrison 2012) and Kenya (Muvea 2011, Niassy et al. 2012).

However, large variations in MI increased trapping efficacy of WFT within trial areas, between seasons and between locations have been recorded. A particular example is that all greenhouse experiments undertaken in southeast Spain have failed to achieve any significant increases in the number of WFT caught on traps baited with MI compared with unbaited traps (M-C Nielsen, unpubl. data). Behavioural characteristics have been found to vary among geographical populations of the same species (Foster and Endler 1999) and variation between insect populations has also been found with respect to other chemical interactions. Indeed, different WFT populations have been found to vary in insecticide resistance (Immaraju et al. 1992, Brødsgaard 1994) and in their response to plant resistance (de Kogel et al. 1997). Insect behaviours are very sensitive to environmental variation, thus resulting in a wide range of behavioural phenotypes to be expressed by genetically identical individuals (Anholt and Mackay 2004). Insects, such as WFT, that occur over a broad geographic range are expected to experience differential selection pressures on both the physiological (survival) and behavioural (host selection) components of foraging (Pannebakker et al. 2008). Although the behavioural response of thrips that leads to increased trap capture is not well understood

(Teulon et al. 1993b, Koschier 2008, Teulon et al. 2008a) it is thought to vary in response to biotic and/or abiotic factors (Teulon et al. 2008a).

A Y-tube laboratory assay carried out by Davidson et al. (2005) identified MI as an effective kairomone lure for WFT, but the assay was limited by being performed on a single long established laboratory population from New Zealand (Davidson et al. 2008). However, despite this results in Chapter 4 showed that different WFT populations responded to MI in a consistent manner irrespective of their background including differences in the age of laboratory culture and feeding history. These results in combination with the greenhouse and field experiments cited above confirm that the response of WFT to MI has been relatively consistent regardless of any difference in the background of the WFT population with respect to feeding history, host plant, rearing conditions and genotype and, to some extent, between different geographic locations. The notable exception is thrips populations from southern Spain. Furthermore, previous studies on WFT response to the thrips attractant *p*-anisaldehyde have shown that thrips behaviour in a Y-tube olfactometer has also been consistent between different laboratories and widely separated thrips populations in the Netherlands (Koschier et al. 2000) and New Zealand (Davidson et al. 2008).

Given the apparent non-responsiveness of southern Spanish WFT to MI, in contrast to WFT from other countries, the objective of this chapter was to investigate these differences. To investigate whether intrinsic factors were involved and whether these differ among populations of WFT, an experiment was designed where extrinsic factors were controlled as much as possible. This was done by rearing WFT populations collected from different geographical locations in New Zealand, The Netherlands, Spain, Kenya and France for several generations under standardised conditions and carrying out Y-tube olfactometer assays under controlled laboratory conditions. The walking responses of the WFT populations were tested using a single dose of MI. The working hypothesis was that the response to MI by WFT from different geographical locations would be similar when extrinsic factors were controlled and if behavioural differences were found, then they would be accounted for by genetic variation among populations. Genetic variation among populations was explored using nuclear and mitochondrial barcoding to compare the thrips populations to establish their position within the global distribution network published by Rugman-Jones et al. (2010) that represents a wide range of the genetic variation among global WFT genotypes.

5.2 Methods and Materials

The experiments were carried out over two summer seasons (July/August 2011 and 2012) at Plant Research International, Wageningen, The Netherlands.

Western flower thrips (WFT). Populations of WFT were sourced from New Zealand, The Netherlands, northeast and southeast Spain, Kenya and France (Table 5.1). All populations were collected from capsicum (*Capsicum annuum* L.) crops, except WFT from Kenya which was sourced from a laboratory colony reared on beans (*Phaseolus vulgaris* L.) at the Mass Rearing Unit at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya.

After collection from their host-plants in their original location, the WFT populations were placed in sealed rearing containers with bean pods (*P. vulgaris* L.) (see full description below) and couriered to Plant Research International, The Netherlands. Upon arrival to the laboratory a subsample from each thrips population was put into ethanol and identified under a stereo microscope (<x100) using the key of Moritz et al. (2004) to verify that they were WFT. All remaining WFT from each population were maintained on green bean (*Phaseolus vulgaris* L.) pods in the laboratory and reared at 25^{o}C, 16L:8D, 60–70% RH in separate (with one exception, see below) controlled environmental chambers (Elbanton, The Netherlands). Extra care was taken during rearing and handling to avoid any cross-contamination of the populations (see below). Because the number of controlled environmental chambers was limited in 2012, two of the Spanish WFT populations (SV and SVI) were kept in the same chamber. To avoid cross-contamination of these two populations within the chamber, the rearing jars were placed inside large sealed clear plastic boxes with lids. In the lid, an opening, approximately 25x13 cm, was cut and the hole covered with thrips proof mesh (80 µm) for ventilation.

When reared on beans at a constant 25^{o}C, the life cycle (egg to egg) of WFT is expected to be approximately 14 days (Gerin et al. 1994, van Rijn et al. 1995, McDonald et al. 1998). Table 5.2 shows the estimated number of generations that would have developed under the rearing conditions described for the different populations

Table 5.1 *Source of western flower thrips populations for Y-tube experiments in 2011 (a) and 2012 (b).*

(a) 2011

Population	Abbr.	Location	Crop/Host plant	Cultivar	Date collected
New Zealand	NZ	Harbor Head Growers Canterbury, New Zealand	Indoor Capsicums	Cupra	09 May 2011
Netherlands I	NLI	Nergena Bennekom, The Netherlands	Indoor Capsicums	Mixed cultivars	09 May 2011
Spanish I	SI	El Algarrobo Almeria, Spain (southeast)	Indoor Capsicums	Mixed cultivars	23 May 2011
Spanish II	SII	Balsa Plata-Nejite C.P. Berja, Almería, Spain (southeast)	Indoor Capsicums	Velero	08 Aug 2011
Spanish III	SIII	Vilassar de Mar Barcelona, Spain (northeast)	Outdoor Capsicums	Amando	12 July 2011

(b) 2012

Population	Abbr.	Location	Crop/Host plant	Cultivar	Date collected
Netherlands II	NLII	PRI Wageningen, The Netherlands	Indoor Capsicums	Mixed cultivars	15 Mar 2012
Spanish IV	SIV	Paraje el Algarrobo El Ejido, Almeria, Spain (southeast)	Indoor Capsicums	Mixed cultivars	20 Apr 2012
Spanish V	SV	Rincón de Mollano Berja, Almería, Spain (southeast)	Indoor Capsicums	Velero	20 Apr 2012
Spanish VI	SVI	Rumeria Balerma, Almeria, Spain (southeast)	Indoor Capsicums	Brito	12 Jul 2012
Kenya	KE	ICIPE Nairobi, Kenya	Laboratory colony French beans	Samantha	12 Mar 2012
France	FR	Route Branquay Eygalieres, France	Indoor Capsicums	Almuden	09 Jul 2012

.

Table 5.2 *Estimated number of generations reared under laboratory conditions (25°C, 16L:8D, 60–70% RH) on beans* (*Phaseolus vulgaris L.*) *for different western flower thrips populations* .

Population	Abbr.	Estimated number of generations in laboratory
New Zealand	NZ	6–7 generations
Netherlands I	NLI	6–7 generations
Netherlands II	NLII	8–9 generations
Spanish I	SI	5–6 generations
Spanish II	SII	5–6 generations
Spanish III	SIII	1–2 generations
Spanish IV	SIV	2–3 generations
Spanish V	SV	5–6 generations
Spanish VI	SVI	5–6 generations
Kenya	KE	8–9 generations
France	FR	1–2 generations

Rearing protocol. The bean pods were purchased fresh weekly, or as needed, from a local grocery shop. They were washed in warm soapy water, rinsed clean and incubated for 72 h at 22–25°C. After incubation the beans were re-washed and rinsed prior to being used for thrips rearing to ensure that if any thrips eggs were present in the beans they would have hatched and been removed. The clean bean pods were stored in a ventilated plastic container in a fridge at 5°C prior to use for thrips rearing. Glass preserving jars (0.5 L) (Leifheit, Germany) served as rearing containers. In the jar lid, a 2–4 cm in diameter opening was cut and covered with thrips proof mesh (80 µm) for ventilation. The bottoms of the jars were lined with a 0.5–1 cm deep layer of cut hand-towel paper pieces to provide a pupation site for the thrips. Clean bean pods (2–3 per jar), for food and for oviposition sites, were placed on the paper and several hundred adult female thrips were added to the jar. After 3 days, adult thrips were gently removed from the bean pods and the pods were transferred into new rearing jars, where the development from eggs laid in the pods to adult was completed. New bean pods were placed back in the original jar with adults for 3 days of further egg laying. This process was repeated every third day to build up each thrips population. When a target number of thrips for each population was reached (several hundred adults in each of 5–8 jars per population) the jars were checked every 48 h and bean pods were added and removed as needed to sustain the colonies. Throughout the rearing process all jars were placed in a large grey plastic tray inside the separate rearing chambers and the tray was covered with a white sheet of paper to protect the thrips from direct light and ensure high humidity around the jars. To avoid cross-contamination between populations only one population was used at a time and all equipment was thoroughly washed after each use. When handling the thrips

populations, jars were placed on a white tabletop so that any thrips that accidentally escaped could easily be seen and killed. The tabletop was wiped with 70% ethanol after use and a minimum period of 2 h was allowed to pass before handling another population. Before disposing used bean pods, they were placed in a tray in the freezer (-20^{o}C) for >72h to kill any thrips that may have be left on or in the pods.

Y-tube olfactometer. The response of walking female WFT to MI was evaluated using a glass Y-tube olfactometer. A full description of the olfactometer is given in Chapter 4, Section 4.2.4. Some variation between methods and materials did occur due to the assay being carried out in different laboratories. The Y-tube was positioned in a wooden box painted solid black on top a solid platform at an acute angle of 25^{o} and covered with black cloth. The assays were carried out under a constant temperature of 22 $\pm$ 1^{o}C. Light coming from a halogen lamp attached to the ceiling of the box illuminated the Y junction of the Y-tube with about 160 lux light intensity. One microliter of pure MI was applied to the filter paper in one vial (treatment vial), while the other vial contained clean filter paper (control vial). Connections between different parts of the apparatus consisted of silicone tubing. The mixture of MI loaded and clean air from the base of the Y-tube was removed from the room by silicon tubing running from the Y-tube to the neighbouring hallway through a small hole in the wall.

***Bioassay procedure*.** A full description of the assay is given in Chapter 4, Section 4.2.4. The adult female WFT were starved by providing water only at room temperature for a minimum of 14 h prior to introducing the thrips into the Y-tube olfactometer. The thrips used for the assay were re-captured from the Y-tube and stored in 96% ethanol for molecular analysis or for a reference collection. Three to six replicates (each comprising 25 thrips making a choice) of the five (2011) or six (2012) WFT populations were tested. All populations were tested in a single day in five (2011) or six (2012) sequential sets and one replicate was run each day. A row-column design was used to randomize and balance the order of the thrips populations within a day using CycDesign (CycSoftware 2009).

5.2.1 Molecular analyses to establish thrips genotypes

The genotypes of a selection of WFT re-captured from the Y-tube experiments were assessed using nuclear-mitochondrial barcoding to compare DNA sequences of nuclear and mitochondrial genes between thrips populations and their cluster position with respect to the genetic variation of a range of global WFT genotypes (see Chapter 1 and Rugman-Jones et al.

2010). Individual thrips, which had been stored in 96% ethanol in Eppendorf tubes, were air-dried on filter paper for 2 min. Rather than grinding the individual thrips specimen, the tip of a sterile pipette was used to lightly damage its abdomen. This was to ensure adequate DNA extraction while trying to keep the specimen intact to serve as a reference after DNA extraction was completed. Genomic DNA was extracted from individual thrips using a prepGEM insect kit (Zygem Ltd, New Zealand). It was impractical in terms of time and cost to test every individual thrips used in the experiment and therefore only a randomly selected subset of thrips were tested (approximately 5% of the total number of thrips recollected from the Y-tube olfactometer assay for each population) (n=5–8). The manufacturer's protocol was followed for the extraction (Zygem Ltd, New Zealand). Polymerase chain reaction (PCR) was used to amplify 456 bp of the D2 domain of 28S (28SD2) nuclear ribosomal DNA (rDNA) and 571 bp of the mitochondrial gene (mtDNA) cytochrome oxidase c subunit one (COI) using the following primer pairs: 28sF3633 (5'-TACCGTGAGGGAAAGTTGAAA-3') with 28sR4076 (5'-AGACTCCTTGGTCCGTGTTT-3') (Choudhury and Werren 2006) and C1-J-1751 (5'-GGATCACCTGATATAGCATTCCC-3') with C1-N-2329 (5'-ACTGTAAATATATG-ATGAGCTCA-3') (Simon et al. 1994). The 28SD2 PCR reaction contained 2.0 µl DNA template, 2.0 µl 10X PCR reaction buffer with $MgCl_2$ (27.5 mM) (Roches Diagnostics, Germany), 2.0 µl dNTP mix 2 µmol (Thermo Scientific, USA), 0.8 µl of each primer (5 µM concentration), 1.2 µl bovine serum albumin (BSA) (New England BioLabs), 11 µl ddH_2O and 0.2 µl ThermoPrime Taq DNA polymerase 5 U/µl (Thermo Scientific, USA); total reaction volume of 20 µl. PCR conditions were: one cycle at 95°C for 2 min, 38 cycles of 30 s at 94^{o}C, 50 s at 58^{o}C, and 1 min 15 s at 72^{o}C; and a final cycle of 10 min at 72^{o}C. The COI PCR reaction contained 2.0 µl DNA template, 2.0 µl 10X DreamTaq PCR reaction buffer with $MgCl_2$ (20 mM) (Fermentas, Thermo Fisher Scientific, USA), 2.0 µl dNTP mix 2 µmol (Thermo Scientific, USA), 0.8 µl of each primer (5 µM concentration), 1.6 µl bovine serum albumin (BSA) (Biolab, England), 0.1 µl $MgCl_2$ (25 mM), 10.5 µl ddH_2O and 0.2 µl DreamTaq™ DNA polymerase 5 U/µl (Fermentas, Thermo Fisher Scientific, USA); total reaction volume of 20 µl. PCR conditions were: one cycle at 95°C for 5 min, 36 cycles of 30 s at 95^{o}C, 40 s at 47^{o}C, and 1 min at 72^{o}C; and a final cycle for 5 min at 72^{o}C. Each PCR included appropriate negative (no template) controls. Each PCR was performed on a thermal cycler and the PCR products were visualised on 1.5% SB buffer agarose gels. The PCR products were purified (Gel Extraction/PCR Clean-up Kit, Dnature Ltd, New Zealand) before being sent for DNA sequence analysis (Sequencing facility at Plant Research International, The Netherlands (28s samples from 2011) and Lincoln University,

New Zealand (28s samples from 2011and COI samples from 2011 and 2012 experiments)). The DNA sequences were edited and aligned using Geneious Pro software (Drummond et al. 2012) and compared with a reference database constructed from sequences obtained from GenBank for western flower thrips (GenBank accessions GU148016–GU148130 from Rugman-Jones et al. 2010). After extraction, the thrips specimens were stored in 96% ethanol at -80^{o}C for record keeping at Plant & Food Research, Lincoln, New Zealand.

5.2.2 Data analysis

Molecular analyses. The DNA sequences were edited and aligned using Geneious Pro software (Drummond et al. 2010) and compared with a reference database constructed from sequences obtained from GenBank (www.ncbi.nlm.nih.gov) for WFT.

Y-tube olfactometer. Data from the Y-tube olfactometer assays was analysed with a binomial generalised linear model (McCullagh and Nelder 1989). Two variables were analysed to test the preference of WFT populations to MI: the percentage of thrips choosing the MI laden arm or the clean air arm or thrips that made no choice and the time taken for the responding thrips that did make a choice to walk to the end of the Y-tube arms. The percentage of thrips not making a choice was also analysed to examine potential differences among the populations that may or may not be relevant to olfactory behaviour. Differences among the thrips population responses to the treatments were investigated explored using contrasts within the analyses. The percentage of thrips choosing the MI laden arm or the clean air arm was made with t-tests of the parameters estimated on the transformed (logit) scale. On the logit scale, 50% has a value of zero, so if the parameter for a treatment is significantly greater than zero on the logit scale, significantly more than 50% of the thrips chose the MI laden arm. For all experiments, 95% confidence limits for the percentage of thrips responding were calculated on the transformed (logit) scale, and then back-transformed. The time taken to make a choice was analysed using two methods involving censoring thrips that did not make a choice. First, differences were examined using the Kaplan-Meier estimate (Collett 2003) of the distribution of time taken to make a choice; and second, Cox proportional hazards modelling of the time distribution (Cox 1972). A scatterplot was used to evaluate if a relationship between thrips not making a choice and the time responding thrips took to make a choice existed. The strength of the linear relationship was measured using the Pearson correlation coefficient. All analyses were carried out with GenStat (GenStat Committee 2011), and graphics were produced using SigmaPlot v.10.

5.3 Results

Molecular analyses. The aligned 28sD2 sequences (456 bp) were identical across all the randomly selected individuals indicating that all WFT populations clustered into one distinct haplotype (cluster one) (Rugman-Jones et al. 2010) discriminated by a 2-bp substitution at nucleotides 252 and 253. This cluster represents the so-called "glasshouse pest strain" (Rugman-Jones et al. 2010). This result was supported by the COI results. Based on a comparison of the overlap of 571 bp between the COI sequences used for haplotype determination (Rugman-Jones et al. 2010) and the COI sequences in this chapter, the thrips populations all belonged to the mtDNA cluster one (Rugman-Jones et al. 2010). However, greater variation was found in the COI sampling and some intra-population genetic diversity was found ($\leq$ 7 base pair substitutions). Table 5.3 shows the results of the mitochondrial haplotype determination and frequency of the haplotypes found in the different thrips populations.

Table 5.3 *Mitochondrial haplotype determination and frequency in western flower thrips populations defined by a 571 bp region of mtCOI gene. The haplotypes are based on the relationship network published by Rugman-Jones et al. (2010). Populations tested were from the Netherlands (NLI, NLII), New Zealand (NZ), Spain (SI, SII, SIII, SIV, SV, SVI), Kenya (KE) and France (FR).*

Population	Abbr.	n*	Haplotype A	Haplotype B	Haplotype D	Haplotype I
New Zealand	NZ	3	100%	-	-	-
Netherlands	NLI	5	80%	-	20%	-
Netherlands	NLII	8	100%	-	-	-
Spanish I	SI	5	20%	40%	-	40%
Spanish II	SII	2	-	-	-	100%
Spanish III	SIII	4	75%	-	-	25%
Spanish IV	SIV	7	71.4%	14.3%	14.3%	-
Spanish V	SV	8	37.5%	37.5%	12.5%	12.5%
Spanish VI	SVI	3	66.7%	-	-	33.3%
Kenya	KE	3	66.7%	-	33.3%	-
France	FR	3	100%	-	-	-

* *Not all thrips tested originally successfully amplified during the PCR process (n =successfully amplified).*

The variability resulted in four different haplotypes (A, B, D and I) (see Appendix I for the global distribution of WFT mtDNA haplotypes). Thrips tested from NZ and FR all belonged to haplotype A while thrips tested from NLI and II samples consisted of both haplotype A

and haplotype D. Thrips tested from Spain consisted of a mixture of all four haplotypes and showed greater variety than any of the other populations. Thrips tested from KE consisted of a mixture of haplotype A and haplotype D. The divergence from haplotype A was 0.17% for both haplotypes B and D, and 0.87% for haplotype I.

2011 Y-tube olfactometer experiments. Only two out of the 700 thrips tested in the 2011 experiment failed to make a choice. Therefore, no further examination of numbers not making a choice was done and statistical analyses were carried out omitting these two thrips. The Kaplan-Meier estimates of the distribution of the time taken to make a choice (Fig. 5.1a), showed that there was comparatively little difference among the populations tested. The Cox proportional hazard modelling showed that the time taken to make a choice was on average shortest for SI and longest for NLI populations ($p = 0.001$ or smaller) (Table 5.4a).

The percentage choosing the MI laden arm varied significantly between thrips populations ($p < 0.001$). Spanish thrips (SI, SII, SIII) were significantly less likely to choose the MI laden arm ($p < 0.001$) compared to the two other populations. The percentage choosing the MI laden arm was not significantly different for the three Spanish populations ($p = 0.818$), and not significantly different between the NZ and NLI populations ($p = 0.796$).

2012 Y-tube olfactometer experiment. Of the 756 thrips tested in the 2012 experiment, 110 (15% of the total) failed to make a choice. The percentage not choosing varied among the five populations tested, from 10% for NLII to 21% for FR. The percentage for FR was significantly larger ($p = 0.016$) than that for NLII but not for any other thrips populations. The Kaplan-Meier estimates of the distribution of the response time (Fig. 5.1b), showed differences among the populations. The Cox proportional hazard modelling confirmed that the response time varied among the populations ($p < 0.0001$) (Table 5.4b). There was a strong positive correlation ($r = 0.80$, $p = 0.05$) between the percentage of thrips not making a choice and the mean time it took for responding thrips to make a choice (Fig. 5.3). NLII and SIV populations were the quickest to respond, with the lowest percentage of non-responding thrips (10 and 12% respectively). KE and FR populations responded the slowest, and had high percentages of non-responding thrips (18 and 21% respectively) (Fig. 5.1b).

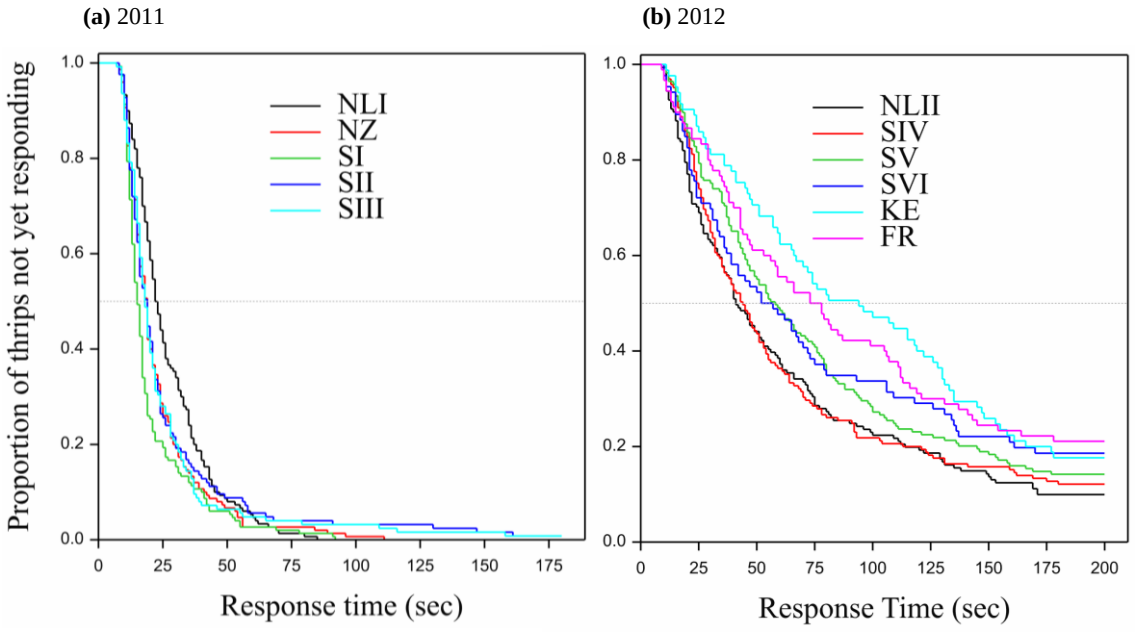


Figure 5.1 *Kaplan-Meier estimates of the distribution of response times (sec) for the different western flower thrips populations for (a) 2011 and (b) 2012 Y-tube experiments containing 1 µl methyl isonicotinate vs. clean air. Populations were from the Netherlands (NLI, NLII), New Zealand (NZ) (n=150)), Spain (SI, SII, SIII, SIV, SV, SVI) (n=150,125, 125, 150,150, 75 respectively), Kenya (KE) (n=70) and France (FR) (n=71).*

Further analyses were carried out omitting the thrips not making a choice. However, given that there were some differences between the treatments in the percentage not making a choice, ignoring these thrips may cause bias in the results, favouring populations with a large number of non-responding thrips. The percentage choosing the MI laden arm varied significantly between thrips populations ($p < 0.001$) (Fig. 5.4). Spanish thrips (SIV, SV, SVI) were significantly less likely to choose the MI laden arm ($p < 0.001$) than the other populations, and the percentage choosing the MI laden arm was not significantly different for SIV, SV and SVI) ($p = 0.836$), with only 47 to 51% choosing the MI laden arm in effect, no more than chance. Significantly more than 50% of the other populations chose the MI laden arm, with over 70% of both NLII and KE thrips choosing the MI laden arm and 60% of FR thrips.

Table 5.4 *Time (sec) taken for different western flower thrips populations to walk to the end of an arm in the Y-tube olfactometer in (a) 2011 and (b) 2012 experiments containing1 µl methyl isonicotinate vs. clean air (time for UQ = upper quartile (75%), median (50%), LQ = lower quartile (25%)). Populations were from the Netherlands (NLI, NLII), New Zealand (NZ), Spain (SI, SII, SIII, SIV, SV, SVI), Kenya (KE) and France (FR).*

(a) 2011

Population	**Abbr.**	**n**	**UQ**	**Median**	**LQ**	**Mean (s.e.)**	**% not choosing****
Netherlands	NLI	150	35.0	22.5	17.0	27.18 (0.678)	0
New Zealand	NZ	150	27.0	19.0	13.0	23.20 (0.775)	0
Spanish I	SI	150	21.0	15.0	12.0	20.24 (0.652)	0
Spanish II	SII	126	26.0	19.0	13.0	26.19 (1.714)*	0.1
Spanish III	SIII	126	28.0	18.0	14.0	24.74 (1.516)*	0.1

**Note: The mean time and its standard error are slightly underestimated as 1 thrips had not chosen within 3 min (censored).*
*** No choice made within 3 min.*

(b) 2012

Population	**Abbr.**	**n**	**UQ**	**Median**	**LQ**	**Mean (s.e.)**	**% not choosing****
Netherlands	NLII	161	90	41	21	63.76 (3.054)	9.94
Spanish IV	SIV	165	91	44	25	66.10 (3.155)	12.12
Spanish V	SV	169	109	58	31	76.36 (3.216)	14.20
Spanish VI	SVI	86	135	57	24	76.70 (5.264)	18.60
Kenya	KE	85	153	94	45	96.82 (5.590)	17.65
France	FR	90	145	78	36	88.72 (5.557)	21.11

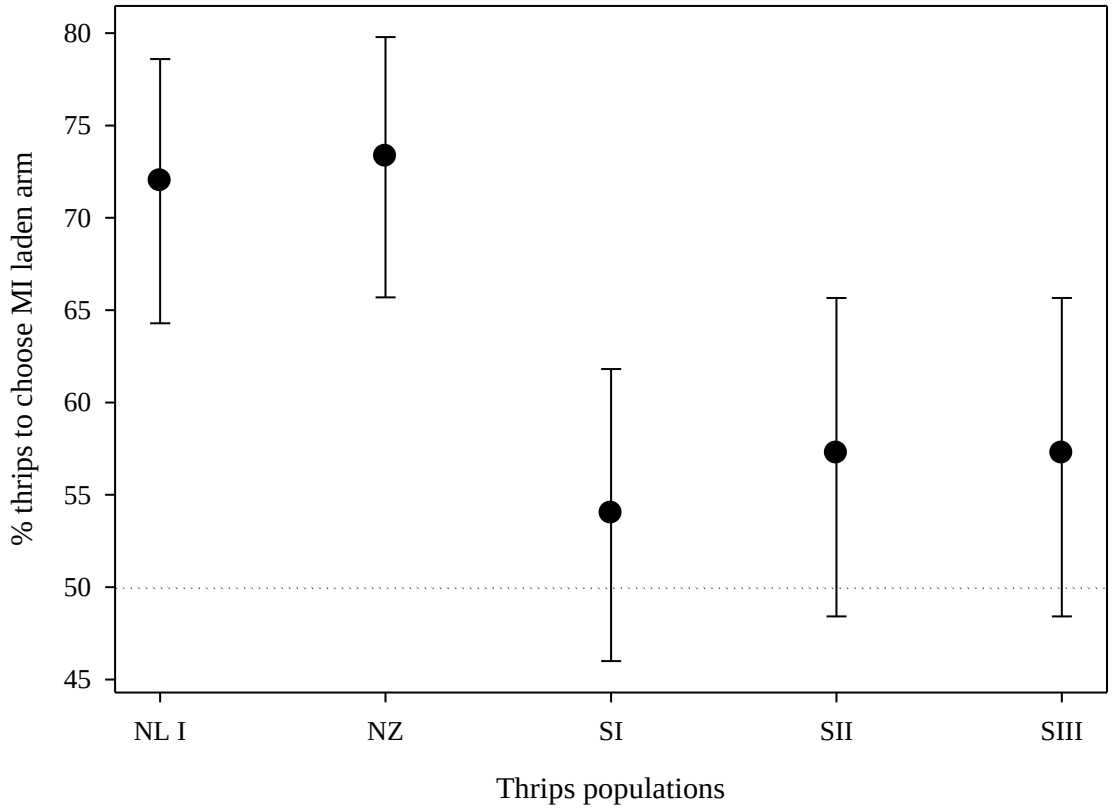


Figure 5.2 *Mean percentage of adult western flower thrips choosing the arm with 1 µl methyl isonicotinate vs. clean air in a Y-tube olfactometer (95% confidence limits). Populations tested were from the Netherlands (NLI) (n=150), New Zealand (NZ) (n=150) and Spain (SI, SII, SIII) (n=150,125, 125 respectively).*

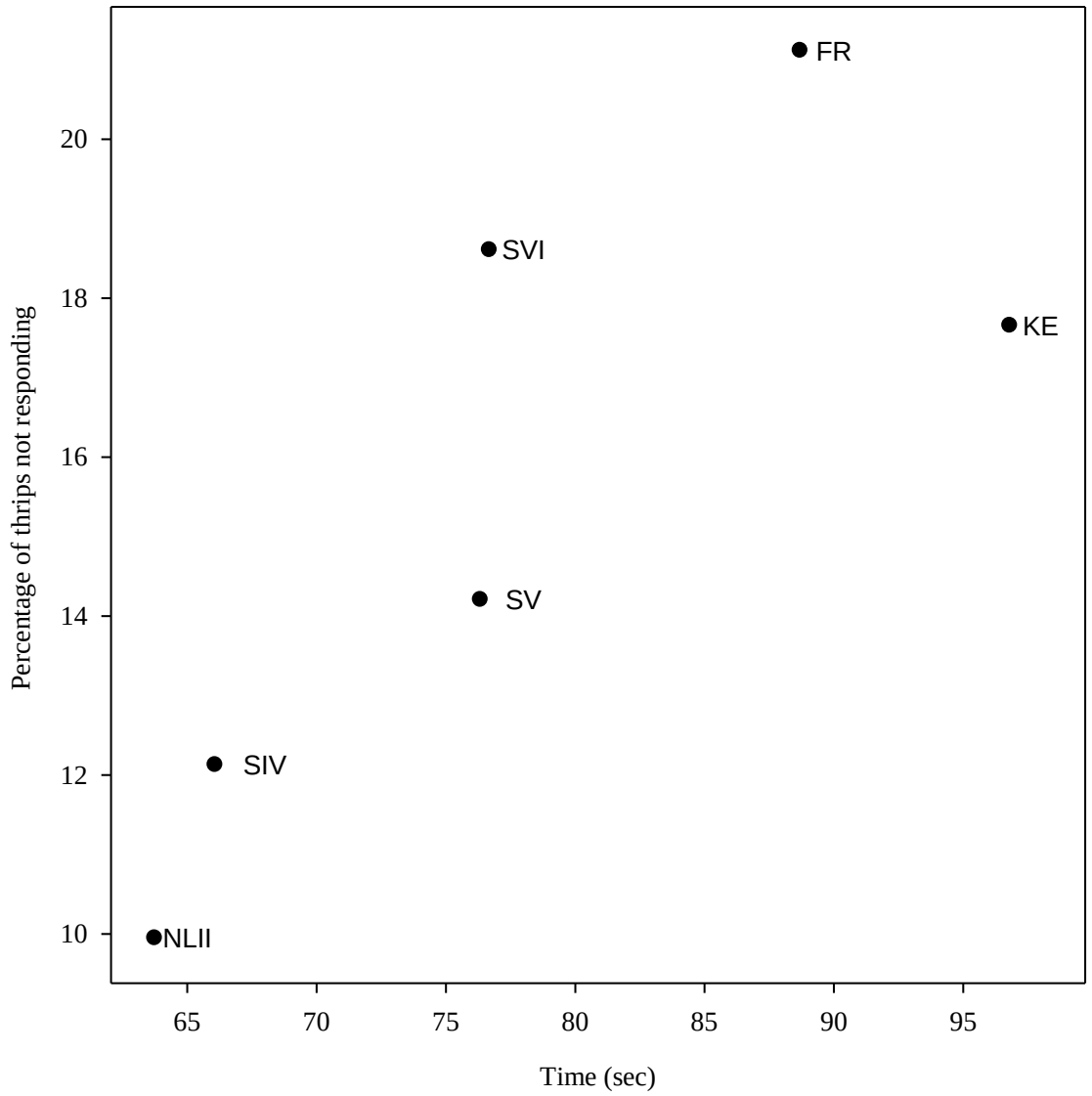


Figure 5.3 *Relationship between the percentage of adult western flower thrips populations not responding (<3 min) in a Y-tube olfactometer with 1 µl methyl isonicotinate vs. clean air and the mean time it took for responding WFT to respond(sec)(r = 0.80, p = 0.05). Populations tested were from the Netherlands (NLII) (n=150), Spain (SIV, SV, SVI) (n=150,150, 75 respectively), Kenya (KE) (n=70) and France (FR) (n=71).*

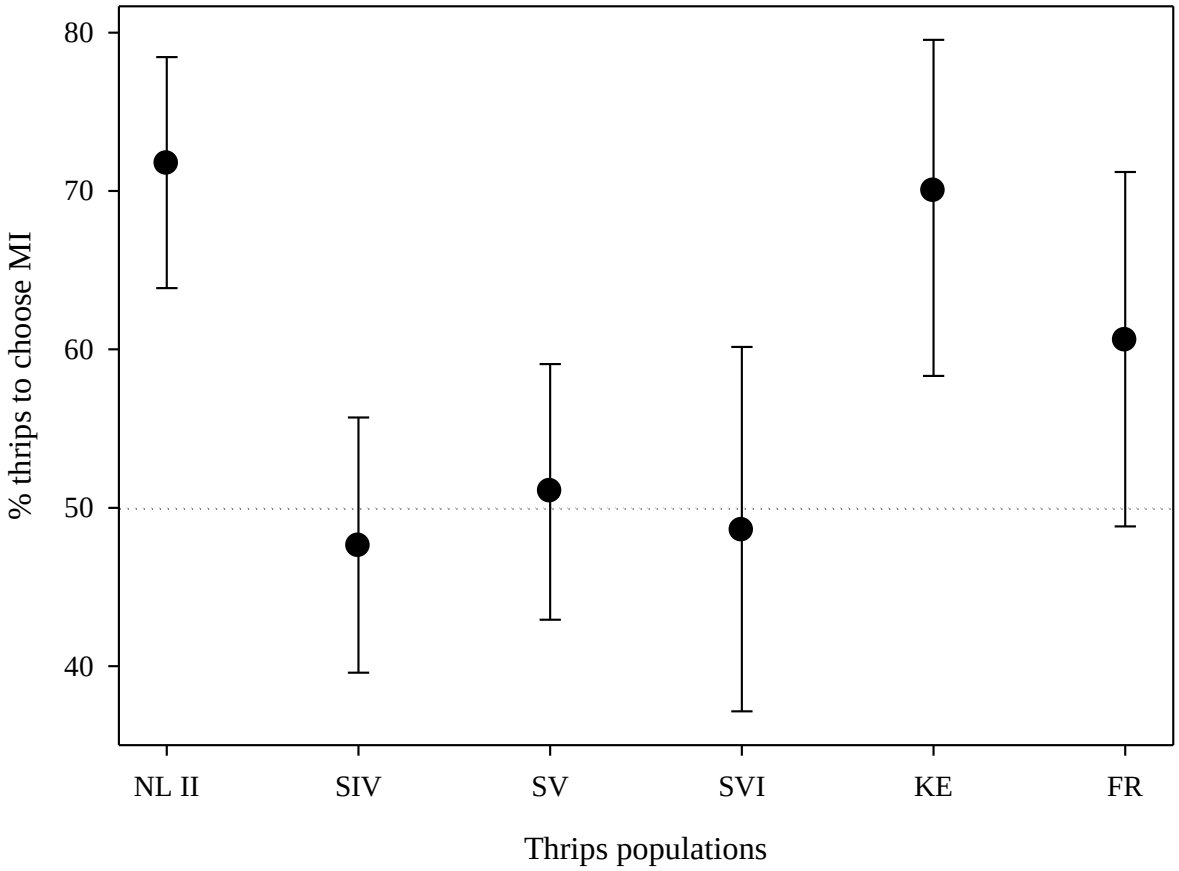


Figure 5.4 *Mean percentage of western flower thrips populations responding to the arm with 1 µl methyl isonicotinate vs. clean air in a Y-tube olfactometer (95% confidence limits). Populations tested were from the Netherlands (NLII) (n=150), Spain (SIV, SV, SVI) (n=150,150, 75 respectively), Kenya (KE) (n=70) and France (FR) (n=71).*

5.4 Discussion

The results of the experiments did not support the hypothesis that WFT from different geographical locations had a similar response to MI in a Y-tube olfactometer choice experiment. Significantly more WFT collected from NZ (73%), NL I (72%), NL II (72%), KE (70%) and FR (61%) preferred the MI laden arm compared to the clean arm. The response for these populations was consistent with results obtained and discussed in Chapter 4. However, WFT populations collected from northeast and southeast Spain (SI, SII, SIII, SIV, SV and SVI) did not show any preference for the MI laden arm, with the percentage choosing the MI laden arm being not significantly different from the percentage choosing the clean arm. The molecular analyses confirmed that all the WFT populations, including SI, SII, SIII, SIV, SV and SVI, all cluster in one group (Cluster 1) and align with other WFT

populations from around the world including WFT tested in Chapter 4 (see Chapter 1 and 4 for definition and description of the cluster). A limited analysis of the populations indicated that a larger number of haplotypes were present in the Spanish populations; however, the majority tested belonged to haplotype A that was the dominant group across the populations. Whether this result could be linked to variation in olfactory sensitivity is unknown. In this chapter only Spanish WFT were represented in haplotype group I and B. However, WFT samples collected from the Netherlands, New Zealand and Australia also belong to haplotype B group, all locations where trapping with MI has been successful previously. The lack of response of the Spanish WFT populations to MI is consistent with field trapping data of WFT populations from Spain (M-C Nielsen, unpubl. data).

Extrinsic factors undoubtedly play an important role in the variability of response to odour that occurs both within and between field and greenhouse populations but these results highlight the importance of intrinsic factors in thrips response to kairomones, at least in understanding the lack of response the Spanish WFT populations. The results in this chapter suggest that the development of a different behavioural phenotype of WFT in Spain has taken place. The question is whether this phenotype is produced by failure of signal detection, transduction or integration.

***Signal detection failure*.** WFT from any of the Spanish populations showed no preference for the MI laden arm in the experiments in this chapter, indicating that no detection or recognition of MI occurs. Perception of semiochemicals is mediated by olfactory receptor neurons (ORNs) compartmentalized in sensilla that are located primarily on the insect antenna (Moritz 1997, Hansson 2002, Bruce et al. 2005). No signal detection in the Spanish WFT could be due to lack of recognition of MI (no complimentary receptor site of the MI chemical structure in the ORNs) or the dose tested is below a detection threshold. Differences in sensitivity in ORNs can differ greatly between populations resulting in behavioural variability (Bernays and Chapman 1994). In geographically different populations of the Colorado potato beetle, *Leptinotarsa decemlineata*, sensitivity varied towards four different plant kairomones (Visser 1983). Behaviours to semiochemicals are extremely sensitive to environmental variation, enabling a wide range of behavioural phenotypes to be produced by genetically identical individuals (Anholt and Mackay 2004). WFT populations collected from Spain may show evidence of differential sensory sensitivity due to specific local adaptation. However, no significant difference in response to MI was found between the southeast (SI,

SII, SIV, SV and SVI) and the northeast (SIII) Spanish WFT populations, two areas with different biophysical environments. This result indicates that there may be a genetic component linked to the variations observed.

Olfactory sensitivity can be governed genetically in insects as demonstrated in *Drosphila melanogaster* (Fuyama 1978, Hoffmann 1983, Richgels and Rollmann 2012) and there is evidence that relatively small changes in the periphery of the sensory system can have a dramatic effect with respect to the semiochemical-guided behaviour. For example, one G to A transition (knock-out mutation) in a receptor gene for the semiochemical diacetyl in the nematode *Caenorhabditis elegans* eliminates the normal positive response to the semiochemical (Sengupta et al. 1996). In the fruit fly *Drosophila melanogaster*, variation in response to synthetic odorants, particularly esters, aldehydes and alcohols, exists across populations (Fuyama 1976, Alcorta and Rubio 1989, Mackay et al. 1996), and a large component of this variation seems to be controlled by genes located in the right arm of the second chromosome (Fuyama 1978). In recent years considerable progress has been made elucidating the molecular mechanisms that underlie odour recognition and odour-guided behaviour in phytophagous insects such as *Drosophila* (Anholt and Mackay 2004, Vosshall and Stocker 2007, Lavagnino et al. 2008). However, this is not so for thrips and no publications have been found on the genetics of the sensory system.

Failure of signal transduction. Spanish WFT may detect and recognise MI but elicit no behaviour response. A lack of response may be linked to variation in processes in the central nervous system (CNS). Behavioural responses (e.g. attraction or repulsion) depend on the processing of peripheral inputs within the CNS. When a semiochemical signal is detected and recognised, the signal is transferred via axons to the antennal lobe of an insect brain (Bernays and Chapman 1994) where the chemical signal is transferred into an electrical signal that inputs directly to the CNS (Hansson 2002). The information that is conducted to the insect's brain can be studied by single sensillum recordings and by electroantennograms, which are believed to reflect the summation of receptor potentials in the antenna (Ma and Visser 1978, Visser 1979, Kaissling 1986). Previous studies have found that some compounds that are recognised and elicit a strong response in the antenna of the insect do not elicit behavioural responses. This is for example found in the moth *Silene latifolia* (Dötterl et al. 2006). Furthermore, distinct abilities for detecting and responding to chemical compounds can also be found between insects forms. Benzaldehyde, a volatile component of the *Prunus padus* L.,

winter host of the bird cherry-oat aphid (*Rhopalosiphum padi* L.), attracted only male *R. padi* with gynoparae and alate virginoparae being unresponsive in the linear-track olfactometer, although both female forms elicited significant EAG responses (Park et al. 2000).

Failure of signal integration. If a signal is registered in the antennal lobe, the olfactory information is further conveyed to the protocerebrum, a premotoric area (Visser and de Jong 1988, Mustaparta 2002). Here the olfactory information is integrated with other sensory modalities such as visual cues, and output elements are connected with the motor system controlling the insect's motor patterns (Boeckh and Ernst 1987). Visual interactions with olfactory cues seem to play a very important part in WFT behaviour. For example, without a visual cue (black water traps) Nielsen et al. (unpubl. data) found no increase in WFT capture when MI was added to the traps located in strawberry or bare ground areas despite a significant increase being found for white water traps when MI was added. In contrast, Teulon et al. (1999) found that by adding the thrips kairomone *p*-anisaldehyde to black water traps in a commercial capsicum glasshouse in the Netherlands a significant increase in WFT trap capture occurred.

In addition to the behavioural factors discussed above, the high number of non-responding thrips and the increased time taken to make a choice for the WFT tested in the second year (2012), suggested other factors influencing the behaviour in the Y-tube experiments. For the 2012 results, a positive relationship was found between the number of thrips that did not make a choice within the 3-minute period and the time it took for the thrips that did make a choice (Figure 6.1). This indicated that some of the variability in response to MI between populations observed might be irrelevant to olfactory behaviour but instead linked to factors such as locomotor activity, dispersal activity and geotaxis, influencing the searching behaviour indirectly. Locomotor activity, dispersal activity and geotaxis could be influenced by potential infections by parasites (Lima-Camara et al. 2011), diet (Sauls et al. 1979) and physiological factors such as mating status (Hawkes and Coaker 1979). However, even if aspects of non-responding behaviour observed in Spanish WFT could be attributed to these factors it seems unlikely to play an important role in the attractiveness of MI to these populations as KE and FR showed a much higher correlation between non-responding thrips and the time taken to make a choice but still show a significant preference of the MI laden arm.

The results obtained in this chapter clearly suggest the development of a different behavioural phenotype of WFT in Spain. Further work was undertaken in Chapter 6 to address whether the observed lack of response in the Spanish WFT populations is due to failure of signal detection, transduction or integration.

Chapter 6

Olfactory response of western flower thrips (*Frankliniella occidentalis*) populations from Spain to thrips semiochemicals

6.1 Introduction

Work undertaken in Chapter 4 showed that there was a robust and consistent response of western flower thrips (WFT) to methyl isonicotinate (MI) irrespective of feeding history, host plant, rearing conditions and genotype in a selection of New Zealand WFT populations. In addition, under laboratory studies, Davidson et al. (2008) showed that MI is attractive to walking WFT over a large range of concentrations (10^{-6}–10^{o} µl) and work undertaken in Chapter 5 showed that different WFT populations from a range of countries (New Zealand, The Netherlands, Kenya and France) displayed similar positive responses towards. The effectiveness of MI on WFT and several other thrips species has been shown in Y-tube olfactometer assays and in greenhouse and field trapping in many of these countries (see Chapter 1). Nevertheless, variations in response of thrips, especially WFT to MI, have been observed (see Chapters 1 and 3). In particular, WFT populations from southern Spain appear to be non-responding to MI in greenhouse trials (Chapter 1) or to MI in Y-tube olfactometer studies (Chapter 5).

The variability in response of WFT to MI both within and between field and greenhouse trials (Table 1.1) is believed to be strongly linked to extrinsic factors. However, in the Y-tube olfactometer, where the effects of extrinsic factors such as trapping system and environment were mostly absent and other factors are kept constant, the non-response in some populations in Spain (Chapter 5) indicates that intrinsic factors play a critical role and underpin the variation in behaviour. In Chapter 5 it was argued that the non-response of WFT populations from southern Spain in the Y-tube olfactometer indicated the presence of a different behavioural phenotype. This phenotype might be associated with the failure of stimuli detection, transduction and/or integration. This chapter describes Y-tube olfactometer experiments and electroantennogram (EAG) recordings to further examine the non-

responsiveness of WFT to MI from Spain with respect to the potential failure of stimuli detection.

As indicated in Chapter 5, failure in response to MI in the Spanish WFT populations could be due to: (i) lack of response to odours in general, (ii) specific lack of recognition of MI (no complimentary receptor site of the MI chemical structure in the olfactory receptor neurons (ORNs)) or (iii) the dose-response relationship is different from the 'responsive' populations). Chapter 5 also indicated that a genetic component may be linked to non-response in the Spanish WFT. However, investigation into locating potential genetic differences was outside the timeframe of the PhD and therefore was not explored further.

To examine the non-response found in Spanish WFT several additional laboratory assays were undertaken. Although MI is found to be attractive to WFT in Y-tube olfactometer studies over a large range of concentrations, different sensitivity and ultimately different behavioural responses appear to occur in the Spanish WFT. To explore if Spanish WFT have a different dose-response relationship than other WFT populations, the response of a non-responsive WFT population to a range of MI concentration in the Y-tube olfactometer was tested. In order to test if the Spanish WFT populations showed a general lack of response to odours two other known attractant semiochemicals were examined in the Y-tube olfactometer: a different kairomone, *p*-anisaldehyde, and the aggregation pheromone of WFT (Kirk and Hamilton 2004, Hamilton et al. 2005). MI has never been found in flowers but is believed to mimic a host plant odour (Teulon et al. 2007b); however, many other kairomones reported to elicit behavioural responses in thrips do occur in flower scents. These include anisaldehydes, eugenol and geraniol (Kirk 1985). *p*-Anisaldehyde is a common component of flower odours of many of WFT hosts and its one of the most studied thrips attractants (Brødsgaard 1990, Hollister et al. 1995, Koschier et al. 2000, den Belder et al. 2001) attracting several flower-inhabiting thrips species (Kirk 1985, Teulon et al. 1993a). *p*-Anisaldehyde has also been described as an attractant for other insects (Morgan and Crumb 1928). Y-tube olfactory experiments with *p*-anisaldehyde have elicited strong responses in WFT over a range of concentrations and in different WFT populations from a range of countries (Smits et al. 2000, de Kogel and Koschier 2001, Koschier et al. 2007, Davidson et al. 2008, Mainali and Lim 2011).

While both MI and *p*-anisaldehyde mediate responses from a wide range of thrips species, a pheromone, which is only known to mediate responses to WFT and *Frankliniella intonsa,* has also been identified (Kirk and Hamilton 2004, Hamilton et al. 2005, Zhu et al. 2012) and found to increase trap capture of WFT in Spanish greenhouses (Sampson et al. 2012). (R)-lavandulyl acetate and neryl (S)-2-methylbutanoate have been identified as the two main compounds of the WFT aggregation pheromone (Kirk and Hamilton 2004, Hamilton et al. 2005) but only neryl (S)-2-methylbutanoate showed activity in field trials (Hamilton et al. 2005). Neryl (S)-2- methylbutanoate is commercially available for WFT monitoring (ThriPher®, Biobest Biological Systems Ltd). Testing a different structured kairomone (*p*-anisaldehyde) and a pheromone (neryl (S)-2- methylbutanoate) in a Y-tube olfactometer would assist in exploring if the lack of response of Spanish WFT populations was general (i.e. for different odours) or specific to MI. EAG recordings would help examine if the potential failures of the thrips to detect MI are due to peripheral changes (i.e. sensing the odour stimuli and encoding it as an electric signal in the neurons) or changes at a higher central level (i.e. the central nervous system (CNS)). After detection by the peripheral processes, olfactory information is relayed to the CNS where the electrical signals are integrated and processed to generate behavioural reactions.

The objectives of this chapter were to establish the walking response of a MI responsive Dutch WFT population and MI non-responsive Spanish WFT populations to different doses and concentrations of MI, to *p*-anisaldehyde and to WFT aggregation pheromone neryl (S)-2-methylbutanoate. In addition, EAG responses of Dutch and Spanish WFT to MI were investigated. The working hypothesis was that different doses and concentrations of MI would elicit a different and/or positive response in Spanish and Dutch WFT populations. Also, when exposed to *p*-anisaldehyde and neryl (S)-2-methylbutanoate both Spanish and Dutch populations of WFT would elicit a positive and similar response

6.2 Methods and Materials

The experiments were carried out during July and August 2012 at Plant Research International, Wageningen, The Netherlands. Populations of WFT were sourced from The Netherlands and southeast Spain (Table 6.1). For further information on WFT populations used in the experiment and rearing protocol see Chapter 5, Section 5.2.

Table 6.1 *Source of western flower thrips populations for Y-tube experiments 2012.*

Populations	Abbr.	Location	Crop/Host plant	Cultivar	Date collected
Netherlands	NLII	PRI Wageningen, The Netherlands	Indoor Capsicums	Mixed cultivars	15 Mar 2012
Spanish IV	SIV	Paraje el Algarrobo El Ejido, Almeria, Spain (southeast)	Indoor Capsicums	Mixed cultivars	20 Apr 2012
Spanish V	SV	Rincón de Mollano Berja, Almería, Spain (southeast)	Indoor Capsicums	Velero	20 Apr 2012

6.2.1 Molecular tests to establish thrips genotypes

The genotypes of a selection of WFT re-captured from the Y-tube experiments were assessed to compare DNA sequences of mitochondrial genes between thrips populations and their cluster position with respect to the genetic variation of a range of global WFT genotypes (see Chapter 1 and Rugman-Jones et al. 2010). The DNA sequences of the mitochondrial genes were also used to confirm that all thrips were WFT. The methods and materials are covered in Chapter 5, Section 5.2.1. Since much greater variation was evident in the DNA sequences of mitochondrial genes (mtDNA) (see results in Chapter 5, Section 5.3) only mitochondrial barcoding (COI) was used. For each treatment described above (excluding EAG) three individual thrips were tested from the NLII (total n=9), SIV (total n=9) and SV (total n=3) populations. It was impractical in terms of time and expense to test every individual thrips used in the experiment and therefore only a small subset were tested to confirm identification. The DNA sequence analysis was undertaken at the sequencing facility at Lincoln University, New Zealand. The DNA sequences were edited and aligned using Geneious Pro software (Drummond et al. 2012) and compared with a reference database constructed from sequences obtained from GenBank (www.ncbi.nlm.nih.gov) for WFT (GenBank accessions GU148016-GU148130 from Rugman-Jones et al. 2010). Reference WFT specimens were deposited at Plant & Food Research, Lincoln, New Zealand.

6.2.2 Y-tube olfactometer assay

For description of the Y-tube olfactometer setup used and the bioassay procedure see Chapter 5, Section 5.2. For each of the assays described below, one experimental run was completed when 25 thrips had actively made a choice regardless the number of ‘no-choice’ recorded. A

complete set of treatments were run in each of four consecutive days (four replicates, n=100/treatment). All experiments were conducted under a constant temperature of 22 $\pm$ 1^{o}C.

Dose and concentration of MI. The experiment was carried out on two WFT populations (NLII and SIV) (Table 6.1). The dose and concentration used were based on Y-tube olfactometer results obtained by Davidson et al. (2008) showing that MI elicited a positive response in WFT ranging from 10^{-6} to 10^{o} µl. To ensure a positive response a low dose and high dose within this range was chosen in addition to repeating the treatment applied in Chapter 5. The treatments tested were: (a) 1 µl of 1% MI (i.e. equivalent of 0.01 µl of pure MI), (b) 1 µl of pure (100%) MI and (c) 10 µl of pure (100%) MI. For treatment (a) hexane was used to dilute MI to a 1% solution. In the Y-tube bioassay 1 µl of 1% MI was applied to the filter paper in one vial (the treatment vial), while the other vial contained 1 µl of pure hexane (the control vial). For treatments (b) and (c) pure MI (1 µl and 10 µl respectively) was applied to the filter paper in one vial (treatment vial), while the other vial contained clean filter paper (control vial). For all treatments tested the treatment vials were replaced after every five thrips tested in the Y-tube to ensure that the added MI had not evaporated fully.

p-Anisaldehyde. The experiment was carried out on three WFT populations (NLII, SIV and SV). Based on positive Y-tube olfactometer results with WFT populations from The Netherlands (Koschier et al. (2000) and New Zealand (Davidson et al. (2008) a 10% solution of *p*-anisaldehyde (98 % purity, Sigma-Aldrich, New Zealand) was used. Undiluted *p*-anisaldehyde (1 µl) did not elicit a positive response in these WFT populations (Koschier et al. 2000, Davidson et al. 2008). Hexane was used to dilute the *p*-anisaldehyde. In the Y-tube bioassay 1 µl of 10% *p*-anisaldehyde (i.e. equivalent to 0.1 µl pure *p*-anisaldehyde) was applied to the filter paper in one vial (treatment vial), while the other vial contained 1µl of pure hexane (control vial). The treatment vials were replaced after every five thrips tested in the Y-tube to ensure that the added *p*-anisaldehyde had not evaporated fully.

ThriPher. The experiment was carried out on two WFT populations (NLII and SIV). Commercially available ThriPher lures (Biobest Biological Systems, Belgium) were used. ThriPher lures consist of a rubber septum and are reported to be impregnated with 30 µg neryl (S)-2-methylbutanoate (R. Pauwels, Biobest N.V., pers. comm. to M-C Nielsen, 24 July 2013). For control, clean (un-impregnated) rubber septa were used (Biobest Biological

Systems, The Netherlands). One set (impregnated and un-impregnated) rubber septa were used per day. The septa were placed with the 4 ml vials and the bioassay procedure was the same as described above and in Chapter 5, Section 5.2.

6.2.3 Bioassay procedure for electroantennogram (EAG) recordings

Adult female WFT (NLII (total n=3) or SIV (total n=3)) of unknown age and mating status were collected at random on three separate days from the rearing jars with an aspirator and each transferred individually into a separate clean petri dish immediately prior to the experiment being started. Females of unknown age and mating status were used to provide a mixed test group of WFT females, similar to groups used in the Y-tube assays, and to avoid the time constraints required to producing thrips individually (i.e. produce individual females of different physiological state) for the experiment. One side of a 2 cm^2 piece of double-sided sticky tape (HEMA B.V., The Netherlands) was used to restrain individual females (ventral surface up) by gently lowering the tape onto the thrips as they walked in the petri dish, and trapping them in the tape glue. The other side of the tape was used to secure the sample to the top of a mobile platform. Thin pieces of copper wire (<0.5 mm in diameter, 2–3 mm in length) were used to further restrain the thrips and the antenna; one piece placed at the base of the antenna and one across the abdomen. A fine tip (tip diameter < 1 µm) glass electrode (GC150F-10, Harvard Apparatus Ltd, UK) filled with hemolymph saline solution (pH 6.5) was inserted into the abdomen to act as the reference electrode. The saline solution (Visser 1979) contained glucose (354 mM), KCl (6.4 mM), KH_2PO_4 (20 mM), $MgCl_2$ (12 mM), $CaCl_2$ (1 mM), NaCl (12 mM), and KOH (9.6 mM). The pH was adjusted to 6.5 with NaOH and HCl as required. A recording glass electrode filled with the saline solution was placed at the tip of the last antennal segment. The last segment on the antennae had been slightly damaged using micro-scissors to remove the outermost part of the antennal tip to create an open connection with the internal fluid of the antenna providing contact between the insect hemolymph and the hemolymph solution of the electrode. AgCl-coated silver wires were inserted into the electrodes and connected to an amplifier via an input probe. Further amplification and processing were completed by the main amplifier and signals stored in a computer with IDAC interface board (EAG version 2.4, Syntech1, The Netherlands). Once a signal baseline was established and stabilised with a low noise level (>100 µV) using slight manipulation of the electrodes, stimulation pulses with MI solutions were introduced into the main airflow. Only 4-6 test series of stimuli were applied per preparation, taking less than 2 h to complete (see below). Longevity of antennal preparations for each individual thrips were

expected to last several hours based on previous studies on aphids (see Park and Hardie (1998)) who employed the same technique (whole insect preparation with intact antenna) and previous testes done on thrips preparations employing this technique (K.C Park and M-C Nielsen unpubl. data).

Odour stimuli. A 10% MI solution was prepared using dichloromethane (CH_2Cl_2) as the solvent. Twenty µl of 10% MI solution (i.e. equivalent of 2.0 µl of pure MI) or pure dichloromethane (control) was applied to a piece of filter paper (0.5×5 cm, Whatman no. 1, UK), which were then placed in Pasteur pipettes (Fisher Scientific, U.S.A.) acting as odour cartridges. The tip of the pipette was placed about 3 mm into a small hole in the wall of a glass tube (13 cm long, 8 mm diameter) oriented towards the prepared thrips antenna (0.3–0.5 cm away). The thrips' antenna was stimulated for 2 s by passing treated (MI) or non-treated air at a rate of 1 ml/s through the Pasteur pipette into the glass tube with a continuous air flow of 40 cm/s (flow-rate: 30 ml/s) over the antenna. At least 1 min was allowed between successive stimulations for antennal recovery. A test series of stimuli (n = 4–6 series/individual thrips) were applied to the antennal preparation for each of the individual thrips using the following order: control, 10% MI, control. Fresh odour cartridges were prepared for each new thrips tested.

6.2.4 Data analyses

Y-tube assay. Two variables were analysed; the percentage of thrips making each choice (treatment laden arm, control arm, no choice) and the time taken for the thrips to make a choice. For details on the analyses methods used see Chapter 5, Section 5.2.2.

EAG. Analysis for population differences of EAG peak responses to 10% MI was done with Analysis of Variance after log transformation of the data.

All analyses were carried out with GenStat (GenStat Committee 2011), and graphics were produced using SigmaPlot v. 10.0.

6.3 Results

Molecular analyses. All thrips tested were molecularly positively identified as WFT. Based on a comparison of the overlap of 571 bp between the COI sequences used for haplotypes determination (Rugman-Jones et al. 2010) and the COI sequences in this study, the thrips

populations all belonged to the mtDNA Cluster 1 (Rugman-Jones et al. 2010). This cluster represents the so-called "glasshouse pest strain" (see Appendix I for the global distribution of WFT mtDNA haplotypes). Variation was found in the COI sampling and some intra-population genetic diversity was found (≤ 7 base pair substitutions). Table 6.2 shows the results of the mitochondrial haplotype determination and frequency of the haplotypes found in the different thrips populations. The divergence from haplotype A was 0.17% for haplotype D and 0.87% for haplotype I.

Table 6.2 *Mitochondrial haplotype determination and frequency in western flower thrips populations defined by a 571 bp region of mtCOI gene. The haplotypes are based on the relationship network published by Rugman-Jones et al. (2010). Populations tested were from The Netherlands (NLII) and Spain (SIV, SV).*

Population	Abbr.	n	Haplotype A	Haplotype D	Haplotype I
Netherlands	NLII	8*	100%	-	-
Spanish IV	SIV	6*	80%	20%	-
Spanish V	SV	3	66.6%	-	33.3%

** Not all thrips tested originally successfully amplified during the PCR process (n = successfully amplified).*

Dose and concentration of MI. The time it took for the thrips to make a choice in the Y-tube did not vary significantly between the two (NLII, SIV) populations ($p = 0.653$) but there were some differences relating to the treatments ($p = 0.028$) (Table 6.3a). When exposed to 1 µl MI, NLII thrips made on average the quickest choice recorded, whereas SIV made the slowest choice recorded when exposed to 10 µl MI. For SIV there was a clear pattern that the time it took for the thrips to make a choice increases with increased concentration and dose. No overall difference was found between the two populations in the percentage of thrips not making a choice. However, there was some evidence that more NLII thrips did not make a choice when the treatments contained 0.01 µl MI compared with the two other treatments with 1 and 10 µl ($p = 0.032$) (Table 6.3a).

Omitting thrips that did not make a choice, the percentage choosing the MI laden arm varied significantly between the two thrips populations ($p < 0.001$) (Fig. 6.1). SIV thrips were significantly less likely to choose the MI laden arm ($p < 0.001$) than NLII thrips. For NLII thrips the percentage choosing the MI laden arm was significantly greater than 50% ($p < 0.001$) for all treatments and the percentage of thrips choosing the MI laden arm increased slightly with increasing concentration and dose (65, 69 and 75% for 0.01 µl MI, 1 µl MI and

10 µl MI, respectively), but the difference was not significant (p = 0.298) (Fig. 6.1). For SIV thrips, the opposite pattern occurred and for none of the treatments did significantly more thrips (> 50%) choose the MI laden arm (p > 0.05) compared with the control. Decreasing numbers of SIV choose the MI laden arm with increasing concentrations and dose (55, 51 and 41% for 0.01 µl MI, 1 µl MI and 10 µl MI, respectively), with the difference being significant between 0.01 µl MI and 10 µl MI (p = 0.032). With 10 µl MI in the odour laden arm significantly less than 50% (p < 0.05) of SIV thrips chose the MI laden arm.

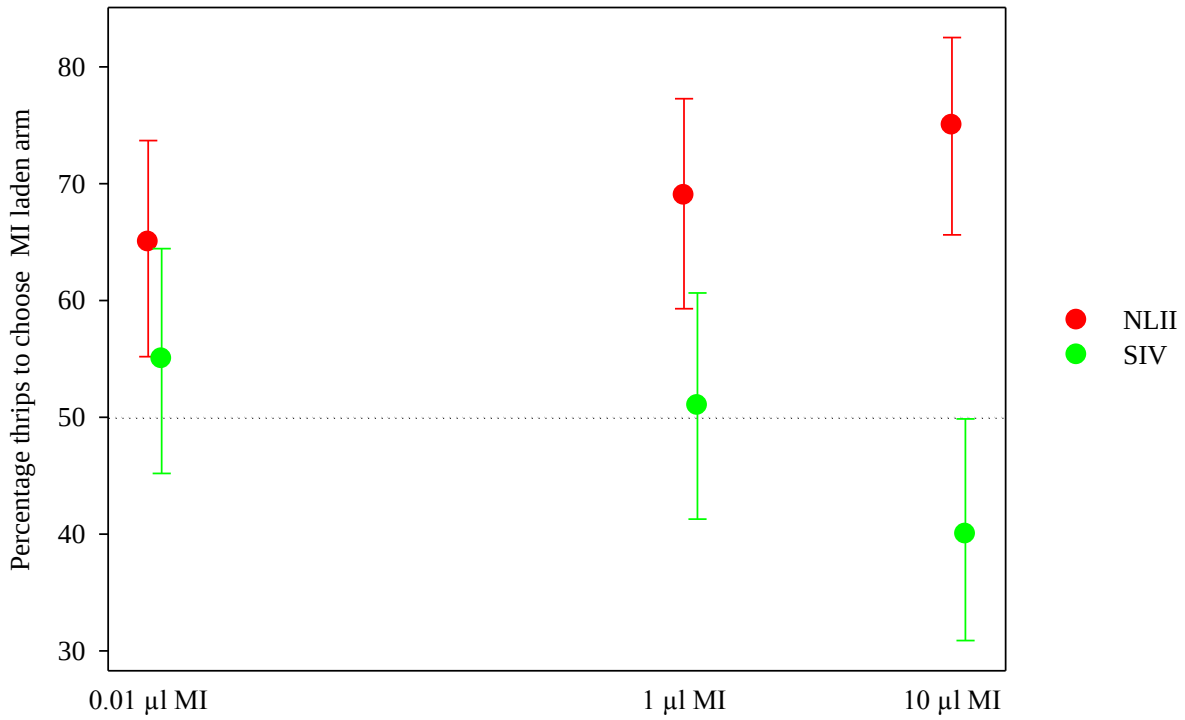


Figure 6.1 *Mean percentage of adult female western flower thrips choosing the MI laden arm vs. arm with clean air or hexane (control) in a Y-tube olfactometer (95% confidence limits). WFT populations tested were from The Netherlands (NLII) and Spain (SIV) against 0.01. 1 and 10 µl MI (n=100 for all treatments). The data is shown on a logarithmic x-axis.*

p-Anisaldehyde. The time it took for the thrips to make a choice in the Y-tube varied significantly among the populations (p < 0.001), with the major difference being between the NLII and the SIV/SV populations (Table 6.3b). Thrips from the SIV and SV populations tended to make a choice more quickly than did thrips from the NLII population. The time to make a choice did not differ significantly between SIV and SV (p = 0.085). Of the total 335 thrips used, 31% did not make a choice. With a total of 15.3% of the NLII thrips not making

a choice, this was significantly higher (p = 0.038) than 8.3% for SIV and 7.4% for SV (Table 6.3b).

Omitting thrips that did not make a choice, the percentage choosing the *p*-anisaldehyde (0.1 µl) laden arm varied significantly between NLII and thrips from the SIV and SV populations (p = 0.004), with little difference between SIV and SV thrips (p = 0.670) (Fig. 6.2). SIV and SV thrips were significantly less likely to choose the *p*-anisaldehyde laden arm than NLII thrips (p < 0.001). Only 47% SIV and 44% SV thrips choose the *p*-anisaldehyde laden arm (p > 0.05), but for NLII thrips the percentage choosing the MI laden arm (63%) was significantly greater than 50% (p = 0.01).

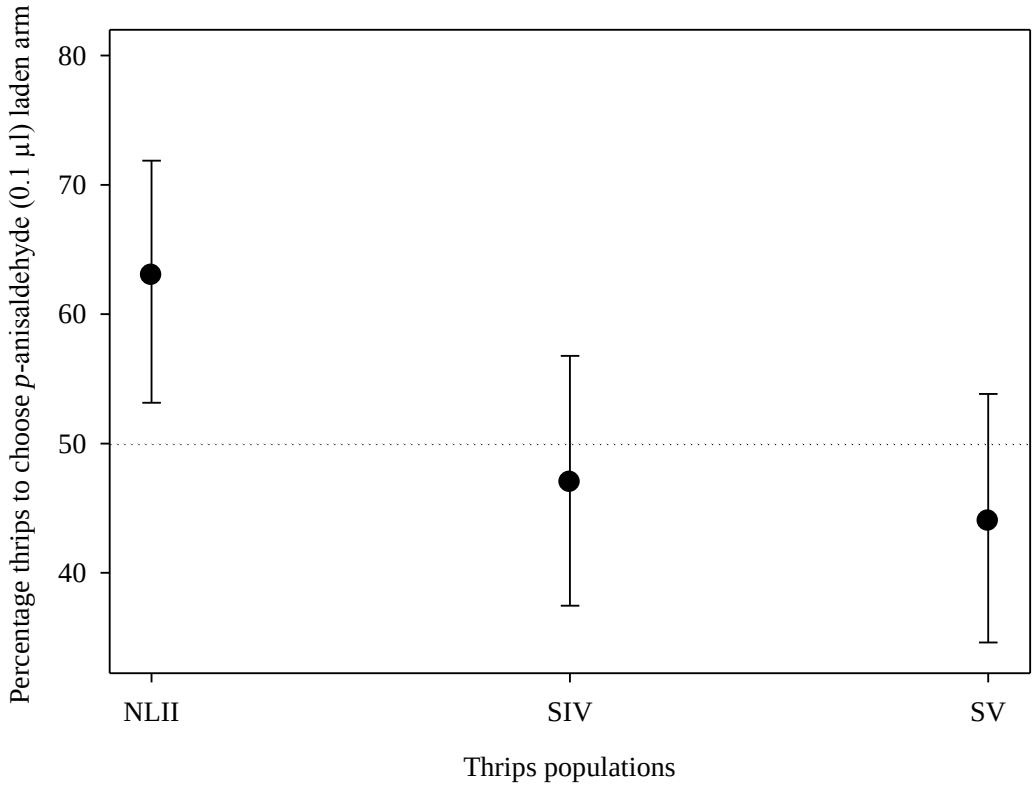


Figure 6.2 *Mean percentage of adult female western flower thrips choosing the arm with 0.1 µl p-anisaldehyde vs. arm with hexane (control) in a Y-tube olfactometer (95% confidence limits). Populations tested were from The Netherlands (NLII) and Spain (SIV and SV) (n=100 for all treatments).*

ThriPher. The time it took for the thrips to make a choice in the Y-tube did not vary significantly between the two populations (p > 0.05) and on average SIV thrips was marginally faster than NLII thrips to make a choice (Table 6.3c). Of the total 228 thrips used, 24.4% did not make a choice. With a total of 15.3% of the NLII thrips not making a choice,

this was higher than the 9.1% recorded from SIV but the difference was not significant (p > 0.05) (Table 6.3c).

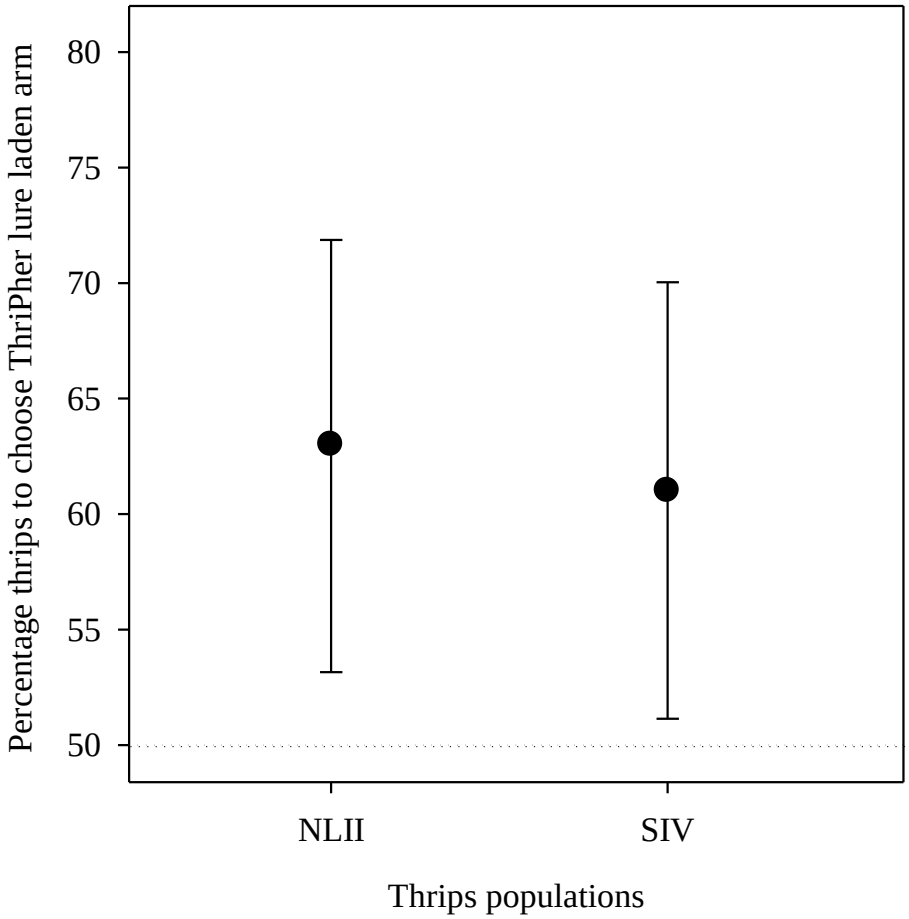


Figure 6.3 *Mean percentage of adult female western flower thrips choosing the arm with ThriPher rubber septa vs. clean air (un-impregnated septa) in a Y-tube olfactometer (95% confidence limits). Populations tested were from The Netherlands (NLII) (n=100) and Spain (SIV) (n=100). ThripPher septa are impregnated with 30 µg neryl (S)-2-methylbutanoate.*

Omitting thrips that did not make a choice, the percentage choosing the ThriPher laden arm (impregnated with 30 µg neryl (S)-2-methylbutanoate) was significantly higher than 50% for both populations (NLII 63%, p = 0.010; SIV 61%, p = 0.029) (Fig. 6.3). No significant difference was found between the two populations (p > 0.0.5).

Table 6.3 *Mean time taken (s) for adult western flower thrips to choose an arm in a Y-tube olfactometer (95% confidence limits) within 3 min containing: (a) 0.01 µl (1 µl of 1% MI) vs. 1 µl hexane, 1µl MI vs. clean air or10 µl MI vs. clean air, (b) 0.1 µl (1 µl 10 % p-anisaldehyde) vs. 1 µl hexane and (c) ThriPher rubber septa vs. clean rubber septa (time for UQ = upper quartile (75%), median (50%), LQ = lower quartile (25%)). Populations used were from The Netherlands (NLII) and Spain (SIV and SV). n=100 for all treatments. ThripPher septa are impregnated with 30 µg neryl (S)-2-methylbutanoate.*

(a) MI (dose and concentration)

Populations	**Abbr.**	**n**	**Dose – concentration (µl)**	**UQ**	**Median**	**LQ**	**Mean (s.e.)**	**% not choosing***
Netherlands	NLII	112	0.01	67.0	39.0	24.0	53.74 (2.99)	10.7
		104	1	49.5	27.0	17.5	38.00 (2.12)	3.9
		103	10	67.0	39.0	22.0	52.90 (3.19)	2.9
Spanish IV	SIV	105	0.01	57.5	35.0	21.0	47.14 (2.89)	4.8
		107	1	60.8	27.0	17.0	49.27 (3.42)	6.5
		107	10	86.3	39.0	22.3	60.88 (3.99)	6.5

(b) *p*-anisaldehyde

Populations	**Abbr.**	**n**	**Dose (µl)**	**UQ**	**Median**	**LQ**	**Mean (s.e.)**	**% not choosing***
Netherlands	NLII	118	0.1	130.0	61.0	32.0	79.14 (4.41)	15.3
Spanish IV	SIV	109	0.1	63.0	31.0	19.0	49.86 (3.19)	8.3
Spanish V	SV	108	0.1	89.0	41.0	26.0	63.41 (3.73)	7.4

(c) ThriPher

Populations	**Abbr.**	**n**	**UQ**	**Median**	**LQ**	**Mean (s.e.)**	**% not choosing***
Netherlands	NLII	118	83.0	40.0	22.0	59.81 (3.47)	15.3
Spanish IV	SIV	110	58.0	31.0	21.0	54.40 (3.60)	9.1

**No choice made within 3 min*

EAG recordings. Stimuli with 2.0 µl MI (20 µl of a 10% MI solution) elicited a significant increase in response in NLII and SIV WFT compared with stimuli with control solvent (CH_2Cl_2) only ($p < 0.001$) (Fig.6.4). On average SIV individuals showed a larger response than NLII individual, but not significantly ($p > 0.1$). There were limited numbers of individuals (n=3/population) and test series per individual thrips (n=4–6) tested, number of test series repeated per individual and large variation among individuals.

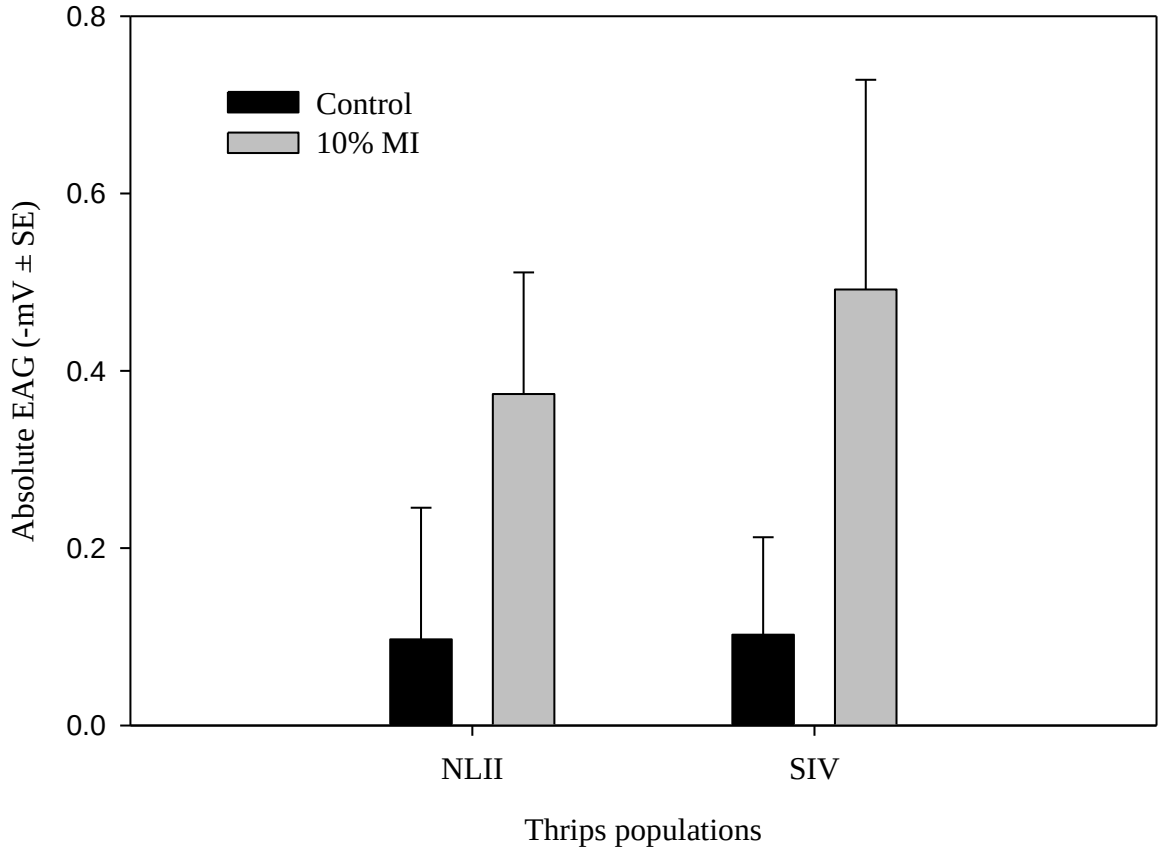


Figure 6.4 *Electroantennogram (EAG) (mean ± SE) responses of adult female western flower thrips to 10% MI (diluted in CH_2Cl_2) and CH_2Cl_2 (control). Populations tested were from The Netherlands (NLII) and Spain (SIV). The stimuli consisted of 20 µl of 10% MI solution or 20 µl pure CH_2Cl_2 (control) applied to a piece of filter paper. The thrips' antenna was stimulated for 2 s by air passing at a rate of 1 ml/s. A total of three individual thrips were used from each population (n=12–18 recordings/treatment for each population).*

6.4 Discussion

The molecular analyses confirmed that all the WFT populations tested cluster in one group (Cluster 1) and align with other WFT populations (see Chapter 1 and 4 for definition and description of the cluster). The majority of WFT from the three populations belonged to haplotype A that was the dominant group across the populations. The results in this chapter partially supported the hypothesis that a positive response to MI would be found with one or more of the different dose and concentrations tested (Section 6.1) but this only occurred in the population sourced from The Netherlands and not from the population sourced from Spain. No positive response was found for SIV WFT to any of the dose or concentrations of MI tested. With increased concentration and dose, fewer SIV choose the MI laden arm and

the time to respond increased. At the highest dose and concentration tested, significantly less than 50% of SIV chose the MI laden arm, whereas NLII showed the strongest response at the highest dose and concentration (75% choose MI laden arm). Differences in attraction to kairomones between populations from different geographic variations have been found in other insects such as mosquitoes (Williams et al. 2006) and fruit flies (Fuyama 1976). Williams et al. (2006) found that although all *Aedes aegypti* populations collected from four different countries were attracted to lactic acid from human skin, there were differences in the threshold dose between the populations. The results of the experiment indicate that SIV WFT does recognise MI but that the dose-response relationship of SIV is different from NLII WFT.

That SIV WFT detects and recognises MI, at least at the peripheral olfactory reception level, is supported by the EAG results. A significant response was recorded toward a 10% MI stimulus in both SIV and NLII WFT compared with the control stimulus. However, EAGs do not distinguish between attraction and inhibitory effects (Schoonhoven 1976). Additionally, an odour stimuli can be detected and elicit an EAG response in an insect but not stimulate any measurable behavioural response and vice versa. The lack of measurable behavioural response, and inversely lack of measurable electrophysical response, between peripheral recognition and the insect's behaviour has previously been recorded. Pow et al. (1999) tested the behavioural response of WFT to a range of compounds collected from chrysanthemum plants and tested their response both electrophysically (EAG) and behaviourally (Pettersson olfactometer). Several compounds elicited an EAG response but only one compound ((*E*)-β-farnesene) at one dose elicited a significant response in the olfactometer. In contrast, ethyl vanillin, a volatile constituent of chocolate, elicited no significant EAG response in female almond moth, *Ephestia cautella*, but did elicit significant upwind flight in a flight tunnel experiment (Olsson et al. 2005). While Pow et al. (1999) did not speculate on why only one compound elicited a behavioural response in the olfactometer despite several showing a electrophysical response, the result does highlight the importance of the dose and aerial concentration of the chemical in the olfactometer. Additional olfactometer bioassays should test lower doses of MI for behavioural activity of Spanish WFT populations.

While olfactory stimuli can elicit different, even antagonistic responses based on the internal physiological state of an individual insect (Martin et al. 2011) this is not believed to have had a bearing on the observed results. Many factors, such as age, time in the circadian cycle, and

feeding and mating status, may change the association of the stimulus in any field or greenhouse setting (Martin et al. 2011). However, for the experiments undertaken a standardised rearing system for all populations was used and the experiment was appropriately randomized and replicated.

It is, however, plausible that the Spanish WFT population's lack of behavioural response to MI in the Y-tube olfactometer could be due to a failure in signal integration in the higher central nervous system as suggested in Chapter 5. Once a kairomone signal is recognised at the periphery, such as in the olfactory receptors in the antennae, multiple steps are required to translate such a signal into measurable behaviour via the olfactory pathway. Odorants activate distinct subsets of ORNs, which converge on glomeruli in the antennal lobe (Vosshall and Stocker 2007). From the antennal lobe the information is relayed to higher brain centres (Vosshall and Stocker 2007) where it is combined with visual inputs. Several studies suggest that behavioural modifications are controlled by the central nervous system (Coss et al. 1980, Erber et al. 1980, Technau 1984). That the lack of response may be due to behavioural differences occurring in the WFT population from Spain at the higher centre of the signal pathway of the brain is supported by the lack of observed response of Spanish WFT to *p*-anisaldehyde in the olfactometer; a chemically distinct kairomone compared to MI (Teulon et al. 2007c). However, it must be stressed that the dose related behaviour of WFT to *p*-anisaldehyde has shown to be dose dependent especially in comparison with WFT responses to MI (Koschier et al. 2000, Davidson et al. 2008). Although *p*-anisaldehyde has shown to be an attractant for WFT in several experiments, pure concentrations of *p*-anisaldehyde have shown to repel WFT in Y-tube olfactometer (Koschier et al. 2000). The lack of response of SVI to *p*-anisaldehyde may be more linked to dose related behaviour. Also, the receptor repertoire in an insect's olfactory system needs to be considered in regards to comparing its response to different odours. It has been shown previously in insects such as Drosophila that some receptors show a strong response to one odorant but no response to a structurally similar odorant, and the opposite, a receptor that responds strongly to the several structural different odorants but not to structurally similar odorant (Hallem and Carlson 2004, 2006). *p*-Anisaldehyde may not have been recognised even at the peripheral olfactory reception level although MI was.

The result obtained in the olfactometer with the aggregation pheromone neryl (S)-2-methylbutanoate also supports the theory that the lack of response of the Spanish WFT

population to MI in the Y-tube olfactometer may be due to failure in the signal pathway of the brain. Peripheral sensory neurons expressing either odorant or pheromone receptors send signals to separate odour- and pheromone-processing centres in the brain to elicit distinct behavioural and neuroendocrinological outputs (Touhara and Vosshall 2009). The response to neryl (S)-2-methylbutanoate was similar for NLII and SIV, identifying no differences in either the peripheral sensory neurones or in the pheromone-processing centres in the brain for the pheromeone tested.

There may be a genetic component linked to the variations observed. Genetic variation in olfactory receptors has been found to contribute to variation in olfactory behaviour in *Drosophilla melangaster* (Richgels and Rollmann 2012). Margolies et al. (1997) demonstrated the presence of genetic components that affect the olfactory response of a commercial strain of the predatory mite *Phytoseiulus persimilis.* The results in this chapter and Chapter 5 strongly suggest that molecular techniques are needed to investigate genetic variation underlying the response to MI and other chemicals behaviours in different WFT populations. However, this is a vast task as previous studies have shown diverse olfactory genes including at least odorant-binding proteins (OBPs), chemosensory proteins (CSPs), sensory neuron membrane proteins (SNMPs), odorant-degrading enzymes (ODEs), ionotropic receptors (IRs) and olfactory receptors involved in different steps in signal transduction pathway (Rützler and Zwiebel 2005, de Bruyne and Baker 2008, Sato and Touhara 2009). To date no references have been found for work undertaken on thrips and olfactory genes and although genome sequencing and molecular studies have together characterized the complete OR repertoires and other olfactory genes in several insect species such as fruit flies (Clyne et al. 1999, Couto et al. 2005, Vosshall and Stocker 2007, Rollmann et al. 2010, Richgels and Rollmann 2012) and mosquitoes (Fox et al. 2001, Zwiebel and Takken 2004, Li et al. 2005, Bohbot et al. 2007, Wang et al. 2010b), comparison between other insects and thrips species may be limited due to species to species differences. A comparison of the OR repertoire of *D. melanogaster* with that of the mosquito *Anopheles gambiae* reveals dramatic sequence divergence between the species (Robertson et al. 2003) and in each insect there are significant species-specific expansions of distinct OR gene subfamilies (Jones et al. 2005).

If a genetic difference has manifested itself in the olfactory pathway of the Spanish WFT it leaves the question as to why this has occurred. Many studies have pointed to existence of

intraspecific variations in the structure and/or performance of sensory organ systems such as chemoreception in insect population living in different environments (Hansson et al. 1990, Chapman and Lee 1991, Opstad et al. 2004). Genetic differences in the olfactory pathway, if present, may translate into differences in sensory behaviour/ performance. Subtle shifts in olfactory perception can generate broad individual variation in sensory perception (Rollmann et al. 2010) and studies conducted on *D. melanogaster* have indicated great capacity of groups of chemosensory genes to alter their expression levels independently under a wide range of external environmental conditions (Zhou et al. 2009). One theory could be that potential differences in historical insecticide use between the southeast region of Spain and The Netherlands have influenced the population of WFT from the different locations. Especially if the changes in the olfactory pathway have taken place in the central nervous system, as several insecticides such as organophosphates and carbamates target the central nervous system. Rafalimanana et al. (2002) showed that chlorpyrifos (organophosphate) exposure in the parasitoid wasp, *Leptopilina boulardi* increased the response of females toward an odour. A genetic change in the olfactory pathway of the central nervous system would likely not be specific to one particular odour and may explain why no response toward *p*-anisaldehyde was found either in the Spanish WFT populations. Also, detection of pheromones and general odours in insects is generally considered to be accomplished by two distinct olfactory pathways (Hansson et al. 1989, Saïd et al. 2005, Deisig et al. 2012). Pheromone information is transmitted by specialized ORNs, whereas host plant odour information is transferred by general ORNs to sexually isomorphic ordinary glomeruli in the antennal lobe (Anton and Homberg 1999). A genetic change in the olfactory pathways for MI, and potentially other kairomones, would likely not affect the perception of a pheromone. The differing responses to kairomones between WFT from Spain and The Netherlands may be evidence of adaptation to local conditions and indicate that the development of kairomone lures for WFT may require some specialization for the region of use. This would involve categorization of the behavioural response to the kairomone lure by different populations targeted under controlled condition prior to use in assays such as the Y-tube olfactometer to establish if a response occur and, if so, the dose and concentration eliciting the strongest response.

One concern raised in Chapter 5 was that the lack of response of the Spanish WFT populations tested in the Y-tube olfactometer could be linked to factors unrelated to olfactory behaviour (e.g. locomotor activity), especially due to high number of non-responders and the

time it took to make a choice in the assays undertaken. However, in this study NLII WFT showed the highest percentage of thrips not making a choice and the time it took for the responding thrips to make a choice was longer than the SIV/SV WFT. It is not known why these patterns seem to have been reversed from experiments undertaken in Chapter 5. The positive response of both SIV and NLII WFT to the aggregation pheromone (ThriPher) does show that this Spanish WFT population is capable of responding to a suitable attractant in the Y-tube olfactometer assay. Given this result the lack of response of Spanish WFT to MI and *p*-anisaldehyde cannot be contributed to some behavioural abnormality hampering the behaviour in the Y-tube.

Chapter 7

General discussion

The overall aim of the research in this study was to investigate how selected intrinsic and extrinsic factors influenced the response of WFT to olfactory cues that are used in host finding by WFT, and to investigate why large variations in the response of WFT to MI occur when used in various trapping situations.

The research has extended existing knowledge of the efficacy of olfactory cues for thrips. In laboratory experiments (Chapters 4, 5 and 6) investigations were undertaken to explore how different genotypes and populations of thrips respond to the same olfactory cues in a controlled environment using a Y-tube olfactometer and electroantennogram techniques. In addition, experiments in Chapter 3 investigated the relationship between release rates of a MI, environmental factors that may affect this and thrips behavioural response in greenhouse environments, and molecular techniques were used to investigate genetic differences in thrips populations to account for differences in behavioural responses.

***Key objectives and findings*.** The following sections briefly summarise the key objectives and main findings for each experimental chapter.

1. Examination of the release kinetics and measure the release rates of MI over time from passive dispensers under constant laboratory conditions to establish the effect dispenser type and abiotic factors have on the release rate (Chapter 2).
    - *All dispensers tested released at a constant rate (zero-order kinetics).*
    - *Temperature is a major determinant of release rate and increasing temperature increased the release rates.*
    - *Increased air flow also increased the release rates, with the effect increasing with temperature.*
    - *Mean release rates established for all dispensers at 15, 25 and 35^{o}C varied according to dispenser type.*
    - *The release rate of MI decreased from the highest to the lowest in the following order for the dispensers tested: cotton dental roll, LUREM-TR, commercial sachet and 150 micron polyethylene bag.*

2. Investigate release rates under greenhouse conditions and determine the effect of different release rates of MI on thrips response in greenhouse environments (Chapter 3).
    - *Abiotic factors, especially temperature, are major determinant of release rates.*
    - *Release rates varied greatly within and between greenhouse experiments.*
    - *The response of thrips to MI was at least partially positively dose related.*
    - *No optimal release rate of MI in regards to thrips response was established.*

3. Investigate the attraction of WFT to MI under controlled laboratory conditions to determine the influence that feeding history, virus status, genetic variation and geographic origin have on the response (Chapters 4 and 5).
    - *Different WFT populations responded consistently to MI independently of their background and feeding history.*
    - *Separate cryptic species of WFT (different genotypes) responded consistently to MI; however, behavioural differences were observed.*
    - *WFT populations from different geographical origins did not respond consistently to MI.*
    - *WFT populations collected from Spain did not show any preference towards MI.*
    - *No information was obtained with regard to the influence the virus status (viruliferous or non-viruliferous) may have on the olfactory response of thrips to MI (no detectable TSWV was present in the putative virus-infected thrips population).*

4. Further investigate the lack of responsiveness of Spanish WFT to MI to determine whether there are intraspecific differences among WFT populations (Chapter 6).
    - *Different behavioural phenotypes of WFT exist among the WFT glasshouse pest' genotype.*
    - *WFT populations collected from Spain did not show any preference towards different doses of MI in Y-tube olfactometer (behaviourally).*
    - *Electroantennogram (EAG) recordings did elicit a response in Spanish WFT towards MI (electrophysically).*
    - *WFT populations collected from Spain did not show any preference towards p-anisaldehyde.*
    - *WFT populations collected from Spain did show a preference towards the WFT aggregation pheromone (neryl (S)-2-methylbutanoate).*
    - *The molecular analyses showed that all WFT populations tested, including WFT from Spain, cluster in one group and align with other WFT populations.*

7.1 Responding and non-responding thrips

The responsiveness of thrips found in this study can be divided into two groups: thrips that either respond (responding) or do not respond (non-responding), and within the responding thrips there are degrees of responsiveness. While work undertaken in this study suggests that underlying generic variation between populations lay at the root of the difference between responding and non-responding thrips populations, intrinsic and extrinsic factors also play a role in the response level archived (Fig. 7.1).

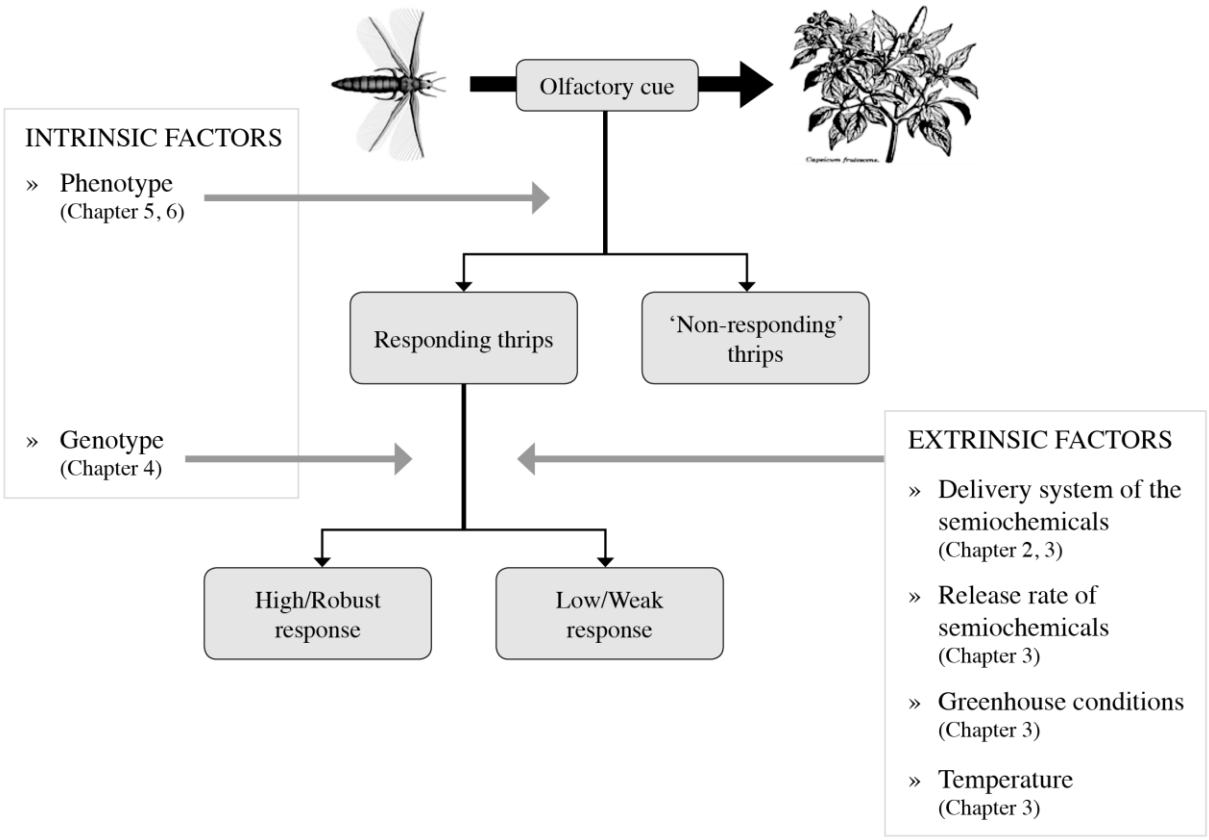


Figure 7.1 *Intrinsic and extrinsic factors identified in this study as influencing the response of thrips to olfactory cues.*

Non-responding thrips. Different behavioural phenotypes of WFT have been shown to exist (Chapters 5 and 6). WFT populations collected from Spain showed no attraction to MI when tested in Y-tube olfactometers, whereas WFT populations from New Zealand, The Netherlands, France and Kenya all did. The lack of attraction of the Spanish WFT populations to MI in olfactometer studies is consistent with field trapping data for WFT populations from Spain. Several greenhouse experiments undertaken in southeast Spain have all failed to achieve any significant increases in the number of WFT caught on traps baited

with MI compared with unbaited traps. The experiments were carried out in different greenhouses and during several different times of year in combination with different passive dispensers (M-C Nielsen, unpubl. data).

The reason for the non-response in the Spanish population has still to be determined. This outcome suggests that WFT populations worldwide show some variation in traits (genetically and behaviorally) notwithstanding the relatively recent worldwide spread of this pest species. WFT is endemic to the western part of North America in an area west of the Rocky Mountains (Bryan and Smith 1956) but since the late 1970s has rapidly invaded most countries throughout the world including Spain (Kirk 2002, Kirk and Terry 2003).

However, recent research has highlighted the genetic complexity among WFT populations worldwide. Rugman-Jones et al. (2010) has revealed the existence of two separate cryptic species of WFT. The WFT populations in their study were represented by 20 different haplotypes forming two distinct clusters separated by 17 nucleotide changes. The results for the molecular analyses undertaken in this study showed that all the WFT populations, including populations from both northeast and southeast Spain, cluster in the same group and align with many other WFT populations worldwide, but that a larger number of haplotypes were represented in the Spanish populations than found by Rugman-Jones et al. (2010). Further work is needed to explore the potential genetic variation among Spanish and other populations of WFT to explore whether there is a potential genetic link to the non-responding behaviour displayed.

Based on laboratory behaviour experiments (Chapter 6) testing different doses and concentrations of MI and other semiochemicals as well as electrophysical experiments, the results of this study suggest that the non-responding behaviour is potentially linked to failed signal integration in the central nervous system. Once an olfactory cue is recognised at the peripheral olfactory reception level, such as in the olfactory receptors in the antennae, multiple steps are required to translate such a signal into measurable behaviour via the olfactory pathway. Several studies suggest that behavioural modifications are controlled by the central nervous system (Coss et al. 1980, Erber et al. 1980, Technau 1984).

The observed variation in behaviour between Spanish WFT and other WFT tested are likely to be linked to a genetic component (Chapters 5 and 6). Genetic variation in olfactory

receptors has been found to contribute to variation in olfactory responses in other arthropods such as *Drosophila melanogaster* (Richgels and Rollmann 2012) and *Phytoseiulus persimilis* (Margolies et al. 1997) demonstrating the presence of genetic components that affect the olfactory response.

With the recent publication of the complete genomic sequence of WFT (Yan et al. 2012), as well as rapid advances in similar projects for both thrips and other insects, the coming years will likely increase our understanding of the molecular genetics basis of thrips olfaction. Currently the complete repertoires of olfactory genes have only been described for a few model species, such as *Drosophila melanogaster* (Kreher et al. 2005), *Anopheles gambiae* (Xia et al. 2008) and the *Bombyx mori* (Tanaka et al. 2009) due to their complete genomes being sequenced previously.

Responding thrips. Within the group of thrips that responded to an olfactory cue there appears to be variation in their responsiveness (see Table 4.2, Chapter 4). The objective of Chapter 4 was to determine the response New Zealand WFT pest greenhouse populations (WFT-G) and a separate cryptic non-pest species of WFT (WFT-L), commonly found outside on lupin in New Zealand, to MI. Although morphologically indistinguishable (Mound and Walker 1982, Mound 2005a) the two cryptic species of WFT display differences in their susceptibility to pesticides (Martin and Workman 1994) and exhibit significant differences in some life history parameters (Nielsen et al. 2010) in addition to being genetically distinct (Nielsen et al. 2010). Compared with the WFT-G populations, WFT-L showed a stronger and quicker positive response to MI in an olfactometer, although these results were only significantly different between WFT-L and one of the WFT-G populations tested. This indicates that genetic differences may be implicated in the olfactory sensitivity and behaviour of WFT. However, the difference in response between WFT-G and WFT-L populations towards MI needs to be considered in light of the molecular differences and basic biology of these populations.

For greenhouse and field trials involving kairomones, several different passive dispensers were used to deliver the MI (Chapter 2). One criterion for effective delivery is that the dispenser can deliver MI at a constant rate and independent of the amount contained in the dispenser under constant temperature and airflow conditions in the laboratory (zero order kinetics). The results showed that all dispensers used during field and greenhouse

experiments delivered MI at a constant rate. However, the laboratory experiments also showed that temperature, and to some extent air flow, are major determinants influencing the release rates from dispensers. Failure to recognise these factors can create two issues under field conditions: depletion of the kairomone prematurely and incorrect release rates in regards to optimum targeted release, both of which can contribute to variation in trapping efficacy. In studies testing the biological efficiency of a semiochemical it is important to know when the release rate declines due to depletion of the compound from the dispenser (Heuskin et al. 2011) thus indicating either the need to end the experiment or replace the dispenser before depletion occurs. Although this issue of release rate is important to keep in mind for experiments when temperatures are high during summer, e.g. in Spain, release rate is not believed to have had an influence on the variation in trapping efficacy recorded in previous greenhouse experiments undertaken in Spain (see Table 1.1, Chapter 1). The majority of the trials in Spain were only run for a few days at a time under autumn/winter conditions using the commercially available thrips lure LUREM-TR, and depletion would not have occurred for at least 12 days (see Fig. 2.3, Chapter 2).

The more influential factor would be the effect of the release rate when there is sufficient MI in the dispenser. One of the key objectives for successful application of a semiochemical for insect pest manipulation and management is to achieve a controlled release rate that elicits an appropriate level of response from the target insect (Byers 1988, Heuskin et al. 2011). Greenhouse trials (Chapter 3) and laboratory experiments (Chapter 2) in this study clearly showed that release rate can vary greatly. The results showed a positive relationship between the total number of thrips caught/trap and the amount of released MI/trap, and this relationship was species-specific. The results signify that the release rate of a semiochemical in a lure like LUREM-TR needs to be calibrated under different temperature and air flow regimes for optimum and effective use in pest management.

The level of MI that may build up within the atmosphere of greenhouses was identified in this study as a potential extrinsic factor influencing the apparent responsiveness of flying thrips to traps with MI (Chapter 3). In outdoor conditions, Teulon et al. (2007a) showed that the closer a unbaited trap was to a baited trap the greater number of thrips the unbaited trap caught indicating that baited traps can influence the capture of thrips in unbaited traps. In indoor conditions, where airflow is considerably reduced, it is quite conceivable that this phenomenon is considerably enhanced. The odour from baited traps could surround unbaited

and increase their efficacy in relation to the baited. There is some evidence for this in Chapter 3 where the difference of thrips numbers caught on baited and unbaited traps reduced over time, possibly in response to a build-up of odour within the greenhouse and surrounding the unbaited traps. Within 2 to 3 days of putting out the MI baited traps in the greenhouse a peak in the number of thrips caught occurred followed by a decrease of thrips caught across all traps over the following days. Within the first days the MI released from the different dispensers attached to the traps were most effective for trapping thrips on baited traps compared with the control traps.

Feeding history and rearing conditions did not have an effect on the response of WFT to MI (Chapter 4). Different New Zealand WFT populations responded to MI in a consistent manner irrespective of their feeding history and rearing conditions. However, a number of thrips semiochemicals are plant-derived (Koschier 2006), and using a plant-derived semiochemical may alter the behaviour and responses as insect behaviours are modifiable through experience (Boller 1972). MI has so far not been found in host plants and therefore presumably has not been experienced previously by any of the populations tested, though MI is structurally similar to benzene carbonyl derivatives that are commonly found in flower scents (Davidson et al. 2008). Using plant-derived semiochemical lures may change the influence that different feeding histories have on the response by different populations depending on previous experience with the semiochemical.

7.2 Directions of future research

Genetic variations between the populations. As stated above, this study was limited in the exploration on genetic variations between the populations. If a genetic difference is believed to be contributing to the differences between responding and non-responding phenotypes of WFT it would be of interest to explore these differences, both to help identify responsive and non-responsive populations and to understand the basis of differences in olfaction between different populations of thrips (see below), and also to explore differences in the overall genetic diversity of the WFT populations. The amplified fragment length polymorphism (AFLP) technique has proven to be a reliable tool to generate highly polymorphic molecular markers used to study genetic divergence in insect populations including thrips (Fang et al. 2005) and could be a valuable technique for evaluating the diversity and genetic relationships between WFT populations. Of special interest would be the exploration of genetic difference

and potential morphological changes accompanied by the genetic differences such as antennae length and number of sensilla that may influence differences in olfaction.

Genetics of the olfactory pathways of thrips. The genetic control of olfactory response in thrips is poorly understood and studies that explore the genetic variability in olfactory response among and within natural populations are needed. The findings of this study indicate the need to elucidate the genetic basis of odour perception in WFT to understanding the relationship between genetic variation and phenotypic variation for quantitative traits. Despite considerable progress in recent years on the genetic makeup of thrips, genetic tools and the genetic classification of the olfactory system in model insects such as *Drosophila* (Carlson 1996, Su et al. 2009, Tunstall et al. 2012), no peer review publications were found on the genetics of the olfactory pathway in thrips. However, with the recent publication of the complete genomic sequence of WFT (Yan et al. 2012) as well as rapid advances in similar projects for both thrips and other insects, an increased understanding of the molecular genetics of thrips olfaction is likely to occur in the future.

Influence of temperature on thrips response. Results obtained in Chapter 3 suggested that temperature may influence both the perception of MI in the thrips directly and the behavioural responses to MI indirectly by influencing the release rate of MI. Laboratory bioassays could be used to explore how temperature affect the perception of a constant concentration of MI and thus the attractiveness of MI to walking thrips, while short trapping periods (e.g. 1-h intervals) in greenhouse situations would allow for more accurate interpretation of temperatures affect on thrips flight response and MI release rate by limiting the temperature range experienced during the trapping interval.

7.3 Conclusion

The work has demonstrated that populations of WFT from southern Spain differ in their response to an olfactory cue depending compared with other WFT populations tested. In Y-tube olfactometer experiments some populations do not respond to the lure at all while others exhibit the expected response. This outcome suggests that WFT populations worldwide show some levels of heterogeneous traits (genetically and behaviorally) regardless of the relatively recent worldwide spread of this pest species. The mechanism for this non-response is still to be determined.

The work has also shown that intrinsic and extrinsic factors play a role in the response level archived. While the definitive cause of this variable response is still unclear, the work has eliminated a number of potential causative factors, and identified where new areas of research will be most profitable. The work has also indentified that intrinsic factors can play a much larger role than previously expected.

The fundamental information obtained from the work will be used to design better tools for monitoring and trapping thrips pests using semiochemicals in New Zealand and worldwide.

Acknowledgments

I owe sincere and earnest thanks to many people who have provided invaluable help, assistance, and encouragement during the course of this study. Although it is not possible to mention all by name, I want to express my gratitude.

First and foremost, I would like to extend a very special thank you to my team of supervisors; Associate Professor Sue Worner (Bio-Protection Research Centre, Lincoln University), Dr Michael Rostas (Bio-Protection Research Centre, Lincoln University), Associate Professor R. Bruce Chapman (Insect Science Solutions, Christchurch), Dr Willem Jan de Kogel (Plant Research International, The Netherlands) and Dr David Teulon (The New Zealand Institute for Plant & Food Research Limited). Your support, knowledge, wisdom, tenacity and invaluable guidance during my research have been invaluable. One simply could not wish for a better team.

Financial support was gratefully received from the Bio-Protection Research Centre (Lincoln University), Plant Research International (The Netherlands), The New Zealand Institute for Plant & Food Research Limited and The Foundation for Research Science and Technology (New Zealand) (now the Ministry of Business, Innovation and Employment). In addition, I would like to acknowledge the financial support provided by the following organisations that enabled me to undertake part of my studies in The Netherlands and to attend overseas conferences during my study: The Royal Society of New Zealand; Marie Curie International Research Staff Exchange Scheme Fellowship within the 7th European Community Framework Program; The New Zealand Institute of Agricultural & Horticultural Science Inc.; The Japan Society for the Promotion of Science; the Ministry of Research, Science and Technology of New Zealand (now the Ministry of Business, Innovation and Employment); MacMillan Brown Agricultural Research Fund; and the New Zealand Plant Protection Society.

I would like to extend a very special thank to my line manager Dr Melanie Davidson, The New Zealand Institute for Plant & Food Research Limited – this study would not have been possible without your encouragement and support. I am also extremely indebted to Dr Ruth

Butler, The New Zealand Institute for Plant & Food Research Limited, for statistical advice and help, and who willingly devoted so much time to advising me.

My thanks to all the staff at Plant Research International for your hospitality and assistance during my visits – especially Gerrie Wiegers, Margriet Huisman-de Lange, Henry van Raaij and Dr Rob van Tol for all their help and support. In addition, I want to thank Jesús Moreno Salmerón, Jaime Gisbert Alborch and Dr José E. Belda from Koppert Biological Systems in Spain; Dr Cristina Castañé and Dr Jordi Riudavets from the Institute for Food and Agricultural Research and Technology (IRTA) in Spain; and Anne-Isabelle Lacordaire and Gisèle Broquier from Koppert Biological Systems in France for providing me with thrips for my experiments.

I thank the following growers for allowing me access to their greenhouses during my studies: David Cara (Spain), Chris Sinnotts (New Zealand) and Ron van Dijk and Sjon de Groot (The Netherlands). Your cooporation and willingness to help was greatly appreciated.

To all the staff at The New Zealand Institute for Plant & Food Research Limited Lincoln, thank you – especially Sarah Thompson, Sarah Murray, Sandi Keenan and Gabrielle Drayton for their assistance on the molecular aspects of my research, and Dr Kye Chung Park for teaching me electroantennogram techniques. Special thanks to Naomi Shaw for her assistance in sourcing numerous articles and with the endless EndNote troubles, and to Catherine Langford for editorial help and advice. Also to Melanie Walker, Nina Jorgensen, Stacey Skill, Jessica Dohmen-Vereijssen, Rachel Warren and Sam Read for always being there to give me a hand or to cheer me up with encouragements and chocolates – I will be forever grateful for your unfailing support.

Finally I would like to express gratitude to my friends and family, for their encouragement, help and understanding throughout the last three years.

References

Achor, D. S., and C. C. Childers. 1995. Fixation techniques for observing thrips morphology and injury with electron microscopy, pp. 595-600. In B. L. Parker, M. Skinner and T. Lewis (eds.), Thrips biology and management. Plenum Press, New York, USA.

Albrectsen, B., and G. Nachman. 2001. Female-biased density-dependent dispersal of a tephritid fly in a fragmented habitat and its implications for population regulation. Oikos 94: 263-272.

Alcorta, E., and J. Rubio. 1989. Intrapopulational variation of olfactory responses in *Drosophila melanogaster*. Behav. Genet. 19: 285-299.

Ananthakrishnan, T. N. 1984. Bioecology of thrips, Indira Publishing House, West Bloomfield, MI, USA.

Anholt, R. R., and D. A. Mackay. 2004. Quantitative genetic analyses of complex behaviours in Drosophila. Nature 5: 838-848.

Annand, P. N. 1926. Thysanoptera and the pollination of flowers. Am. Nat. 60: 177-182.

Antignus, Y., N. Mor, R. B. Joseph, M. Lapidot, and S. Cohen. 1996. Ultraviolet-absorbing plastic sheets protect crops from insect pests and from virus diseases vectored by insects. Environ. Entomol. 25: 919-924.

Antignus, Y., D. Nestel, S. Cohen, and M. Lapidot. 2001. Ultraviolet-deficient greenhouse environment affects whitefly attraction and flight-behavior. Environ. Entomol. 30: 394-399.

Anton, S., and U. Homberg. 1999. Antennal lobe structure, pp. 98-125. In B. S. Hansson (ed.), Insect olfaction. Springer, Berlin, Germany.

Ayyub, C., J. Paranjape, V. Rodrigues, and O. Siddiqi. 1990. Genetics of olfactory behavior in *Drosophila melanogaster*. J. Neurogenet. 6: 243-262.

Baker, T. C., W. Francke, J. G. Millar, C. Lofstedt, B. Hans Son, J.-W. Du, P. L. Phelan, R. S. Vetter, R. Youngman, and J. L. Todd. 1991. Identification and bioassay of sex pheromone components of carob moth, *Ectomyelois ceratoniae* (Zeller). J. Chem. Ecol. 17: 1973-1988.

Baoyu, H., and H. Baohong. 2007. EAG and behavioral responses of the wingless tea aphid *Toxoptera aurantii* (Homoptera: Aphididae) to tea plant volatiles. Acta Ecol. Sin. 27: 4485-4490.

Baptista, F. J., B. J. Bailey, J. M. Randall, and J. F. Meneses. 1999. Greenhouse ventilation rate: theory and measurement with tracer gas techniques. J. Agric. Eng. Res. 72: 363-374.

Bartzanas, T., T. Boulard, and C. Kittas. 2004. Effect of vent arrangement on windward ventilation of a tunnel greenhouse. Biosys. Eng. 88: 479-490.

Bautista, R. C., and R. F. L. Mau. 1994. Preferences and development of western flower thrips (Thysanoptera, Thripidae) on plant hosts of tomato spotted wilt tospovirus in Hawaii. Environ. Entomol. 23: 1501-1507.

Bell, W. L. 1990. Searching behavior patterns in insects. Annu. Rev. Entomol. 35: 447-467.

Berenguel, M., L. Yebra, and F. Rodríguez. 2003. Adaptive control strategies for greenhouse temperature control. In European Conference Control, 1-4 September 2003, Cambridge, UK.

Berlinger, M. J., S. Lebiush-Mordechi, D. Fridja, and N. Mor. 1993. The effect of types of greenhouse screens on the presence of western flower thrips: a preliminary study, pp. 13-16. In Proc. Working Group Integrated Control in Glasshouses Apr. 25-29 1993, Pacific Grove, CA, USA. IOBC/WPRS Bulletin.

Bernays, E. A., and R. F. Chapman. 1994. Host-plant selection by phytophagous insects, Chapman & Hall, New York, USA.

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

Bhasin, A., A. J. Mordue, and W. Mordue. 2001. Field studies on efficacy of host odour baits for the biting midge *Culicoides impunctatus* in Scotland. Med. Vet. Entomol. 15: 147-156.

Bielza, P., V. Quinto, J. Contreras, M. Torné, A. Martín, and P. J. Espinosa. 2007. Resistance to spinosad in the western flower thrips, *Frankliniella occidentalis* (Pergande), in greenhouses of south-eastern Spain. Pest Manage. Sci. 63: 682-687.

Bielza, P., V. Quinto, C. Gravalos, J. Abellan, and E. Fernandez. 2008. Lack of fitness costs of insecticide resistance in the western flower thrips (Thysanoptera: Thripidae). J. Econ. Entomol. 101: 499-503.

Bierl-Leonhardt, B. A., E. D. DeVilbiss, and J. R. Plimmer. 1979. Rate of release of disparlure from laminated plastic dispensers. J. Econ. Entomol. 72: 319-321.

Birkett, M. A., C. A. M. Campbell, K. Chamberlain, E. Guerrieri, A. J. Hick, J. L. Martin, M. Matthes, J. A. Napier, J. Pettersson, J. A. Pickett, G. M. Poppy, E. M. Pow, B. J. Pye, L. E. Smart, G. H. Wadhams, L. J. Wadhams, and C. M. Woodcock. 2000. New roles for cis-jasmone as an insect semiochemical and in plant defense. Proc. Natl. Acad. Sci. USA 97: 9329-9334.

Blackmer, J. L., and P. L. Phelan. 1992. Chemical generalists and behavioral specialists - a comparison of host finding by *Stelidota geminata* and *Stelidota octomaculata*. Entomol. Exp. Appl. 63: 249-257.

Blum, M. S. 1991. Chemical ecology of the Thysanoptera, pp. 95-112. In Towards Understanding Thysanoptera. Proceedings International Conference on Thrips, 21-23 February, 1989, 1991, Burlington, Vermont, USA.

Boeckh, J., and K. D. Ernst. 1987. Contribution of single unit analysis in insects to an understanding of olfactory function. J. Comp. Physiol. A. 161: 549-565.

Bohbot, J., R. J. Pitts, H. W. Kwon, M. Rützler, H. M. Robertson, and L. J. Zwiebel. 2007. Molecular characterization of the *Aedes aegypti* odorant receptor gene family. Insect Mol. Biol. 16: 525-537.

Boller, E. F. 1972. Behavioural aspects of mass-rearing of insects. Entomophaga 17: 9-25.

Boulard, T., and B. Draoui. 1995. Natural ventilation of a greenhouse with continuous roof vents: measurements and data analysis. J. Agric. Eng. Res. 61: 27-35.

Bradley, S. J., D. M. Suckling, K. G. McNaughton, C. H. Wearing, and G. Karg. 1995. A temperature-dependent model for predicting release rates of pheromone from a polyethylene tubing dispenser. J. Chem. Ecol. 21: 745-760.

Brevault, T., and S. Quilici. 2010. Interaction between visual and olfactory cues during host finding in the tomato fruit fly *Neoceratitis cyanescens*. J. Chem. Ecol. 36: 249-259.

Brødsgaard, H. 1990. The effect of anisaldehyde as a scent attractant for *Frankliniella occidentalis* (Thysanoptera: Thripidae) and the response mechanism involved. SROP/WPRS Bulletin XIII: 36-38.

Brødsgaard, H. F. 1989. Coloured sticky traps for *Frankliniella occidentalis* (Pergande) (Thysanoptera, Thripidae) in glasshouses. J. Appl. Entomol. 107: 136-140.

Brødsgaard, H. F. 1994. Insecticide resistance in European and African strains of western flower thrips (Thysanoptera: Thripidae) tested in a new residue-on-glass test. J. Econ. Entomol. 87: 1141-1146.

Broughton, S., and J. Harrison. 2012. Evaluation of monitoring methods for thrips and the effect of trap colour and semiochemicals on sticky trap capture of thrips

(Thysanoptera) and beneficial insects (Syrphidae, Hemerobiidae) in deciduous fruit trees in Western Australia. Crop Protect. 42: 156-163.

Brown, D. F., A. L. Knight, J. F. Howell, C. R. Sell, J. L. Krysan, and M. Weiss. 1992. Emission characteristics of a polyethylene pheromone dispenser for mating disruption of codling moth (Lepidoptera: Tortricidae). J. Econ. Entomol. 85: 910-917.

Bruce, T. J. A., L. J. Wadhams, and C. M. Woodcock. 2005. Insect host location: a volatile situation. Trends Plant Sci. 10: 269-274.

Brunner, P. C., E. K. Chatzivassiliou, N. I. Katis, and J. E. Frey. 2004. Host-associated genetic differentiation in *Thrips tabaci* (Insecta; Thysanoptera), as determined from mtDNA sequence data. Heredity 93: 364-370.

Brunner, P. C., and J. E. Frey. 2010. Habitat-specific population structure in native western flower thrips *Frankliniella occidentalis* (Insecta, Thysanoptera). J. Evol. Biol. 23: 797-804.

Bryan, D. E., and R. F. Smith. 1956. The *Frankliniella occidentalis* (Pergande) complex in California (Thysanoptera: Thripidae). University of California Entomological Publications 10: 359-410.

Buitenhuis, R., and J. L. Shipp. 2006. Factors influencing the use of trap plants for the control of *Frankliniella occidentalis* (Thysanoptera: Thripidae) on greenhouse potted chrysanthemum. Environ. Entomol. 35: 1411-1416.

Burger, B. V., and Z. Munro. 1986. Headspace gas analysis: quantitative trapping and thermal desorption of volatiles using fused-silica open tubular capillary traps. J. Chromatogr. 370: 449-464.

Byers, J. A. 1988. Novel diffusion-dilution method for release of semiochemicals: testing pheromone component ratios on western pine beetle. J. Chem. Ecol. 14: 199-212.

Byers, J. A. 1995. Host tree chemistry affecting colonization in bark beetles, pp. 154-213. In R. T. Carde and W. J. Williams (eds.), Chemical ecology of insects, 2 ed. Chapman and Hall, New York, USA.

Campbell, C. A. M., J. Pettersson, J. A. Pickett, L. J. Wadhams, and C. M. Woodcock. 1993. Spring migration of damson-hop aphid, *Phorodon humuli* (Homoptera, Aphididae), and summer host plant-derived semiochemicals released on feeding. J. Chem. Ecol. 19: 1569-1576.

Cardé, R., and M. Willis. 2008. Navigational strategies used by insects to find distant, wind-borne sources of odor. J. Chem. Ecol. 34: 854-866.

Carlson, J. R. 1996. Olfaction in Drosophila: from odor to behavior. Trends Genet. 12: 175-180.

Castane, C., O. Alomar, and J. Riudavets. 1996. Management of western flower thrips on cucumber with *Dicyphus tamaninii* (Heteroptera: Miridae). Biol. Control 7: 114-120.

Chaisuekul, C., and D. G. Riley. 2005. Host plant, temperature, and photoperiod effects on ovipositional preference of *Frankliniella occidentalis* and *Frankliniella fusca* (Thysanoptera: Thripidae). J. Econ. Entomol. 98 2107-2113.

Chambers, J. 1990. Overview on stored-product insect pheromones and food attractants. J. Kans. Entomol. Soc. 63: 490-499.

Chapman, R. F., and J. C. Lee. 1991. Environmental-effects on numbers of peripheral chemoreceptors on the antennae of a grasshopper. Chem. Senses 16: 607-616.

Cheah, C. A., and T. H. Coaker. 1992. Host finding and discrimination in *Diglyphus isaea*, a parasitoid of the chrysanthemum leaf miner, *Chromatomyia syngenesiae*. Biocontrol Sci. Technol. 2: 109-118.

Chermenskaya, T. D., V. N. Burov, S. P. Maniar, E. M. Pow, N. Roditakis, O. G. Selytskaya, I. V. Shamshev, L. J. Wadhams, and C. M. Woodcock. 2001. Behavioural responses of western flower thrips, *Frankliniella occidentalis* (Pergande), to volatiles from three aromatic plants. Insect Sci. Applic. 21: 67-72.

Childers, C. C., and J. K. Brecht. 1996. Colored sticky traps for monitoring *Frankliniella bispinosa* (Morgan) (Thysanoptera: Thripidae) during flowering cycles in citrus. J. Econ. Entomol. 89: 1240-1249.

Chiu, Y. J., and F. J. Messina. 1994. Effect of experience on host preference in *Callosobruchus-maculatus* (Coleoptera, Bruchidae) - variability among populations. J. Insect Behav. 7: 503-515.

Choudhury, R., and J. H. Werren. 2006. Unpublished primers. Available: http://troi.cc.rochester.edu/~wolb/FIBR/downloads.html#protocols, Accessed Dec 2010.

Chyzik, R., S. Dobrinin, and Y. Antignus. 2003. Effect of a UV-deficient environment on the biology and flight activity of *Myzus persicae* and its hymenopterous parasite *Aphidius matricariae*. Phytoparasitica 31: 467-477.

Cloyd, R. A. 2009. Western flower thrips (*Frankliniella occidentalis*) management on ornamental crops grown in greenhouses: have we reached an impasse? Pest Technol. 3: 1-9.

Clyne, P. J., C. G. Warr, M. R. Freeman, D. Lessing, J. Kim, and J. R. Carlson. 1999. A novel family of divergent seven-transmembrane proteins: candidate odorant receptors in Drosophila. Neuron 22: 327-338.

Collett, D. 2003. Modelling survival data in medical research, 2nd ed. Chapman Hall/ CRC Press, Boca Raton, FL, USA.

Combes, C. 1991. Ethological aspects of parasite transmission. Am. Nat. 138: 866-880.

Contreras, J., P. J. Espinosa, V. Quinto, J. Abellán, C. Grávalos, E. Fernández, and P. Bielza. 2010. Life-stage variation in insecticide resistance of the western flower thrips (Thysanoptera: Thripidae). J. Econ. Entomol. 103: 2164-2168.

Coss, R. G., J. G. Brandon, and A. Globus. 1980. Changes in morphology of dendritic spines on honeybee calycal interneurons associated with cumulative nursing and foraging experiences. Brain Res. 192: 49-59.

Costa, H. S., and K. L. Robb. 1999. Effects of ultraviolet-absorbing greenhouse plastic films on flight behavior of *Bemisia argentifolii* (Homoptera: Aleyrodidae) and *Frankliniella occidentalis* (Thysanoptera: Thripidae). J. Econ. Entomol. 92: 557-562.

Costa, H. S., K. L. Robb, and C. A. Wilen. 2002. Field trials measuring the effects of ultraviolet-absorbing greenhouse plastic films on insect populations. J. Econ. Entomol. 95: 113-120.

Couto, A., M. Alenius, and B. J. Dickson. 2005. Molecular, anatomical, and functional organization of the Drosophila olfactory system. Curr. Biol. 15: 1535-1547.

Cox, D. R. 1972. Regression models and life-tables. J. R. Stat. Soc. Ser. B. (Stat. Method.) 34: 187-220.

Cunningham, J. P., C. J. Moore, M. P. Zalucki, and B. W. Cribb. 2006. Insect odour perception: recognition of odour components by flower foraging moths. Proc. R. Soc. Biol. Sci. Ser. B 273: 2035-2040.

Curtis, C. E., and J. D. Clark. 1978. Responses of navel orangeworm moths to attractants evaluated as oviposition stimulants in an almond orchard. Environ. Entomol. 8: 330-333.

CycSoftware. 2009. CycDesigN 4.0 A package for the computer generation of experimental designs. Version 4.0. CycSoftware Ltd, Hamilton, New Zealand.

Czencz, K. 1987. The role of colour traps in collecting thrips fauna, pp. 9-14. In International Symposium of Thysanoptera, 14-17 July 1987, Godollo, Hungary.

Dame, A., D. B. Woodard, H. R. Ford, and D. E. Weidhaas. 1964. Field behavior of sexually sterile *Anopheles quadrimaculatus* males. Mosquito News 24: 6-14.

Davidson, M. M., D. A. J. Teulon, and N. B. Perry. 2005. Insect behavior modifying compounds. International Publication Number: WO 2005/046330, AL. Australian Patent Office, Woden, Australia.

Davidson, M. M., R. C. Butler, and D. A. J. Teulon. 2006. Starvation period and age affect the response of female *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) to odor and visual cues. J. Insect Physiol. 52: 729-736.

Davidson, M. M., R. C. Butler, S. Winkler, and D. A. J. Teulon. 2007. Pyridine compounds increase trap capture of *Frankliniella occidentalis* (Pergande) in a covered crop. N. Z. Plant Prot. 60: 56-60.

Davidson, M. M., N. B. Perry, L. Larsen, V. C. Green, R. C. Butler, and D. A. J. Teulon. 2008. 4-pyridyl carbonyl compounds as thrips lures: effectiveness for western flower thrips in Y-tube bioassays. J. Agric. Food Chem. 56: 6554-6561.

Davidson, M. M., R. C. Butler, and D. A. J. Teulon. 2009. Pyridine compounds increase thrips (Thysanoptera: Thripidae) trap capture in an onion crop. J. Econ. Entomol. 102: 1468-1471.

Davidson, M. M., R. C. Butler, and D. A. J. Teulon. 2012a. Response of female *Frankliniella occidentalis* (Pergande) to visual cues and para-anisaldehyde in a flight chamber. J. Insect Behav. 25: 297-307.

Davidson, M. M., S. M. Skill, R. C. Butler, M.-C. Nielsen, S. Keenan, and S. Bulman. 2012b. Virus status of western flower thrips does not affect their response to a thrips lure in a Y-tube olfactometer. N. Z. Plant Prot. 65: 120-125.

Davies, K. M. 2004. An introduction to plant pigments in biology and commerce, pp. 1-18. In K. M. Davies (ed.), Plant pigments and their manipulation. Annual plant reviews, vol. 14. Blackwell Publishing, Oxford, UK.

de Bruyne, M., and T. Baker. 2008. Odor detection in insects: Volatile codes. J. Chem. Ecol. 34: 882-897.

de Kogel, W. J., M. van der Hoek, and C. Mollema. 1997. Variation in performance of western flower thrips populations on susceptible and partially resistant cucumber. Entomol. Exp. Appl. 83: 73-80.

de Kogel, W. J., E. H. Koschier, and J. H. Visser. 1999. Y-tube olfactometer to determine the attractiveness of plant volatiles to western flower thrips. Proc. Exper. Appl. Entomol. 10: 131-135.

de Kogel, W. J., and E. H. Koschier. 2001. Thrips response to odours, pp. 189-190. In Proceeding of the 7th International Symposium on Thysanoptera. Reggio Calabria, Italy, 1-8 July 2001.

de Kogel, W. J., and D. A. J. Teulon. 2007. Thrips seduced by smell. FlowerTECH 10: 30-31.

de Moraes, C. M., M. C. Mescher, and J. H. Tumlinson. 2001. Caterpillar-induced nocturnal plant volatiles repel nonspecific females. Nature 410: 577-580.

de Weerdt, C. J., and F. J. Kelling. 2001. Electrophysiological characteristics of olfactory receptors of different strains of *Musca domestica*. Proc. Exper. Appl. Entomol. 12: 71-73.

Deisig, N., J. Kropf, S. Vitecek, D. Pevergne, A. Rouyar, J.-C. Sandoz, P. Lucas, C. Gadenne, S. Anton, and R. Barrozo. 2012. Differential interactions of sex pheromone and plant odour in the olfactory pathway of a male moth. PLoS ONE 7.

den Belder, E., J. Elderson, and I. Agca. 2001. Is olfactory orientation of *Thrips tabaci* disrupted by a nonhost plant? Proc. Exper. Appl. Entomol. 12: 61-64.

Desouhant, E., S. Navel, E. Foubert, D. Fischbein, M. Thery, and C. Bernstein. 2010. What matters in the associative learning of visual cues in foraging parasitoid wasps: colour or brightness? Anim. Cogn. 13: 535-543.

Diaz-Montano, J., M. Fuchs, B. A. Nault, J. Fail, and A. M. Shelton. 2011. Onion thrips (Thysanoptera: Thripidae): a global pest of increasing concern in onion. J. Econ. Entomol. 104: 1-13.

Dobrowski, S. Z., J. C. Pusknik, S. L. Ustin, and P. J. Zarco-Tejada. 2005. Simple reflectance indices track heat and water stress induced changes in steady state chlorophyll fluorescence. Remote Sens. Environ. 97: 403-414.

Dötterl, S., A. Jürgens, K. Seifert, T. Laube, B. Weißbecker, and S. Schütz. 2006. Nursery pollination by a moth in *Silene latifolia*: the role of odours in eliciting antennal and behavioural responses. New Phytol. 169: 707-718.

Drummond, A. J., B. Ashton, M. Cheung, J. Heled, M. Kearse, R. Moir, S. Stones-Havas, T. Thierer, and A. Wilson. 2010. Geneious v5.5 [computer program], Available at: http://www.geneious.com/.

Drummond, A. J., B. Ashton, S. Buxton, M. Cheung, A. Cooper, C. Duran, J. Heled, M. Kearse, S. Markowitz, R. Moir, S. Stones-Havas, S. Sturrock, F. Swidan, T. Thierer, and A. Wilson. 2012. Geneious v5.6 [computer program], Available at: http://www.geneious.com/.

Duan, J. J., and R. J. Prokopy. 1994. Apple maggot fly response to red sphere traps in relation to fly age and experience. Entomol. Exp. Appl. 73: 279-287.

Dudareva, N., and E. Pichersky. 2000. Biochemical and molecular genetic aspects of floral scents. Plant Physiol. 122: 627-634.

Dunkelblum, E., S. Gothilf, and M. Kehat. 1980. Identification of the sex pheromone of the cotton bollworm, *Heliothis armigera*, in Israel. Phytoparasitica 8: 209-211.

Eigenbrode, S. D., H. Ding, P. Shiel, and P. H. Berger. 2002. Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). Proc. R. Soc. Biol. Sci. Ser. B 269: 455-460.

El-Sayed, A. M., J. A. Byers, L. M. Manning, A. Jurgens, V. J. Mitchell, and D. M. Suckling. 2008. Floral scent of Canada thistle and its potential as a generic insect attractant. J. Econ. Entomol. 101: 720-727.

El-Sayed, A. M., V. J. Mitchell, G. F. McLaren, L. M. Manning, B. Bunn, and D. M. Suckling. 2009. Attraction of New Zealand flower thrips, *Thrips obscuratus*, to cis-jasmone, a volatile identified from Japanese honeysuckle flowers. J. Chem. Ecol. 35: 656-663.

Erber, J., T. Masuhr, and R. Menzel. 1980. Localization of short-term memory in the brain of the bee, *Apis mellifera*. Physiol. Entomol. 5: 343-358.

Espinosa, P. J., P. Bielza, J. Contreras, and A. Lacasa. 2002. Insecticide resistance in field populations of *Frankliniella occidentalis* (Pergande) in Murcia (south-east Spain). Pest Manage. Sci. 58: 967–971.

Fang, J., A. Kritzman, N. Yonash, A. Gera, N. Pollak, and U. Lavi. 2005. Genetic variation of thrips populations assessed by amplified fragment length polymorphism (Thysanoptera: Thripidae). Ann. Entomol. Soc. Am 98: 351-358.

Fereres, A., and A. Moreno. 2009. Behavioural aspects influencing plant virus transmission by homopteran insects. Virus Res. 141: 158-168.

Finch, S., and R. H. Collier. 2000. Host-plant selection by insects - a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. Entomol. Exp. Appl. 96: 91-102.

Finch, S., and R. H. Collier. 2002. Host-plant selection by insects, Marcel Dekker Inc, New York, USA.

Finch, S., H. Billiald, and R. H. Collier. 2003. Companion planting - do aromatic plants disrupt host-plant finding by the cabbage root fly and the onion fly more effectively than non-aromatic plants? Entomol. Exp. Appl. 109: 183-195.

Fletcher, L. W., H. V. Claborn, J. P. Turner, and E. Lopez. 1968. Difference in response of two strains of screw-worm flies to male pheromones. J. Econ. Entomol 61: 1386-1388.

Forbes, I., and E. W. Beck. 1954. A rapid biological technique for screening blue lupine populations for low-alkaloid plants. Agron. J. 46: 528-529.

Foster, S. A., and J. A. Endler. 1999. Geographic variation in behavior, Oxford University Press, New York, USA.

Foster, S. P., and A. J. Howard. 1998. Influence of stimuli from *Camellia japonica* on ovipositional behavior of generalist herbivore *Epiphyas postvittana*. J. Chem. Ecol. 24: 1251-1275.

Fox, A. N., R. J. Pitts, H. M. Robertson, J. R. Carlson, and L. J. Zwiebel. 2001. Candidate odorant receptors from the malaria vector mosquito *Anopheles gambiae* and evidence of down-regulation in response to blood feeding. Proc. Natl. Acad. Sci. USA 98: 14693-14697.

Francis, F., G. Lognay, and E. Haubruge. 2004. Olfactory responses to aphid and host plant volatile releases: (E)-ß-farnesene an effective kairomone for the predator *Adalia bipunctata*. J. Chem. Ecol. 30: 741-755.

Frey, J. E., T. J. Bierbaum, and G. L. Bush. 1992. Differences among sibling species *Rhagoletis mendax* and *R. pomonella* (Diptera: Ephritidae) in their antennal sensitivity to host fruit compounds. J. Chem. Ecol. 18: 2011-2024.

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

Funderburk, J., and J. Stavisky. 2004. Biology and economic importance of flower thrips. Entomology and Nematology Department, Florida Cooperative Extension Service; Institute of Food and Agricultural Sciences, University of Florida, USA.

Futuyma, D. J., and S. C. Peterson. 1985. Genetic-variation in the use of resources by insects. Annu. Rev. Entomol. 30: 217-238.

Fuyama, Y. 1976. Behavior genetics of olfactory responses in Drosophila. I. Olfactometry and strain differences in *Drosophila melanogaster*. Behav. Genet. 6: 407-420.

Fuyama, Y. 1978. Behavior genetics of olfactory responses in Drosophila. II. An odorant-specific variant in a natural population of *Drosophila melanogaster*. Behav. Genet. 8: 399-414.

Gailey, D. A., R. C. Lacaillade, and J. C. Hall. 1986. Chemosensory elements of courtship in normal and mutant, olfaction-deficient *Drosophila melanogaster*. Behav. Genet. 16: 375-405.

Gast, R. T. 1968. Mass rearing of insects: its concept, methods, and problems, pp. 59-67. In IAEA (ed.), Radiation, radioisotopes and rearing methods in the control of insect pests. International Atomic Energy Agency, Vienna, Austria.

Gaum, W. G., J. H. Giliomee, and K. L. Pringle. 1994. Life history and life tables of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), on English cucumbers. Bull. Entomol. Res. 84 (2): 219-224

GenStat Committee. 2010. The Guide to GenStat Release 13 - Parts 1-3, VSN International, Oxford, UK.

GenStat Committee. 2011. The Guide to GenStat Release 14 - Parts 1-3, VSN International, Oxford, UK.

GenStat Committee. 2012. The Guide to GenStat Release 15 - Parts 1-3, VSN International, Oxford, UK.

Gerin, C., T. Hance, and G. V. Impe. 1994. Demographical parameters of *Frankliniella occidentalis* (Pergande) (Thysanoptera, Thripidae). J. Appl. Entomol. 118: 370-377.

Gillings, M. R., D. Rae, G. A. Herron, and G. A. C. Beatti. 1995. Tracking thrips populations using DNA based methods. Proceedings of 1995 Australian & New Zealand Thrips Workshop: 97-103.

Glenn, E. P. 1984. Seasonal effects of radiation and temperature on growth of greenhouse lettuce in a high insolation desert environment. Sci. Hortic. 22: 9-21.

Glinwood, R., and J. Pettersson. 2000. Host choice and host leaving in *Rhopalosiphum padi* (Hemiptera: Aphididae) emigrants and repellency of aphid colonies on the winter host. Bull. Entomol. Res. 90: 57-61.

Golub, M., J. Weatherston, and M. H. Benn. 1983. Measurement of release rates of gossyplure from controlled release formulations by mini-airflow method. J. Chem. Ecol. 9: 323-333.

Gopinathan, K., R. Varatharajan, and T. N. Ananthakrishan. 1981. Incidence of *Microephalothrips abdmominalis* (Crawford) (Thysanoptera: Insecta) in relation to the pollination biology of the weed *Ageratum conyzoides* Linn (Compositae). Proc. Indian Natl. Sci. Acad. B Biol. Sci. 4: 505–509.

Gothilf, S., M. Kehat, M. Jacobson, and R. Galun. 1978. Screening pheromone analogues by EAG technique for biological activity on males of *Earias insulana, Heliothis armigera,* and *Spodoptera littoralis*. Environ. Entomol. 7: 31-35.

Grout, T. G., and G. I. Richards. 1990. Monitoring citrus thrips, *Scirtothrips aurantii* Faure (Thysanoptera, Thripidae), with yellow card traps and the effect of latitude on treatment thresholds. J. Appl. Entomol. 109: 385-389.

Gruber, M. Y., N. Xu, L. Grenkow, X. Li, J. Onyilagha, J. J. Soroka, N. D. Westcott, and D. D. Hegedus. 2009. Responses of the crucifer flea beetle to brassica volatiles in an olfactometer. Environ. Entomol. 38: 1467-1479.

Guedes, N. M. P., R. N. C. Guedes, G. H. Ferreira, and L. B. Silva. 2009. Flight take-off and walking behavior of insecticide-susceptible and resistant strains of *Sitophilus zeamais* exposed to deltamethrin. Bull. Entomol. Res. 99: 393-400.

Hallem, E. A., and J. R. Carlson. 2004. The odor coding system of Drosophila. Trends Genet. 20: 453-459.

Hallem, E. A., and J. R. Carlson. 2006. Coding of odors by a receptor repertoire. Cell 125: 143-160.

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

Hammack, L., and R. J. Petroski. 2004. Field capture of northern and western corn rootworm beetles relative to attractant structure and volatility. J. Chem. Ecol. 30: 1809-1825.

Han, B., and Z. Chen. 2002. Behavioral and electrophysiological responses of natural enemies to synomones from tea shoots and kairomones from tea aphids, *Toxoptera aurantii*. J. Chem. Ecol. 28: 2203-2219.

Hansen, E. A., J. E. Funderburk, S. R. Reitz, S. Ramachandran, J. E. Eger, and H. McAuslane. 2003. Within-plant distribution of *Frankliniella species* (Thysanoptera: Thripidae) and *Orius insidiosus* (Heteroptera: Anthocoridae) in field pepper. Environ. Entomol. 32: 1035-1044.

Hansson, B. S., J. N. C. Van der Pers, and J. A. N. Löfqvist. 1989. Comparison of male and female olfactory cell response to pheromone compounds and plant volatiles in the turnip moth, *Agrotis segetum*. Physiol. Entomol. 14: 147-155.

Hansson, B. S., M. Toth, C. Löfstedt, G. Szöcs, M. Subchev, and J. Löfqvist. 1990. Pheromone variation among eastern European and a western Asian population of the turnip moth *Agrotis segetum*. J. Chem. Ecol. 16: 1611-1622.

Hansson, B. S. 2002. A bug's smell – research into insect olfaction. Trends Neurosci. 25: 270–274.

Hawkes, C., and T. H. Coaker. 1979. Factors affecting the behavioural responses of the adult cabbage root fly, *Delia brassicae*, to host plant odour. Entomol. Exp. Appl. 25: 45-58.

Hern, A., and S. Dorn. 2004. A female-specific attractant for the codling moth, *Cydia pomonella*, from apple fruit volatiles. Naturwissenschaften 91: 77-80.

Herron, G. A., and B. J. Langfield. 2011. An increase in spinosad resistance detected in Australian *Frankliniella occidentalis* (Pergande) (Thripidae: Thysanoptera). Gen. Appl. Entomol. 40: 7-8.

Herzig, A. L. 1995. Effects of population-density on long-distance dispersal in the goldenrod beetle *Trirhabda-virgata*. Ecology 76: 2044-2054.

Heuskin, S., F. J. Verheggen, E. Haubruge, J. P. Wathelet, and G. Lognay. 2011. The use of semiochemical slow-release devices in integrated pest management strategies. Biotechnol Agron. Soc. Environ. 15: 459-470.

Hoffmann, A. A. 1983. Bidirectional selection for olfactory response to acetaldehyde and ethanol in *Drosophila melanogaster*. Genet. Sel. Evol. 15: 501-518.

Hofmann, R. W., B. D. Campbell, S. J. Bloor, E. E. Swinny, K. R. Markham, K. G. Ryan, and D. W. Fountain. 2003. Responses to UV-B radiation in *Trifolium repens* L. – physiological links to plant productivity and water availability. Plant Cell Environ. 26: 603-612.

Hofmeyr, J. H., and B. V. Burger. 1995. Controlled-release pheromone dispenser for use in traps to monitor flight activity of false codling moth. J. Chem. Ecol. 21: 355-363.

Hollister, B., E. A. Cameron, and D. A. J. Teulon. 1995. Effect of *p*-anisaldehyde and a yellow color on behavior and capture of western flower thrips, pp. 571-574. In B. L. Parker, M. Skinner and T. Lewis (eds.), Thrips biology and management. Plenum Press, New York, USA.

Hopper, K. R., R. T. Roush, and W. Powell. 1993. Management of genetics of biological-control introductions. Annu. Rev. Entomol. 38: 27-51.

Howlett, F. M. 1914. A trap for thrips. J. Econ. Biol. 9: 21-23.

Imai, T., M. Maekawa, and T. Murai. 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). Appl. Entomol. Zool. 36: 475-478.

Immaraju, J. A., T. D. Paine, J. A. Bethke, K. L. Robb, and J. P. Newman. 1992. Western flower thrips (Thysanoptera: Thripidae) resistance to insecticides in coastal Californian greenhouses. J. Econ. Entomol. 85: 9-14.

Ingwell, L. L., S. D. Eigenbrode, and N. A. Bosque-Perez. 2012. Plant viruses alter insect behavior to enhance their spread. Sci. Rep. 2: 1-6.

Iwama, S. 1977. A influência de factores climáticos na actividade externa de *Tetragonisca angustula* (Apidae, Meliponinae). Bolm. Zool. S. Paulo 2: 189-201.

Jacobson, A. L., J. S. Johnston, D. Rotenberg, A. E. Whitfield, W. Booth, E. L. Vargo, and G. G. Kennedy. 2013. Genome size and ploidy of Thysanoptera. Insect Mol. Biol. 22: 12-17.

Jaenike, J., and R. D. Holt. 1991. Genetic-variation for habitat preference - evidence and explanations. Am. Nat. 137: 67-90.

James, D. G. 2005. Further field evaluation of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. J. Chem. Ecol. 31: 481-495.

Jermy, T., Á. Szentesi, and J. Horváth. 1988. Host plant finding in phytophagous insects: the case of the Colorado potato beetle. Entomol. Exp. Appl. 49: 83-98.

Jones, R. L. 1996. Semiochemicals in host and mate finding behavior of *Macrocentrus grandii* Goidanich (Hymenoptera: Braconidae). Fla. Entomol. 79: 104-108.

Jonsson, P., and O. Anderbrant. 1993. Weather factors influencing catch of *Neodiprion sertifer* (Hymenoptera: Diprionidae) in pheromone traps. Environ. Entomol. 22: 445-452.

Kaissling, K.-E. 1986. Chemo-electrical transduction in insect olfactory receptors. Annu. Rev. Neurosci 9: 121-145.

Karunaratne, W. S., V. Kumar, J. Pettersson, and N. S. Kumar. 2009. Density dependence and induced resistance or behavioural response of the shot-hole borer of tea, *Xyleborus fornicatus* (Coleoptera:Scolytidae) to conspecifics and plant odours. Acta Agric. Scand. Section B - Plant Soil Sci. 59: 357-361.

Kasina, J., J. Nderitu, G. Nyamasyo, F. Olubayo, C. Waturu, E. Obudho, and D. Yobera. 2006. Diurnal population trends of *Megalurothrips sjostedti* and *Frankliniella occidentalis* (Thysanoptera: Thripidae) and their natural enemies on French bean *Phaseolus vulgaris* (Fabaceae). Int. J. Trop. Insect Sci. 26: 2-7.

Kawai, A., and C. Kitamura. 1987. Studies on population ecology of *Thrips palmi* Karny, Evaluation of effectiveness of control methods using a simulation model. Appl. Entomol. Zool. 22: 292-302.

Kennedy, J. S. 1977. Olfactory responses to distant plants and other odour sources, pp. 67-91. In H. H. Shorey and J. J. McKelvey Jr. (eds.), Chemical control of insect behavior, theory and application. Wiley, New York, USA.

Kiers, E., W. J. De Kogel, A. Balkema-Boomstra, and C. Mollema. 2000. Flower visitation and oviposition behavior of *Frankliniella occidentalis* (Tysan., Thripidae) on cucumber plants. J. Appl. Entomol. 124: 27-32.

Kigathi, R., and H. M. Poehling. 2012. UV-absorbing films and nets affect the dispersal of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). J. Appl. Entomol. 136: 761-771.

Kikuchi, T. 1973. Genetic alteration of olfactory functions in *Drosophila melanogaster*. Jpn J. Genet 48: 105-118.

Kirk, W. D. J. 1984. Ecologically selective colored traps. Ecol. Entomol. 9: 35-41.

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

Kirk, W. D. J. 1987. Effects of trap size and scent on the catches of *Thrips imiganis* Bagnall (Thysanoptera: Thripidae) J. Aust. Entomol. Soc. 26: 299-302.

Kirk, W. D. J. 1997. Distribution, abundance and population dynamics, pp. 217-258. In T. Lewis (ed.), Thrips as crop pests. CAB International, Wallingford, UK.

Kirk, W. D. J. 2001. The pest and vector from the west: *Frankliniella occidentalis*, pp. 33-42. In Proceeding of the 7th International Symposium on Thysanoptera, 1-8 July 2001, Reggio Calabria, Italy.

Kirk, W. D. J. 2002. The pest and vector from the west: *Frankliniella occidentalis*, pp. 33-42. In Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera, 1-8 July 2001, 2002, Reggio Calabria, Italy.

Kirk, W. D. J., and L. I. Terry. 2003. The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). Agric. For. Entomol. 5: 301-310.

Kirk, W. D. J., and J. G. C. Hamilton. 2004. Evidence for a male-produced sex pheromone in the western flower thrips *Frankliniella occidentalis*. J. Chem. Ecol. 30: 167-174.

Kittas, C., T. Boulard, M. Mermier, and G. Papadakis. 1996. Wind induced air exchange rates in a greenhouse tunnel with continuous side openings. J. Agric. Eng. Res. 65: 37-49.

Knight, A. L., and D. M. Light. 2005. Dose–response of codling moth (Lepidoptera: Tortricidae) to ethyl (E, Z)-2,4-decadienoate in apple orchards treated with sex pheromone dispensers. Environ. Entomol. 34: 604-609.

Kolosova, N., D. Sherman, D. Karlson, and N. Dudareva. 2001. Cellular and subcellular localization of S-adenosyl-L-methionine: Benzoic acid carboxyl methyltransferase, the enzyme responsible for biosynthesis of the volatile ester methylbenzoate in snapdragon flowers. Plant Physiol. 126: 956-964.

Koschier, E. H., W. J. De Kogel, and J. H. Visser. 2000. Assessing the attractiveness of volatile plant compounds to western flower thrips *Frankliniella occidentalis*. J. Chem. Ecol. 26: 2643-2655.

Koschier, E. H. 2006. Plant allelochemicals in thrips control strategies, pp. 221-249. In M. Rai and M. C. Carpinella (eds.), Naturally Occurring Bioactive Compounds, vol. 3. Elsevier, Amsterdam, The Netherlands.

Koschier, E. H., D. Hoffmann, and J. Riefler. 2007. Influence of salicylaldehyde and methyl salicylate on post-landing behaviour of *Frankliniella occidentalis* Pergande. J. Appl. Entomol. 131: 362-367.

Koschier, E. H. 2008. Essential oil compounds for thrips control - A review. Nat. Prod. Commun. 3: 1171-1182.

Kraan, C., and A. Ebbers. 1990. Release rates of tetradecen-1-ol acetates from polymeric formulations in relation to temperature and air velocity. J. Chem. Ecol. 16: 1041-1058.

Kreher, S. A., J. Y. Kwon, and J. R. Carlson. 2005. The molecular basis of odor coding in the Drosophila larva. Neuron 46: 445-456.

Kurtovic, A., A. Widmer, and B. J. Dickson. 2007. A single class of olfactory neurons mediates behavioural responses to a Drosophila sex pheromone. Nature 446: 542-546.

Kyamanywa, S., C. W. Baliddawa, and K. J. O. Ampofo. 1993. Effect of maize plants on colonization of cowpea plants by bean flower thrips, *Megalurothrips sjostedti*. Entomol. Exp. Appl. 69: 61-68.

Landolt, P., and O. Molina. 1996. Host-finding by cabbage looper moths (Lepidoptera: Noctuidae): learning of host odor upon contact with host foliage. J. Insect Behav. 9: 899-908.

Laubertie, E. A., S. D. Wratten, and J. R. Sedcole. 2006. The role of odour and visual cues in the pan-trap catching of hoverflies (Diptera : Syrphidae). Ann. Appl. Biol. 148: 173-178.

Lavagnino, N. J., R. R. H. Anholt, and J. J. Fanara. 2008. Variation in genetic architecture of olfactory behaviour among wild-derived populations of *Drosophila melanogaster*. J Evol Biol 21: 988-996.

Lee, Y., J. A. Nelder, and Y. Pawitan. 2006. Generalized linear models with random effects: unified analysis via H-likelihood, Chapman & Hall/CRC Press, London, UK.

Leong, J. M., and R. W. Thorp. 1999. Colour-coded sampling: the pan trap colour preferences of oligolectic and nonoligolectic bees associated with a vernal pool plant. Ecol. Entomol. 24: 329-335.

Leskey, T. C., and R. J. Prokopy. 2003. Influence of barometric pressure on odor discrimination and oviposition by adult plum curculios (Coleoptera: Curculionidae). Eur. J. Entomol. 100: 517-520.

Lewis, T. 1963. The effect of weather on emergence and take-off of overwintering *Limothrips cerealium* Haliday (Thysanoptera). Ann. Appl. Biol. 51: 489-502.

Lewis, T., and L. R. Taylor. 1965. Diurnal periodicity of flight by insects. Trans. R. Entomol. Soc. London 116: 393-435.

Lewis, T. 1973. Thrips their biology, ecology and economic importance, Academic Press, London, UK.

Lewis, T. 1997a. Pest thrips in perspective, University Press, Cambridge, UK.
Lewis, T. 1997b. Thrips as crop pests, University Press, Wallingford, UK.
Lewis, W. J., L. E. M. Vet, J. H. Tumlinson, J. C. van Lenteren, and D. R. Papaj. 1990. Variations in parasitoid foraging behavior: essential element of a sound biological control theory. Environ Entomol 19: 1183-1193.
Li, Z. X., J. A. Pickett, L. M. Field, and J. J. Zhou. 2005. Identification and expression of odorant-binding proteins of the malaria-carrying mosquitoes *Anopheles gambiae* and *Anopheles arabiensis*. Arch. Insect Biochem. Physiol. 58: 175-189.
Liang, X. H., Z. R. Lei, J. Z. Wen, and M. L. Zhu. 2010. The diurnal flight activity and influential factors of *Frankliniella occidentalis* in the greenhouse. Insect Sci. 17: 535-541.
Light, D. M., A. L. Knight, C. A. Henrick, D. Rajapaska, B. Lingren, D. J.C., K. M. Reynolds, R. Buttery, G. Merrill, J. Roitman, and B. C. Campbell. 2001. A pear-derived kairomone with pheromonal potency that attracts male and female codling moth, *Cydia pomonella* (L.). Naturwissenschaften 88: 333-338.
Lima-Camara, T. N., R. V. Bruno, P. M. Luz, M. G. Castro, R. Lourenço-de-Oliveira, M. H. F. Sorgine, and A. A. Peixoto. 2011. Dengue infection increases the locomotor activity of *Aedes aegypti* females. PLoS ONE 6: e17690.
Linn, C. E., M. G. Campbell, and W. L. Roelofs. 1988. Temperature modulation of behavioural thresholds controlling male moth sex pheromone response specificity. Physiol. Entomol. 13: 59-67.
Liu, S. Z., H. Yong, Y. B. Zhang, and X. W. Miao. 2005. Prediction and analysis model of temperature and its application to a natural ventilation multi-span plastic greenhouse equipped with insect-proof screen. J. Zhejiang Univ. Sci. B 6: 523-529.
Loughrin, J. H., T. R. Hamiltonkemp, H. R. Burton, R. A. Andersen, and D. F. Hildebrand. 1992. Glycosidically bound volatile components of *Nicotiana sylvestris* and *N.suaveolens* flowers. Phytochemistry 31: 1537-1540.
Loxdale, H. D., and G. Lushai. 1999. Slaves of the environment: the movement of herbivorous insects in relation to their ecology and genotype. Philos. Trans. R. Soc. London, Ser. B 354: 1479-1495.
Lublinkhof, J., and D. E. Foster. 1977. Development and reproductive capacity of *Frankliniella occidentalis* (Thysanoptera: Thripidae) reared at three temperatures. J. Kans. Entomol. Soc. 50: 313-316.
Ma, W. C., and J. H. Visser. 1978. Single unit analysis of odour quality coding by the olfactory antennal receptor system of the Colorado beetle. Entomol Exp. Appl 24: 520-533.
MacDonald, K. M., J. G. C. Hamilton, R. Jacobson, and W. D. J. Kirk. 2002. Effects of alarm pheromone on landing and take-off by adult western flower thrips. Entomol. Exp. Appl. 103: 279-282.
Mackay, T. F. C., J. B. Hackett, R. F. Lyman, M. L. Wayne, and R. R. H. Anholt. 1996. Quantitative genetic variation of odor-guided behavior in a natural population of *Drosophila melanogaster*. Genetics 144: 727-735.
Maeda, T., and N. Hinomoto. 2006. Effects of laboratory rearing conditions on the predatory mite *Neoseiulus womersleyi* (Schicha) (Acari: Phytoseiidae): II. Olfactory response. Int. J. Acarol. 32: 99-102.
Mainali, B., and U. Lim. 2011. Behavioral response of western flower thrips to visual and olfactory cues. J. Insect Behav. 24: 436-446.
Margolies, D. C., M. W. Sabelis, and J. E. Boyer. 1997. Response of a phytoseiid predator to herbivore-induced plant volatiles: selection on attraction and effect on prey exploitation. J. Insect Behav. 10: 695-709.

Martin, J. P., A. Beyerlein, A. M. Dacks, C. E. Reisenman, J. A. Riffell, H. Lei, and J. G. Hildebrand. 2011. The neurobiology of insect olfaction: sensory processing in a comparative context. Prog. Neurobiol. 95: 427-447.

Martin, N. A., and P. J. Workman. 1994. Confirmation of pesticide-resistant strain of western flower thrips in New Zealand. N. Z. Plant Prot. 47: 144-148.

Mateus, C., and A. Mexia. 1995. Western flower thrips response to color, pp. 567-570. In B. L. Parker, M. Skinner and T. Lewis (eds.), Thrips biology and management. Plenum Press, New York, USA.

Mathieu, F., L. O. Brun, C. Marcillaud, and B. Frerot. 1997. Trapping of the coffee berry borer within a mesh-enclosed environment: interaction of olfactory and visual stimuli. J. Appl. Entomol. 121: 181-186.

Matteson, N. A., and L. I. Terry. 1992. Response to color by male and female *Frankliniella occidentalis* during swarming and non-swarming behavior. Entomol. Exp. Appl. 63: 187-201.

McCullagh, P., and J. A. Nelder. 1989. Generalized linear models, Chapman & Hall, London, UK.

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

Metcalf, R. L., and E. R. Metcalf. 1992. Plant kairomones in insect ecology and control, Chapman and Hall, New York, USA.

Miller, D. R., and J. H. Borden. 1990. β-Phellandrene: kairomone for pine engraver, *Ips pini* (Say) (Coleoptera: Scolytidae). J. Chem. Ecol. 16.

Miller, D. R., J. H. Borden, and B. S. Lindgren. 1995. Verbenone: dose-dependent interruption of pheromone-based attraction of three sympatric species of pine bark beetles (Coleoptera: Scolytidae). Environ. Entomol. 24: 692–696.

Mirnezhad, M., N. Schidlo, P. G. L. Klinkhamer, and K. A. Leiss. 2012. Variation in genetics and performance of Dutch western flower thrips populations. J. Econ. Entomol. 105: 1816-1824.

Mochizuki, M., and E. Yano. 2007. Olfactory response of the anthocorid predatory bug *Orius sauteri* to thrips-infested eggplants. Entomol. Exp. Appl. 123: 57-62.

Moffit, H. R. 1964. A colour preference of the western flower thrips, *Frankliniella occidentalis*. J. Econ. Entomol. 57: 604-605.

Montero, J. I., N. Castilla, E. Gutierrez de Ravé, and F. Bretones. 1985. Climate under plastic in the Almeria area. Acta Hortic. 170: 227-234.

Morgan, A. C., and S. E. Crumb. 1928. Notes on the chemotropic responses of certain insects. J. Econ. Entomol. 21: 913-920.

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

Moritz, G. M., D. Morris, and L. A. Mound. 2001. Thrips ID: pest thrips of the world (CD). ACIAR. CSIRO Publishing, Collingwood, Australia.

Moritz, G. M., L. A. Mound, D. C. Morris, and A. Goldarazena. 2004. Pest thrips of the world: visual and molecular identification of pest thrips (CD-ROM), CBIT, University of Brisbane, Australia, ISBN-1-86499-781-8.

Morse, J. G., and M. S. Hoddle. 2006. Invasion biology of thrips. Annu. Rev. Entomol. 51: 67-89.

Morse, S., and W. Buhler. 1997. Integrated pest management: ideals and realities in developing countries, Lynne Rienner Publishers, Boulder, CO, USA.

Morsello, S. C., R. L. Groves, B. A. Nault, and G. G. Kennedy. 2008. Temperature and precipitation affect seasonal patterns of dispersing tobacco thrips, *Frankliniella fusca*,

and onion thrips, *Thrips tabaci* (thysanoptera: thripidae) caught on sticky traps. Environ. Entomol. 37: 79-86.
Mound, L. A., and A. K. Walker. 1982. Terebrantia (Insecta: Thysanoptera), Science Information Division, DSIR, Wellington, New Zealand.
Mound, L. A., and D. A. J. Teulon. 1995. Thysanoptera as phytophagous opportunists, pp. 3-20. In B. L. Parker, M. Skinner and T. Lewis (eds.), Thrips biology and management. Plenum Press, New York, USA.
Mound, L. A. 1996. The Thysanoptera vector species of tospoviruses. Acta Hortic. 431: 298-306.
Mound, L. A., and R. Marullo. 1996. The thrips of central and south America: an introduction, Associated Publishers, Gainesville, FL, USA.
Mound, L. A. 1997. Biological diversity, pp. 197-215. In T. Lewis (ed.), Thrips as crop pests. CAB International, Wallingford, UK.
Mound, L. A. 2002. So many thrips-so few tospoviruses, pp. 3-5. In Thrips and Tospoviruses: Proceedings of the 7th International Symposium of Thysanoptera, 1-8 July 2001, 2002, Reggio Calabria, Italy.
Mound, L. A. 2005a. Thysanoptera: diversity and interactions. Annu. Rev. Entomol. 50: 247-269.
Mound, L. A. 2005b. Fighting, flight and fecundity: behavioural determinants of Thysanoptera structural diversity, pp. 81-106. In T. N. Ananthakrishnan and D. Whitman (eds.), Insect phenotypic plasticity: diversity of responses. Science Publishers Inc., Enfield, USA.
Mound, L. A., and D. C. Morris. 2007. The insect order Thysanoptera: classification versus systematics. Zootaxa 1668: 395-411.
Mound, L. A., and A. A. Azidah. 2009. Species of the genus *Thrips* (Thysanoptera) from Peninsular Malaysia, with a checklist of recorded Thripidae Zootaxa 2023: 55-68.
Murai, T., and T. Ishii. 1982. Simple rearing method for flower thrips on pollen. Jpn J. Appl. Entomol. Zool 26: 149-154.
Murai, T. 1990. Rearing method and biology of thrips parasitoid, *Ceranisus menes*. IOBC/ WPRS Bull. 13: 142-146.
Murai, T., T. Imai, and M. Maekawa. 2000. Methyl anthranilate as an attractant for two thrips species and the thrips parasitoid *Ceranisus menes*. J. Chem. Ecol. 26: 2557-2565.
Murlis, J., J. S. Elkinton, and R. T. Carde. 1992. Odor plumes and how insects use them. Annu. Rev. Entomol. 37: 505-532.
Mustaparta, H. 2002. Encoding of plant odour information in insects: peripheral and central mechanisms. Entomol. Exp. Appl. 104: 1-13.
Muvea, A. M. 2011. The potential of coloured sticky traps with kairomonal attractants (LUREM-TR) in management of thrips on tomato and French beans. Master Thesis, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.
Nagai, T. 1983. On the relationship between the electroantennogram and simultaneously recorded single sensillum response of the european corn borer, *Ostrinia nubilalis*. Arch. Insect Biochem. Physiol. 1: 85-91.
Najar-Rodriguez, A. J., C. G. Galizia, J. Stierle, and S. Dorn. 2010. Behavioral and neurophysiological responses of an insect to changing ratios of constituents in host plant-derived volatile mixtures. J. Exp Biol 213: 3388-3397.
Nguyen, T. H. N., C. Borgemeister, J. Max, and H. M. Poehling. 2009. Manipulation of ultraviolet light affects immigration behavior of Ceratothripoides claratris (Thysanoptera: Thripidae). J. Econ. Entomol. 102: 1559-1566.
Niassy, S., N. K. Maniania, S. Subramanian, L. M. Gitonga, and S. Ekesi. 2012. Performance of a semiochemical-baited autoinoculation device treated with

Metarhizium anisopliae for control of *Frankliniella occidentalis* on French bean in field cages. Entomol. Exp. Appl. 142: 97-103.

Nielsen, M. C., D. A. J. Teulon, R. B. Chapman, R. C. Butler, G. M. Drayton, and H. Philipsen. 2010. Comparison of life history parameters of two *Frankliniella occidentalis* (Thysanoptera: Thripidae) strains in New Zealand. Environ. Entomol. 39: 303-311.

Njiru, B. N., W. R. Mukabana, W. Takken, and B. G. J. Knols. 2006. Trapping of the malaria vector *Anopheles gambiae* with odour-baited MM-X traps in semi-field conditions in western Kenya. Malar. J 5: 39.

Nordlund, D. A., R. L. Jones, and W. J. Lewis. 1981. Semiochemicals. Their role in pest control, John Wiley & Sons, New York, USA.

Nylin, S., G. H. Nygren, J. J. Windig, N. Janz, and A. Bergstrom. 2005. Genetics of host-plant preference in the comma butterfly *Polygonia album* (Nymphalidae), and evolutionary implications. Biol. J. Linn. Soc. 84: 755-765.

Olsson, P. O. C., O. Anderbrant, C. Löfstedt , A. K. Borg-Karlson, and I. Liblikas. 2005. Electrophysiological and behavioral response to chocolate volatiles in both sexes of the pyralid moths *Ephestia cautella* and *Plodia interpunctella*. J. Chem. Ecol. 31: 2947-2961.

Olsson, S. B., C. E. Linn, Jr., and W. L. Wendell. 2006. The chemosensory basis for behavioral divergence involved in sympatric host shifts II: olfactory receptor neuron sensitivity and temporal firing pattern to individual key host volatiles. J. Comp. Physiol. A 192: 289-300.

Opstad, R., S. M. Rogers, S. T. Behmer, and S. J. Simpson. 2004. Behavioural correlates of phenotypic plasticity in mouthpart chemoreceptor numbers in locusts. J. Insect Physiol. 50: 725-736.

Pannebakker, B. A., N. R. T. Garrido, B. J. Zwaan, and J. J. M. van Alphen. 2008. Geographic variation in host-selection behaviour in the Drosophila parasitoid *Leptopilina clavipes*. Entomol. Exp. Appl. 127: 48-54.

Papadaki, M., V. Harizanova, and A. Bournazakis. 2008. Influence of host plant on the population density of *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) on different vegetable cultures in greenhouses. Bulg. J. Agric. Sci. 14: 454-459.

Park, K. C., D. Elias, B. Donato, and J. Hardie. 2000. Electroantennogram and behavioural responses of different forms of the bird cherry-oat aphid, *Rhopalosiphum padi*, to sex pheromone and a plant volatile. J. Insect Physiol. 46: 597-604.

Parker, B. L., M. Skinner, and T. Lewis. 1995. Thrips biology and management, Plenum Press, New York, USA.

Pearsall, I. A., and J. H. Myers. 2001. Spatial and temporal patterns of dispersal of western flower thrips (Thysanoptera: Thripidae) in nectarine orchards in British Columbia. J. Econ. Entomol. 94: 831-843.

Pearsall, I. A. 2002. Daily flight activity of the western flower thrips (Thysan., Thripidae) in nectarine orchards in British Columbia, Canada. J. Appl. Entomol. 126: 293-302.

Penman, D. R., G. O. Osborne, S. P. Worner, R. B. Chapman, and G. F. McLaren. 1982. Ethyl nicotinate: a chemical attractant for *Thrips obscuratus* (Thysanoptera: Thripidae) in stonefruit in New Zealand. J. Chem. Ecol. 8: 1299-1303.

Pergande, T. 1895. Observations on certain Thripidae. Insect Life 7: 390-395.

Pettersson, J. 1970. An aphid sex attractant 1. Biological studies. Insect Syst & Evol. Suppl 1: 63-73.

Phillips, T. W. 1997. Semiochemicals of stored-product insects: Research and applications. J. Stored Prod. Res. 33: 17-30.

Pichersky, E., and J. Gersherzon. 2002. Plant odyssey - volatile substances. Biofutur: 22-26.

Pickett, J. A., and R. T. Glinwood. 2007. Chemical ecology, pp. 235-260. In H. F. van Emden and R. Harrington (eds.), Aphids as crop pests. CABI, Wallingford, UK.

Pivnick, K. A., B. J. Jarvis, and G. P. Slater. 1994. Identification of olfactory cues used in host-plant finding by diamondback moth, *Plutella-xylostella* (Lepidoptera, Plutellidae). J. Chem. Ecol. 20: 1407-1427.

Plettner, E., and R. Gries. 2010. Agonists and antagonists of antennal responses of gypsy moth (*Lymantria dispar*) to the pheromone (+)-disparlure and other odorants. J. Agric. Food Chem. 58: 3708-3719.

Pompanon, F., P. Fouillet, and M. Bouletreau. 1999. Physiological and genetic factors as sources of variation in locomotion and activity rhythm in a parasitoid wasp (*Trichogramma brassicae*). Physiol. Entomol. 24: 346-357.

Pow, E. M., A. M. Hooper, M. C. Luszniak, J. A. Pickett, and L. J. Wadhams. 1998. Novel strategies for improving biological control of western flower thrips on protected ornamentals - attraction of western flower thrips to verbena plants. , pp. 417-422. In The 1998 Brighton Conference - Pest & Diseases, 16 - 19 November 1998, The Brighton Centre & The Stakis Brighton Metropole Hotel, Brighton, UK.

Pow, E. M., J. A. Bennison, M. A. Birkett, M. J. Luszniak, M. Manjunatha, J. A. Pickett, I. S. Segers, L. J. Wadhams, L. R. Wardlow, and C. M. Woodcock. 1999. Behavioural responses of western flower thrips (*Frankliniella occidentalis)* to host plant volatiles, pp. 121-128. In Proceedings of the Sixth International Symposium on Thysanoptera, April 27–May 1 1998, 1999, Antalya, Turkey.

Prokopy, R. J., and E. D. Owens. 1983. Visual detection of plants by herbivorous insects. Annu. Rev. Entomol. 28: 337-364.

Prokopy, R. J., C. Bergweiler, L. Galarza, and J. Schwerin. 1994. Prior experience affects the visual ability of *Rhagoletis pomonella* flies (Diptera, Tephritidae) to find host fruit. J. Insect Behav. 7: 663-677.

Qiu, Y. T., R. C. Smallegange, H. Smid, J. J. A. van Loon, A. Galimard, P. M. Posthumus, T. van Beek, and W. Takken. 2004. GC-EAG analysis of human odours that attract the malaria mosquito *Anopheles gambiae* sensu stricto. Proc. Neth. Entomol. Soc. Meet. 15: 59-64.

Quiroz, A., and H. M. Niemeyer. 1998. Olfactometer-assessed responses of aphid *Rhopalosiphum padi* to wheat and oat volatiles. J. Chem. Ecol. 24: 113-124.

Rafalimanana, H., L. Kaiser, and J. M. Delpuech. 2002. Stimulating effects of the insecticide chlorpyrifos on host searching and infestation efficacy of a parasitoid wasp. Pest Manag Sci 58: 321-328.

Ramakers, P., and W. van Wensveen. 2008. Verbetering tripsbestrijding in paprika, pp. 48-49, Onder glas Horti-Text BV, Nootdorp, The Netherlands.

Raviv, M., and Y. Antignus. 2004. UV radiation effects on pathogens and insect pests of greenhouse-grown crops. Photochem. Photobiol. 79: 219-226.

Reddy, G. V. P., R. Fettkother, U. Noldt, and K. Dettner. 2005. Enhancement of attraction and trap catches of the old-house borer, *Hylotrupes bajulus* (Coleoptera : Cerambycidae), by combination of male sex pheromone and monoterpenes. Pest Manage. Sci. 61: 699-704.

Reitz, S. R. 2009. Biology and ecology of the western flower thrips (Thysanoptera: Thripidae): The making of a pest. Fla. Entomol. 92: 7-13.

Reitz, S. R., G. Yu-lin, and L. Zhong-ren. 2011. Thrips: pests of concern to China and the United States. Agric. Sci. Chin. 10: 867-892.

Remund, U. E., F. Boller, A. P. Economopoulos, and J. A. Tsitsipis. 1977. Flight performance of *Dacus oleae* reared on olives and artificial diet. Z. Aug. Entomol. 82: 330-339.

Rhainds, M., G. Gries, and P. S. Chew. 1997. Adaptive significance of density-dependent ballooning by bagworm larvae, *Metisa plana* (Walker) (Lepidoptera: Psychidae). Can. Entomol. 129: 927-931.

Rhainds, M., and L. Shipp. 2003. Dispersal of adult western flower thrips (Thysanoptera: Thripidae) on chrysanthemum plants: impact of feeding-induced, senescence of inflorescences. Environ. Entomol. 32: 1056-1065.

Rhainds, M., and L. Shipp. 2004. Dispersal of adult western flower thrips (Thysanoptera: Thripidae) in greenhouse crops. Can. Entomol. 136: 241-254.

Richgels, P. K., and S. M. Rollmann. 2012. Genetic variation in odorant receptors contributes to variation in olfactory behavior in a natural population of *Drosophila melanogaster*. Chem. Senses 37: 229-240.

Riedell, W. E., and T. M. Blackmer. 1999. Leaf reflectance spectra of cereal aphid-damaged wheat. Crop Sci. 39: 1835-1840.

Robb, K. L., and M. P. Parrell. 1991. Western flower thrips, a serious pest of floricultural crops, pp. 343-358. In Towards Understanding Thysanoptera. Proceedings International Conference on Thrips, 21-23 February, 1989, 1991, Burlington, Vermont, USA.

Robb, K. L., J. Newman, J. K. Virzi, and M. P. Parrell. 1995. Insecticide resistance in western flower thrips, pp. 341-346. In B. L. Parker, M. Skinner and T. Lewis (eds.), Thrips biology and management. Plenum Press, New York, USA.

Roberts, C. A., R. G. Dietzgen, L. A. Heelan, and D. J. Maclean. 2000. Real-time RT-PCR fluorescent detection of tomato spotted wilt virus. J. Virol. Methods 88: 1-8.

Roditakis, N. E., and D. P. Lykouressis. 1996. Prospects for the use of volatile chemicals and a new pyrrole in integrated pest management of western flower thrips. Acta Hortic. 431: 513-520.

Rodriguez-Saona, C. R., and L. L. Stelinski. 2009. Behavior-modifying strategies in IPM: theory and practice pp. 263-315. In R. Peshin and A. K. Dhawan (eds.), Integrated pest management: innovation-development process. Springer, Amsterdam, The Netherlands.

Rojas, J. C., and T. D. Wyatt. 1999. Role of visual cues and interaction with host odour during the host-finding behaviour of the cabbage moth. Entomol. Exp. Appl. 91: 59-65.

Rollmann, S. M., P. Wang, P. Date, S. A. West, T. F. Mackay, and R. R. Anholt. 2010. Odorant receptor polymorphisms and natural variation in olfactory behavior in *Drosophila melanogaster*. Genetics 186: 687-697.

Ross, D. W., and G. Daterman. 1998. Pheromone-baited traps for *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae): Influence of selected release rates and trap designs. J. Econ. Entomol. 91: 500-506.

Rousse, P., F. Gourdon, M. Roubaud, F. Chiroleu, and S. Quilici. 2009. Biotic and abiotic factors affecting the flight activity of *Fopius arisanus*, an egg-pupal parasitoid of fruit fly pests. Environ. Entomol. 38: 896-903.

Rugman-Jones, P. F., M. S. Hoddle, L. A. Mound, and R. Stouthamer. 2006. Molecular identification key for pest species of Scirtothrips (Thysanoptera: Thripidae). J. Econ. Entomol. 99: 1813-1819.

Rugman-Jones, P. F., M. S. Hoddle, and R. Stouthamer. 2010. Nuclear-mitochondrial barcoding exposes the global pest western flower thrips (Thysanoptera: Thripidae) as two sympatric cryptic species in its native California. J. Econ. Entomol. 103: 877-886.

Ruisinger, M. 2008. Förderung des biologischen Pflanzenschutzen im Zierpflanzenbaum, pp. 78-79, Jahresbuch Pflanzenschutzdienst der Landwirtschaftskammer Nordrhein-Westfalen. Pflanzenschutzdiens, Bonn, Germany.

Rützler, M., and L. J. Zwiebel. 2005. Molecular biology of insect olfaction: recent progress and conceptual models. J. Comp. Physiol. A 191: 777-790.

Saïd, I., M. Renou, J.-P. Morin, J. S. Ferreira, and D. Rochat. 2005. Interactions between acetoin, a plant volatile, and pheromone in *Rhynchophorus palmarum*: behavioral and olfactory neuron responses. J. Chem. Ecol. 31: 1789-1805.

Saïd, I., R. Torre, J.-P. Morin, and D. Rochat. 2006. Adaptation of a four-arm olfactometer for behavioural bioassays of large beetles. Chemoecology 16: 9-16.

Sakimura, K. 1962. *Frankliniella occidentalis (*Thysanoptera: Thripidae), a vector of the tomato spotted wilt virus, with special reference to the color forms. Ann. Entomol. Soc. Am. 55: 387-389.

Salguero Navas, V. E., J. E. Funderburk, R. J. Beshear, S. M. Olson, and T. P. Mack. 1991. Seasonal patterns of *Frankliniella* spp. (Thysanoptera: Thripidae) in tomato flowers. J. Econ. Entomol. 84: 1818-1822.

Sampson, C., J. G. C. Hamilton, and W. D. J. Kirk. 2012. The effect of trap colour and aggregation pheromone on trap catch of *Frankliniella occidentalis* and associated predators in protected pepper in Spain. IOBC/WPRS Bulletin 80: 313-318.

Samways, M. J. 1986. Spatial distribution of *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae) and threshold level for one per cent. damage on citrus fruit based on trapping with fluorescent yellow sticky traps. Bull. Entomol. Res. 76: 649-659.

Sase, S. 2006. Air movement and climate uniformity in ventilated greenhouses. Acta Hortic. 719: 313-324.

Sato, K., and K. Touhara. 2009. Insect olfaction: receptors, signal transduction, and behavior, pp. 203-220. In S. Korsching and W. Meyerhof (eds.), Chemosensory systems in mammals, fishes, and insects, vol. 47. Springer, Berlin, Germany.

Sauls, C., D. A. Nordlund, and W. J. Lewis. 1979. Kairomones and their use for management of entomophagous insects VIII. Effect of diet on the kairomonal activity of frass from *Heliothis zea* (Boddie) larvae for *Microplitis croceipes* (Cresson). J. Chem. Ecol. 5: 363-369.

Schmera, D., M. Toth, M. Subchev, I. Sredkov, I. Szarukan, T. Jermy, and A. Szentesi. 2004. Importance of visual and chemical cues in the development of an attractant trap for *Epicometis (Tropinota) hirta* Poda (Coleoptera : Scarabaeidae). Crop Protect. 23: 939-944.

Schneider, D. 1957. Elektrophysiologische untersuchungen von chemo- und mechanorezeptoren der antenne des seidenspinners *Bombyx mori* L. Zeitschift für Vergleichende Physiologie 40: 8-41.

Schoonhoven, L. M. 1976. Electroantennograms (EAG) as a tool in the analysis of insects attractants, pp. 59-63. In Studies of the shootborer, *Hypsipyla grandella* (Zeller) Lep. Pyralidae, 1976, Turrialba, Costa Rica. CATIE Misc. Publ. No. 1.

Schoonhoven, L. M., J. J. A. v. Loon, and M. Dicke. 2005. Insect-plant biology, University Press, Oxford, UK.

Sedy, K. A., and E. H. Koschier. 2003. Bioactivity of carvacrol and thymol against *Frankliniella occidentalis* and *Thrips tabaci*. J. Appl. Entomol. 127: 313-316.

Sengupta, P., J. H. Chou, and C. I. Bargmann. 1996. Odr-10 encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl. Cell 84: 875-887.

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

Shaver, T. N., P. D. Lingren, and H. F. Marshall. 1997. Nighttime variation in volatile content of flowers of the night blooming plant *Gaura drummondii*. J. Chem. Ecol. 23: 2673-2682.

Shem, P. M., P. M. Shiundu, N. K. Gikonyo, A. H. Ali, and R. K. Saini. 2009. Release kinetics of a synthetic tsetse allomone derived from waterbuck odour from a Tygon silicon dispenser under laboratory and semi field conditions. Am-Eurasian. J. Agric. Environ. Sci. 6: 625-636.

Shimoda, T., J. Takabayashi, W. Ashihara, and A. Takafuji. 1997. Response of predatory insect *Scolothrips takahashii* toward herbivore-induced plant volatiles under laboratory and field conditions. J. Chem. Ecol. 23: 2033-2048.

Shipp, J. L., and N. Zariffa. 1991. Spatial patterns of and sampling methods for western flower thrips (Thysanoptera: Thripidae) on greenhouse sweet pepper. Can. Entomol. 123: 989-1000.

Shipp, J. L., and T. J. Gillespie. 1993. Influence of temperature and water-vapor pressure deficit on survival of *Frankliniella occidentalis* (Thysanoptera, Thripidae). Environ. Entomol. 22: 726-732.

Shipp, J. L., M. R. Binns, X. Hao, and K. Wang. 1998. Economic injury levels for western flower thrips (Thysanoptera: Thripidae) on greenhouse sweet pepper. J. Econ. Entomol. 91: 671-677.

Shipp, J. L., and Y. Zhang. 1999. Using greenhouse microclimate to improve the efficacy of insecticide application for *Frankliniella occidentalis* (Thysanoptera: Thripidae). J. Econ. Entomol. 92: 201-206.

Sihag, R. C., and D. P. Abrol. 1986. Correlation and path-coefficient analysis of environmental factors influencing flight activity of *Apis florea* F. J. Apic. Res. 25: 202-208.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Amer. 87: 651-701.

Skill, S. M., M. M. Davidson, and R. C. Butler. 2012. The relative importance of visual and olfactory cues to onion thrips (*Thrips tabaci*) in host and non-host crops. N.Z. Plant Prot. 65: 294.

Smith, J. G. 1976. Influence of crop backgrounds on aphids and other phytophagous insects on brussels sprouts. Ann. Appl. Biol. 83: 1-13.

Smits, P. H., P. van Deventer, and W. J. de Kogel. 2000. Western flower thrips: reactions to odours and colours. Proc. Exper. Appl. Entomol. 11: 175-180.

Southwood, T. R. E. 1978. Ecological methods, with particular reference to the study of insect populations, 2nd ed. Chapman & Hall, London, UK.

Stavisky, J., J. Funderburk, B. V. Brodbeck, S. M. Olson, and P. C. Andersen. 2002. Population dynamics of *Frankliniella* spp. and tomato spotted wilt incidence as influenced by cultural management tactics in tomato. J. Econ. Entomol. 95: 1216-1221.

Stenberg, J. A., and L. Ericson. 2007. Visual cues override olfactory cues in the host-finding process of the monophagous leaf beetle *Altica engstroemi*. Entomol. Exp. Appl. 125: 81-88.

Stipanovic, A. J., P. J. Hennessy, F. X. Webster, and Y. Takahashi. 2004. Microparticle dispensers for the controlled release of insect pheromones. J. Agric. Food Chem. 52: 2301-2308.

Su, C.-Y., K. Menuz, and J. R. Carlson. 2009. Olfactory perception: receptors, cells, and circuits. Cell 139: 45-59.

Suckling, D. M. 2000. Issues affecting the use of pheromones and other semiochemicals in orchards. Crop Protect. 19: 677-683.

Sweeney, J., J. M. Gutowski, J. Price, and P. De Groot. 2006. Effect of semiochemical release rate, killing agent, and trap design on detection of *Tetropium fuscum* (f.) and other longhorn beetles (Coleoptera: Cerambycidae). Environ. Entomol. 35: 645-654.

Tanaka, K., Y. Uda, Y. Ono, T. Nakagawa, M. Suwa, R. Yamaoka, and K. Touhara. 2009. Highly selective tuning of a silkworm olfactory receptor to a key mulberry leaf volatile. Curr. Biol. 19: 881-890.

Tanny, J., M. Teitel, and M. Barak. 2005. Airflow patterns through roof openings of a naturally ventilated greenhouse. Acta Hortic. 691: 662-670.

Tapia, S., F. Pardo, F. Perich, and A. Quiroz. 2005. Clover root borer *Hylastinus obscurus* (Marsham) (Coleoptera: Scolytidae) has no preference for volatiles from root extracts of disease infected red clover. Acta Agric. Scand. Section B - Plant Soil Sci. 55: 158-160.

Tatemoto, S., and T. Shimoda. 2008. Olfactory responses of the predatory mites (*Neoseiulus cucumeris*) and insects (*Orius strigicollis*) to two different plant species infested with onion thrips (*Thrips tabaci*). J. Chem. Ecol. 34: 605-613.

Tavella, L., R. Tedeschi, G. Mason, and P. Roggero. 2001. Efficiency of north-western Italian thrips populations in transmitting tospoviruses. In Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera, 1-8 July 2001, Reggio Calabria, Italy.

Technau, G. M. 1984. Fiber number in the mushroom bodies of adult *Drosophila melanogaster* depends on age, sex and experience. J. Neurogenet. 1: 113-126.

Teitel, M., and J. Tanny. 1999. Natural ventilation of greenhouses: experiments and model. Agric. Forest Meteorol. 96: 59-70.

Teitel, M., G. Ziskind, O. Liran, V. Dubovsky, and R. Letan. 2008. Effect of wind direction on greenhouse ventilation rate, airflow patterns and temperature distributions. Biosys. Eng. 101: 351-369.

Terry, I., and M. Schneider. 1993. Copulatory behaviour and mating frequency of the western flower thrips, *Frankliniella occidentalis* (Insecta: Thysanoptera). J. Pure Appl. Zool. 4: 339–354.

Terry, L. I. 1997. Host selection, communication and reproductive behaviour, pp. 65-118. In T. Lewis (ed.), Thrips as crop pests. CAB International, Wallingford, UK.

Teulon, D. A. J., and P. M. J. Ramakers. 1990. A review of attractants for trapping thrips with particular reference to glasshouses. SROP/WPRS Bulletin 13: 212-214.

Teulon, D. A. J. 1992. Laboratory technique for rearing western flower thrips (Thysanoptera: Thripidae). J. Econ. Entomol. 85: 895-899.

Teulon, D. A. J., and D. R. Penman. 1992. Colour preferences of New Zealand thrips (Terebrantia: Thysanoptera). N. Z. Entomol. 1: 8-13.

Teulon, D. A. J., B. Hollister, and E. A. Cameron. 1993a. Behavioural responses of western flower thrips to anisaldehyde, and implications for trapping in greenhouses. Bulletin OILB/SROP 16: 177-180.

Teulon, D. A. J., D. R. Penman, and P. M. J. Ramakers. 1993b. Volatile chemicals for thrips (Thysanoptera, Thripidae) host-finding and applications for thrips pest-management. J. Econ. Entomol. 86: 1405-1415.

Teulon, D. A. J., B. Hollister, R. C. Butler, and E. A. Cameron. 1999. Colour and odour responses of flying western flower thrips: wind tunnel and greenhouse experiments. Entomol. Exp. Appl. 93: 9-19.

Teulon, D. A. J., and M. C. Nielsen. 2005. Distribution of western (glasshouse strain) and Intonsa flower thrips in New Zealand. N. Z. Plant Prot. 58: 208-212.

Teulon, D. A. J., R. C. Butler, D. E. James, and M. M. Davidson. 2007a. Odour-baited traps influence thrips capture in proximal unbaited traps in the field. Entomol. Exp. Appl. 123: 253-262.

Teulon, D. A. J., M. M. Davidson, D. I. Hedderley, D. E. James, C. D. Fletcher, L. Larsen, V. C. Green, and N. B. Perry. 2007b. 4-pyridyl carbonyl and related compounds as thrips lures: effectiveness for onion thrips and New Zealand flower thrips in field experiments. J. Agric. Food Chem. 55: 6198-6205.

Teulon, D. A. J., M. C. Nielsen, D. E. James, S. Winkler, A. R. G. McLachlan, and N. B. Perry. 2007c. Combination of two odour chemical lures does not increase thrips capture in field bioassays. N. Z. Plant Prot. 60: 61-66.

Teulon, D. A. J., M. M. Davidson, M. C. Nielsen, N. B. Perry, R. W. H. M. van Tol, and W. J. de Kogel. 2008a. The potential use of lures for thrips biological control in greenhouses: practice and theory, pp. 301–308. In Proceedings of the Third International Symposium on Biological Control of Arthropods, 8-13 February, 2008 2008a, Christchurch, New Zealand.

Teulon, D. A. J., M. C. Nielsen, W. J. de Kogel, R. W. H. M. van Tol, and M. M. Davidson. 2008b. A new lure for *Thrips major*. N. Z. Plant Prot. 61: 386.

Teulon, D. A. J., M. M. Davidson, N. B. Perry, M. C. Nielsen, R. W. H. M. van Tol, and W. J. de Kogel. 2011. Recent developments with methyl isonicotinate, a semiochemical used in thrips pest management. N. Z. Plant Prot. 64: 287.

Till, C. M., R. C. Butler, P. A. Horne, N. Hives, and D. A. J. Teulon. 2009. Using LUREM-TR to trap thrips in glasshouse crops in Victoria, Australia. N. Z. Plant Prot. 62: 398.

Torr, S. J., D. R. Hall, R. J. Phelps, and G. A. Vale. 1997. Methods for dispensing odour attractants for tsetse flies (Diptera: Glossinidae). Bull. Entomol. Res. 87: 299-311.

Touhara, K., and L. B. Vosshall. 2009. Sensing odorants and pheromones with chemosensory receptors. Annu. Rev. Physiol. 71: 307-332.

Tunstall, N. E., A. Herr, M. de Bruyne, and C. G. Warr. 2012. A screen for genes expressed in the olfactory organs of *Drosophila melanogaster* identifies genes involved in olfactory behaviour. PLoS ONE 7: e35641.

Turlings, T. C. J., A. C. Davison, and C. Tamo. 2004. A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. Physiol. Entomol. 29: 45-55.

Utchida, M. 1973. Pesticidal apparatus containing anisaldehyde and cinnamaldehyde. Japanese patent 73-06, 537.

van de Wetering, F., M. van der Hoek, R. Goldbach, C. Mollema, and D. Peters. 1999a. Variation in tospovirus transmission between populations of *Frankliniella occidentalis* (Thysanoptera: Thripidae). Bull. Entomol. Res. 89: 579-588.

van de Wetering, F., M. van der Hoek, R. Goldbach, and D. Peters. 1999b. Differences in tomato spotted wilt virus vector competency between males and females of *Frankliniella occidentalis*. Entomol. Exp. Appl. 93: 105-112.

Van der Pers, J. N. C., and A. K. Minks. 1993. Pheromone monitoring in the field using single sensillum recording. Entomol. Exp. Appl. 68: 237-245.

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

van Tol, R., D. E. James, W. J. de Kogel, and D. A. J. Teulon. 2007. Plant odours with potential for a push-pull strategy to control the onion thrips, *Thrips tabaci*. Entomol. Exp. Appl. 122: 69-76.

van Tol, R. W. H. M., A. de Bruin, R. C. Butler, M. M. Davidson, D. A. J. Teulon, and J. de Kogel. 2012. Methyl isonicotinate induces increased walking and take-off

behaviour in western flower thrips, *Frankliniella occidentalis*. Entomol. Exp. Appl. 142: 181-190.
Vassiliou, V. A. 2010. Ecology and behavior of *Pezothrips kellyanus* (Thysanoptera: Thripidae) on citrus. J. Econ. Entomol. 103: 47-53.
Venzon, M., A. Janssen, and M. W. Sabelis. 1999. Attraction of a generalist predator towards herbivore-infested plants. Entomol. Exp. Appl. 93: 305-314.
Vernon, R. S., and D. R. Gillespie. 1990. Spectral responsiveness of *Frankliniella occidentalis* (Thysanoptera: Thripidae) determined by trap catches in greenhouses. Environ. Entomol. 19: 1229-1241.
Vet, L. E. M., J. C. van Lenteren, M. Heymans, and E. Meelis. 1983. An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. Physiol. Entomol. 8: 97-106.
Vet, L. E. M., W. J. Lewis, D. R. Papaj, and J. C. van Lenteren. 1990. A variable-response model for parasitoid foraging behavior. J. Insect Behav. 3: 471-490.
Via, S. 1990. Ecological genetics and host adaptation in herbivorous insects - the experimental-study of evolution in natural and agricultural systems. Annu. Rev. Entomol. 35: 421-446.
Vierbergen, G. 2002. Occurrence of glasshouse Thysanoptera in the open in the Netherlands, pp. 359-362. In Thrips and Tospoviruses: Proceedings of the 7th International Symposium on Thysanoptera, 1-8 July 2001, 2002, Reggio Calabria, Italy.
Visser, J. H. 1979. Electroantennogram responses of the Colorado beetle, *Leptinotarsa decemlineata*, to plant volatiles. Entomol. Exper. Appl. 25: 86-97.
Visser, J. H. 1983. Differential sensory perceptions of plant compounds by insects, pp. 215-230. In P. A. Hedin (ed.), Plant resistance to insects, vol. 208. American Chemical Society, Washington, USA.
Visser, J. H. 1986. Host odor perception in phytophagous insects. Annu. Rev. Entomol. 31: 121-144.
Visser, J. H., and J. W. Taanman. 1987. Odour-conditioned anemotaxis of apterous aphids (*Cryptomyzus korschelti)* in response to host plants. Physiol. Entomol. 12: 473-479.
Visser, J. H. 1988. Host-plant finding by insects - orientation, sensory input and search patterns. J. Insect Physiol. 34: 259-268.
Visser, J. H., and R. de Jong. 1988. Olfactory coding in the perception of semiochemicals. J. Chem. Ecol. 14: 2005-2018.
Visser, J. H., H. den Ouden, and S. Voerman. 1990. The perception of attractants and repellents by insects. Proc. Exper. Appl. Entomol. 1: 37-41.
Vosshall, L. B., and R. F. Stocker. 2007. Molecular architecture of smell and taste in Drosophila. Annu. Rev. Neurosci. 30: 505-533.
Wackers, F. L., and W. J. Lewis. 1994. Olfactory and visual learning and their combined influence on host site location by the parasitoid *Microplitis croceipes* (Cresson). Biol. Control 4: 105-112.
Wang, C. L., F. C. Lin, Y. C. Chiu, and H. T. Shih. 2010a. Species of *Frankliniella* Trybom (Thysanoptera: Thripidae) from the Asian-Pacific area. Zool. Stud. 49: 824-838.
Wang, G., A. F. Carey, J. R. Carlson, and L. J. Zwiebel. 2010b. Molecular basis of odor coding in the malaria vector mosquito *Anopheles gambiae*. Proc. Natl. Acad. Sci. USA 107: 4418-4423.
Wang, H., W.-F. Guo, P.-J. Zhang, Z.-Y. Wu, and S.-S. Liu. 2008. Experience-induced habituation and preference towards non-host plant odors in ovipositing females of a moth. J. Chem. Ecol. 34: 330-338.
Wang, S., T. Boulard, and R. Haxaire. 1999. Air speed profiles in a naturally ventilated greenhouse with a tomato crop. Agric. Forest Meteorol. 96: 181-188.

Wellington, W. G. 1946. The effects of variations is atmospheric pressure upon insects. Can. J. Res. Dis 24: 51-70.

Wenninger, E. J., L. L. Stelinski, and D. G. Hall. 2009. Roles of olfactory cues, visual cues, and mating status in orientation of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) to four different host plants. Environ. Entomol. 38: 225-234.

Wieczorek, H. 1976. The glycoside receptor of the larvae of *Mamestra brassicae* L. (Lepidoptera, Noctuidae). J. Comp. Physiol. 106: 153-176.

Williams, C. R., R. Bergbauer, M. Geier, D. L. Kline, U. R. Bernier, R. Russell, and S. A. Ritchie. 2006. Laboratory and field assessment of some kairomone blends for host-seeking *Aedes aegypti*. J. Am. Mosq. Control Assoc. 22: 641-647.

Wogin, M. J., R. C. Butler, D. A. J. Teulon, and M. M. Davidson. 2010. Field response of onion thrips and New Zealand flower thrips to single and binary blends of thrips lures. Can. Entomol. 142: 75-79.

Workman, P. J., G. P. Walker, and S. Winkler. 2007. Incidence and control of thrips on outdoor lettuce at Pukekohe. N. Z. Plant Prot. 60: 42-49.

Xia, Y., G. Wang, D. Buscariollo, R. J. Pitts, H. Wenger, and L. J. Zwiebel. 2008. The molecular and cellular basis of olfactory-driven behavior in *Anopheles gambiae* larvae. Proceedings of the National Academy of Sciences 105: 6433-6438.

Yan, D., Y. Tang, X. Xue, M. Wang, F. Liu, and J. Fan. 2012. The complete mitochondrial genome sequence of the western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae) contains triplicate putative control regions. Gene 506: 117-124.

Yudin, L. S., W. C. Mitchell, and J. J. Cho. 1987. Color preference of thrips (Thysanoptera: Thripidae) with reference to aphids and leafminers in Hawaiian lettuce farms. J. Econ. Entomol. 80: 51-55.

Yunis, H., Y. Elad, and Y. Mahrer. 1990. Effects of air temperature, relative humidity and canopy wetness on gray mold of cucumbers in unheated greenhouses. Phytoparasitica 18: 203-215.

Zhang, G. F., X. Q. Meng, L. Min, W. N. Qiao, and F. H. Wan. 2011. Rapid diagnosis of the invasive species, *Frankliniella occidentalis* (Pergande): a species-specific COI marker. J. Appl. Entomol. 136: 410-420.

Zhang, S., C. Xiao, Z. Li, X. Jiang, H. Tai, C. Hu, and X. Wang. 2007a. Attraction of the volatiles from mango fruits for oriental fruit fly, *Dacus dorsalis* (Hendel). J. Yunnan. Agric. Univ. 22: 659-664.

Zhang, Z. J., Q. J. Wu, X. F. Li, Y. J. Zhang, B. Y. Xu, and G. R. Zhu. 2007b. Life history of western flower thrips, *Frankliniella occidentalis* (Thysan., Thripae), on five different vegetable leaves. J. Appl. Entomol. 131: 347-354.

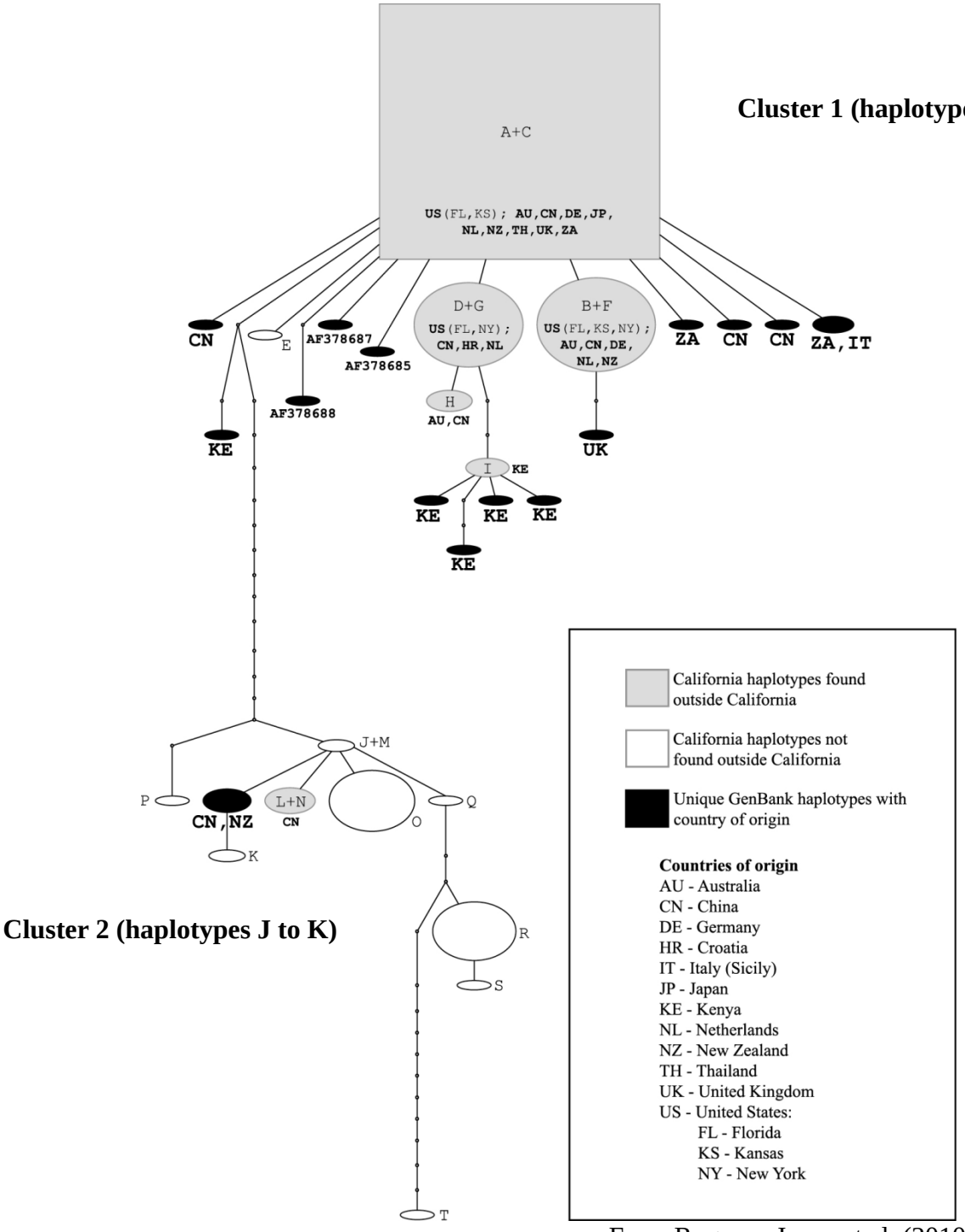
Zhou, S., E. A. Stone, T. F. C. Mackay, and R. R. H. Anholt. 2009. Plasticity of the chemoreceptor repertoire in *Drosophila melanogaster*. PLoS Genet 5.

Zhu, X. Y., P. J. Zhang, and Y. B. Lu. 2012. Isolation and identification of the aggregation pheromone released by male adults of *Frankliniella intonsa* (Thysanoptera: Thripidae). Acta Entomol. Sin. 55: 376-385.

Zwiebel, L. J., and W. Takken. 2004. Olfactory regulation of mosquito–host interactions. Insect Biochem. Mol. Biol. 34: 645-652.

Appendices

Appendix I: Global distribution of WFT mtDNA haplotypes.

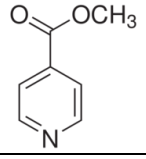


Cluster 1 (haplotypes A to I)

Cluster 2 (haplotypes J to K)

From Rugman-Jones et al. (2010)

Appendix II: Physical and chemical properties of methyl isonicotinate (MI).

Empirical formula	$C_7H_7NO_2$
Structure	
Form (appearance)	Liquid
Colour	Dark brown
Molecular mass	137.14 g/mol
Melting point	8–8.5^{o}C
Boiling point	207–209^{o}C
Flash point	82^{o}C
Density	1.161 g/cm^3 at 25^{o}C
pH	No data available
Ignition temperature	No data available
Explosion limits (upper/lower)	No data available
Vapour pressure	No data available
Water solubility	No data available
Partition coefficient: n-octanol/water	No data available
Relative vapour density	No data available
Odour	No data available
Odour threshold	No data available
Evaporation rate	No data available

The information is obtained from the supplier's webpage and available MSDS sheet (go to http://www.sigmaaldrich.com for further information (product number M52950)).

Appendix III: Morphological features used to differentiate thrips in the *Frankliniella* genus.

The genus *Frankliniella* includes about 180 described species. Most of these are known only from the neotropics, but ***F. occidentalis, F. Schultzei*** and ***F. Williamsi*** have been widely introduced around the world (Kirk and Terry 2003), and more recently ***F. intonsa*** (Vierbergen 2002, Moritz et al. 2004, Teulon and Nielsen 2005, Mound and Azidah 2009, Wang et al. 2010a).

The genus can be distinguished by the following morphological structures:

- The presence of three pairs of ocellar setae
- Complete rows of setae on both first and second veins of the forewing
- Pronotum with five pairs of elongated setae*
- Paired ctenidia laterally on the abdominal tergites.

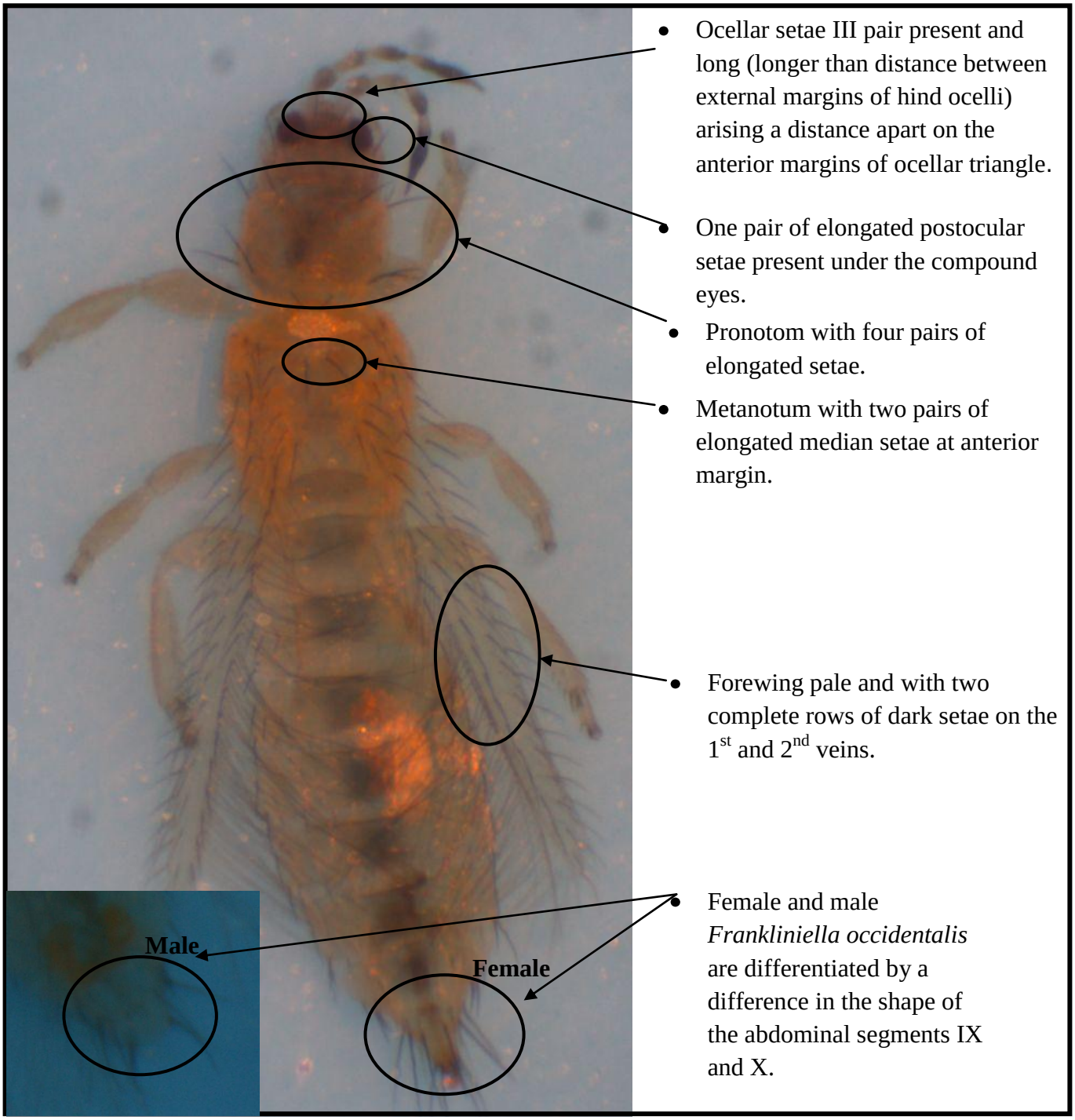
In Europe four pest species of *Frankliniella* are known: *F. occidentalis, F. intonsa, F. schultzei and F. tenuicornis* (Moritz et al. 2004). The table below highlights the relevant morphological details that allow differentiation of *F. occidentalis* (WFT) from the other species under a stereomicroscope (>x100).

F. occidentalis (WFT)	***F. intonsa***	***F. schultzei***	***F. tenuicornis***
Ocellar setae III pair present and long (longer than distance between external margins of hind ocelli), arising a distance apart on the anterior margins of ocellar triangle.	Ocellar pair III sorter (only slightly longer than side of ocellar triangle).	Close placement of ocellar setae III within the ocellar triangle.	
One pair of elongated postocular setae present under the compound eyes.	No elongated setae present.		No elongated seta present.
Pronotum with five pairs of major elongated setae			Anteromarginal setae noticeably shorter than anteroangulars setae.
Metanotum with two pairs of elongated median setae at anterior margin.			
Mainly dark yellow with brown areas medially.	Body colour brown.		Body colour dark brown.

**This criterion distinguishes Frankliniella spp. from all other pest species of thrips in Moritz et al. (2004) except Kakothrips pisivorus, a common pest on peas. Criteria such as main body colour, forewing colour and shading, and lack of minor setae medially on the posterior margin allowed for quick discrimination between the Frankliniella species and K. pisivorus.*

Appendix IV: Morphological features used to identify western flower thrips (WFT).

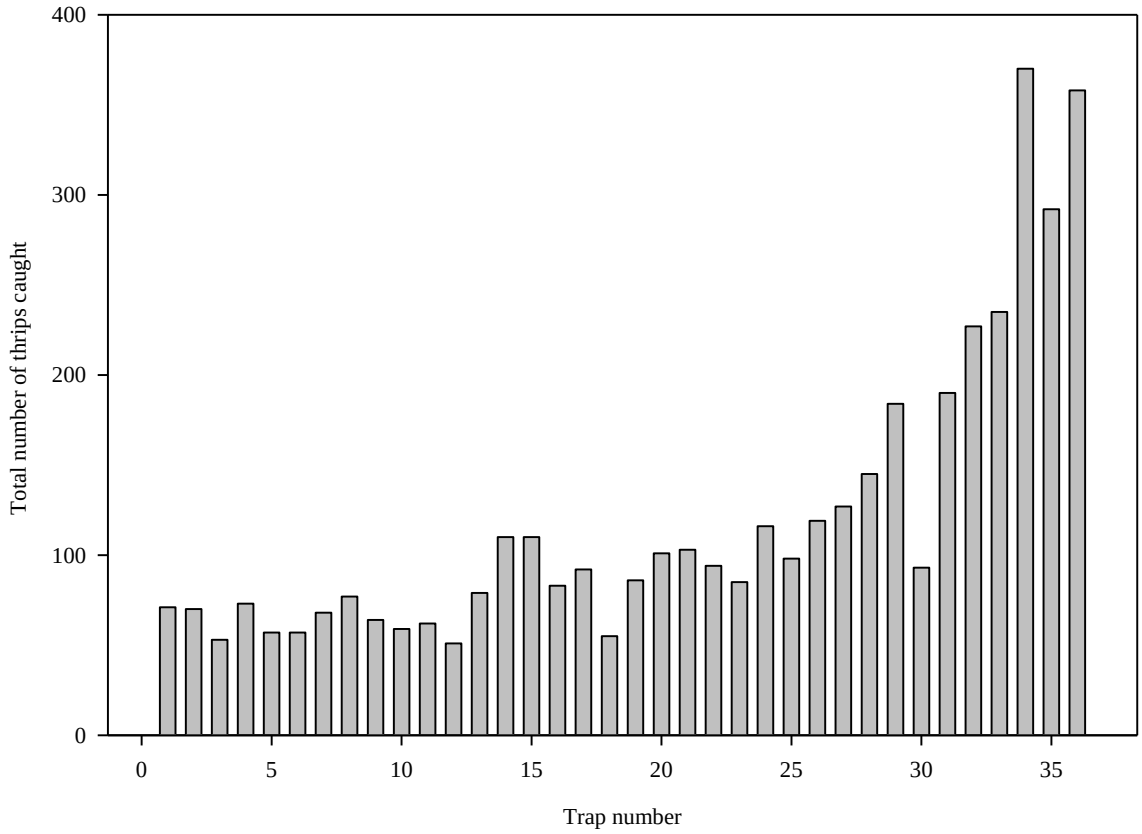
Morphological features used to identification WFT directly on tricky traps under stereo microscope (>x100) based on the description in Mound and Walker et al. (1982) and Moritz et al. (2004).



- Ocellar setae III pair present and long (longer than distance between external margins of hind ocelli) arising a distance apart on the anterior margins of ocellar triangle.
- One pair of elongated postocular setae present under the compound eyes.
- Pronotom with four pairs of elongated setae.
- Metanotum with two pairs of elongated median setae at anterior margin.
- Forewing pale and with two complete rows of dark setae on the 1st and 2nd veins.
- Female and male *Frankliniella occidentalis* are differentiated by a difference in the shape of the abdominal segments IX and X.

Female Western flower thrips (WFT), Frankliniella occidentalis – trapped on ventral side (dorsal aspects) on blue HORIVER sticky traps (photo M-C Nielsen).

Appendix V: Total catches of thrips on blue sticky traps during base-line trapping. The traps were left out for 24 h in greenhouse experiment in August, 2012 to examine the thrips density in the trapping area. The 36 traps were laid out in a 6x6 Latin square.



Appendix VI: Average catches of WFT total (A), female (B) and male (C) on blue sticky traps baited with MI released from different passive dispensers (n=6/treatment) (95% confidence limits). The traps and dispensers were left out for six days in greenhouse experiment in August, 2012. The dispensers were: 150 micron thick polyethylene bags (P150), 50 micron thick polyethylene bags (P50), ChemTica low release (CmLow), ChemTica high release (CmHigh) each with 2.5 ml of MI added and LUREM-TR. For control traps (Control) a 150 micron thick polyethylene bag with no MI added was used.

(A) WFT total

Day	Control	P150	CmLow	P50	LUREM-TR	CmHigh
1	109.7 (64.1,187.6)	121.2 (71.2,206.3)	146.0 (86.4,246.7)	122.3 (71.9,208.2)	143.0 (84.6,241.8)	111.2 (65.0,190.0)
2	111.8 (64.7,193.3)	113.0 (65.4,195.3)	169.8 (99.5,289.7)	132.2 (76.9,227.1)	186.5 (109.6,317.4)	131.7 (76.6,226.3)
3	94.2 (54.3,163.4)	101.0 (58.4,174.6)	138.3 (81.1,236.0)	143.8 (84.4,245.1)	147.7 (86.7,251.4)	123.8 (72.3,212.2)
4	99.7 (59.9,165.7)	89.5 (53.6,149.4)	124.5 (75.4,205.7)	121.8 (73.7,201.4)	123.7 (74.8,204.3)	113.5 (68.5,188.0)
5	89.5 (49.9,160.4)	69.2 (38.1,125.5)	111.7 (62.8,198.4)	79.3 (44.0,143.0)	112.5 (63.3,199.8)	88.3 (49.3,158.4)
6	70.7 (39.9,125.2)	57.5 (32.0,103.3)	101.5 (58.4,176.5)	84.8 (48.4,148.8)	111.7 (64.5,193.4)	91.5 (52.4,159.9)
1-6	575.6 (332.8,995.6)	551.4 (319.7,954.4)	791.8 (463.6,1344.0)	684.2 (399.3,1173.6)	825.1 (483.5,1408.1)	660.0 (3841.1,1134.8)

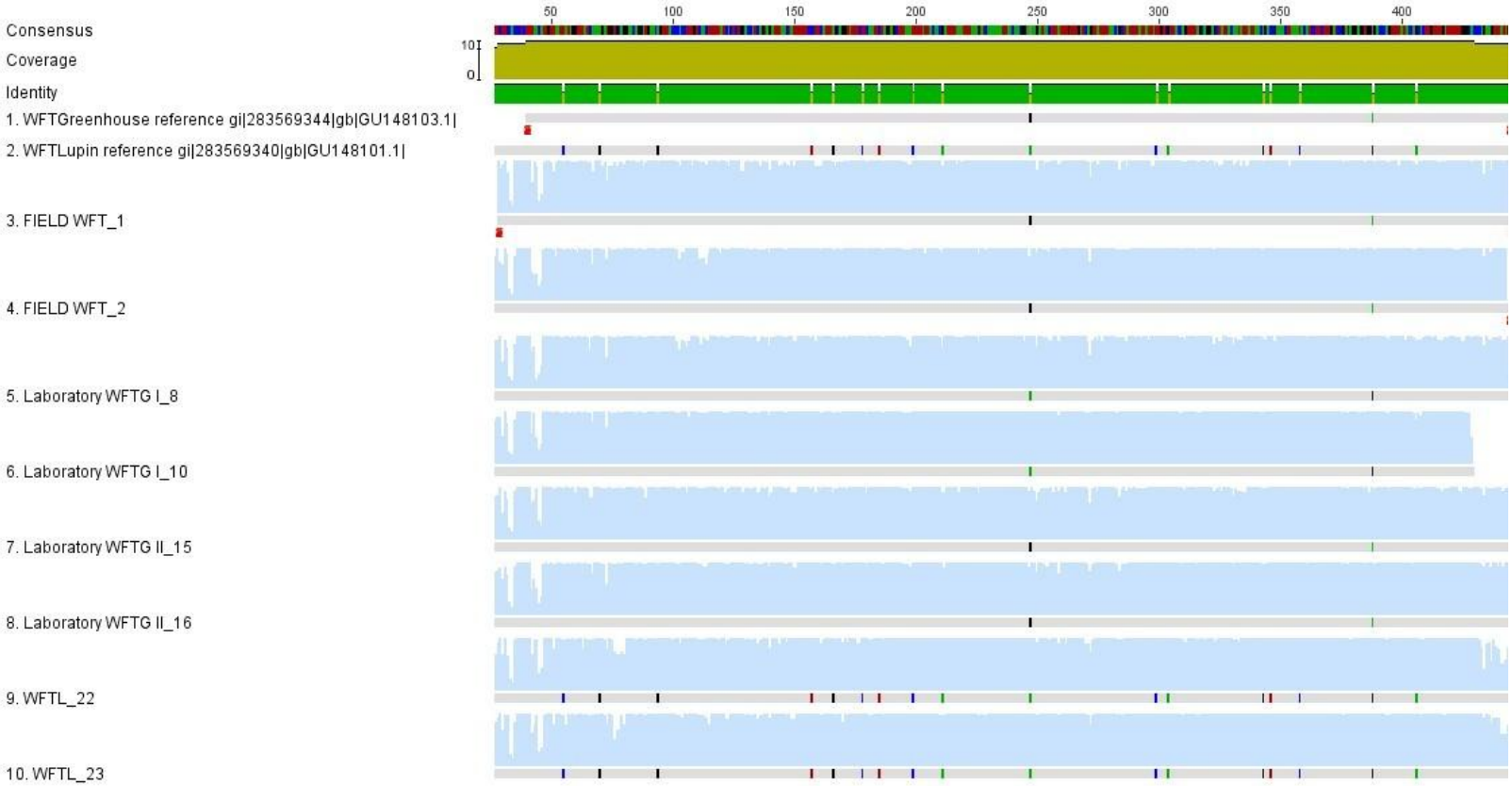
(B) WFT female

Day	Control	P150	CmLow	P50	LUREM-TR	CmHigh
1	31.1 (18.2,53.0)	41.5 (25.0,68.9)	44.3 (26.5,74.0)	44.4 (26.8,73.5)	50.8 (30.7,83.9)	43.6 (26.3,72.1)
2	35.8 (22.7,56.4)	46.3 (29.9,71.7)	56.2 (36.2,87.3)	48.2 (31.0,74.9)	74.7 (48.6,114.8)	48.3 (31.2,74.9)
3	31.0 (17.6,54.6)	42.5 (24.7,72.9)	46.5 (26.9,80.4)	53.1 (31.0,91.0)	58.8 (34.5,100.2)	45.1 (26.2,77.9)
4	25.6 (15.5,42.2)	34.1 (21.3,54.6)	39.5 (24.6,63.4)	41.3 (25.8,66.0)	41.4 (25.9,66.2)	37.0 (23.1,59.4)
5	22.8 (11.5,45.3)	24.0 (12.5,46.2)	32.5 (16.7,63.3)	24.5 (12.5,48.0)	44.0 (23.1,83.7)	34.4 (18.0,66.0)
6	24.0 (11.8,48.8)	20.7 (10.2,41.8)	33.2 (16.6,66.6)	35.7 (18.3,69.8)	46.8 (24.1,90.7)	40.7 (20.9,79.5)
1-6	170.3 (97.3,300.3)	209.1 (123.6,356.1)	252.2 (147.5,435.2)	247.2 (145.4,432.2)	316.5 (186.9,539.6)	249.1 (145.7,429.8)

(C) WFT male

Day	Control	P150	CmLow	P50	LUREM-TR	CmHigh
1	78.5 (46.0,134.0)	80.4 (47.1,137.2)	77.9 (45.3,134.0)	70.1 (40.8,120.4)	84.4 (49.2,144.6)	60.6 (35.1,104.6)
2	77.8 (43.2,140.0)	66.7 (36.9,120.5)	94.7 (52.5,170.9)	77.0 (42.6,139.1)	106.3 (58.9,191.6)	66.0 (36.4,119.6)
3	61.7 (33.7,112.8)	52.8 (28.5,97.7)	70.0 (38.1,128.4)	76.6 (41.9,140.2)	78.8 (43.0,144.3)	62.4 (33.8,115.2)
4	66.3 (37.4,117.7)	51.3 (28.8,91.3)	63.0 (35.3,112.5)	72.9 (41.1,129.3)	80.3 (45.5,141.9)	61.5 (34.6,109.5)
5	58.3 (33.4,101.9)	38.3 (21.8,67.4)	53.6 (30.3,94.7)	45.1 (25.5,79.8)	50.2 (28.3,88.8)	42.1 (23.7,75.0)
6	41.4 (24.0,71.6)	29.2 (16.7,51.0)	47.9 (27.7,82.9)	39.9 (23.0,69.1)	55.5 (32.4,95.3)	37.4 (21.4,65.4)
1-6	384.0 (217.7,678.0)	318.7 (179.8,565.1)	407.1 (229.2,723.4)	381.6 (214.9,677.9)	455.5 (257.3,806.5)	330.0 (185,589.3)

Appendix VII: Sequence alignment of WFT-G and WFT-L (mtDNA) samples.



Appendix VIII: Result of subsequent tomato spotted wilt virus (TSWV) titer tests of leaf samples collected from the chrysanthemum host plants used to rear the putative virus-infected thrips (Laboratory WFT-G I). Stick A, B and C are batches of 5–6 leaves collected from chrysanthemum plants (10–14 days in rearing setup). Stick D are leaf samples from a TSWV infected control plant.

(Photo: J. Fletcher, Plant & Food Research Limited Lincoln)