Developing an integrated management system for western flower thrips, *Frankliniella occidentalis* (Pergande), on deciduous fruit, using semiochemicals in a push-pull strategy

Elleunorah Allsopp

Dissertation presented for the degree of
Doctor of Philosophy
in the Faculty of Agrisciences at
Stellenbosch University

Promoter: Dr. Pia Addison
Co-promoter: Dr. Sarah Y. Dewhirst
(Department of Conservation Ecology and Entomoloty)

December 2016

DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

December 2016

i

ABSTRACT

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), causes both feeding (russetting and silvering) and oviposition (pansy spot) damage to fruit. Despite routine insecticide applications from 20% bloom until petal fall, pansy spot and pitting damage was still being reported, particularly on plums. This study was initiated to determine the reason for the apparent failure of chemical control and the cause of pitting damage, and to investigate the feasibility of developing a push-pull system to minimize economic WFT damage by using deterrent plant essential oils and trap crops.

Field trials in commercial plum orchards in the Western Cape confirmed that WFT oviposition causes pitting damage. The apparent failure of insecticide applications to prevent pansy spot and pitting damage was due to the fact that WFT entered plum blossoms even before the petals opened, where they were protected from contact insecticides applied at 20% bloom. No treatment threshold could be determined because no consistent significant relationship was found between blue sticky trap counts and WFT oviposition damage to plums. Sticky trap counts thus only serve to indicate presence or absence of WFT in an orchard. To reduce WFT oviposition damage, monitoring must start as soon as flower buds begin to swell, some blue sticky traps should be hung closer to the ground during the early season and, if WFT are present, the first spray application should be made as soon as blossoms reach balloon stage.

To provide the "push" in a push-pull system, the potential of three plant essential oils to reduce WFT oviposition rate on plum blossoms was investigated. This study was the first to demonstrate that suspensions of thymol (10%), methyl salicylate (1% and 10%) and carvacrol (1% and 5%) significantly reduced WFT oviposition rate when applied to individual plum blossoms in laboratory bioassays. Significant results could not be obtained in semi-field trials using potted plum trees, mainly because the suspensions were unable to provide sustained release of the volatile essential oils at behaviourally effective concentrations. Phytotoxic damage to blossoms was encountered at higher concentrations of the essential oils. While thymol, methyl salicylate and carvacrol were shown to have potential as oviposition deterrents for WFT on plum blossoms, they could only be considered for commercial use if stable suspensions can be developed to deliver sustained release of behaviourally effective concentrations with no phytotoxic effects.

An effective trap crop that provides the "pull" should be as attractive, or more attractive to WFT than plum blossoms. White clover, *Trifolium repens* L., was selected for investigation. The attractiveness of flower volatiles of clover flowers and plum blossoms, collected by means of

air entrainment, was evaluated using a Y-tube glass olfactometer. Results showed that the volatiles of clover flowers and plum blossoms are both very attractive to WFT females. White clover shows potential as a trap crop for WFT, but a control system on heavily infested clover should be implemented to remove WFT and clover flowers should be cut before honeybees are brought in to ensure effective pollination.

This study provided crucial information to improve the efficacy of early-season chemical control of WFT. Three essential oils were identified as potential oviposition deterrents for WFT on plum blossoms and white clover was identified as a potential trap crop. Development of suitable formulations of the essential oils is required before a push-pull system to manage WFT more sustainably in deciduous fruit orchards can be implemented.

OPSOMMING

Westelike blomblaaspootjie (WBB), *Frankliniella occidentalis* (Pergande), veroorsaak beide vreetskade (skilverruwing en versilwering) en eierleggingskade ("pansy spot") by vrugte. Ten spyte van roetine insekdoder aanwendings vanaf 20% blom tot blomblaarval, is "pansy spot" en kuiltjieskade, veral by pruime, toenemend aangemeld. Hierdie studie is geloods om te bepaal waarom chemiese beheer nie skade tydens die blomstadium doeltreffend beheer nie, om die oorsaak van die kuiltjieskade te bepaal, en om die ontwikkeling van 'n afweer-aanlok stelsel te ondersoek, wat ekonomiese WBB skade minimiseer deur gebruik van afwerende essensiële plantolies en vangoeste.

Veldproewe in kommersiële pruimboorde in die Weskaap het bevestig dat eierlegging deur WBB kuiltjieskade veroosaak. Die skynbare onvermoë van insekdoders om "pansy spot" en kuiltjieskade te voorkom, was te wyte aan die feit dat WBB pruimbloeisels binnedring nog voordat die blomblare oopmaak, waar hulle beskerm was teen kontakmiddels wat op 20% blom toegedien is. Geen drumpelwaarde vir beheer kon bepaal word nie, aangesien daar geen konsekwent betekenisvolle verwantskap tussen blou lokvaltellings en WBB eierleggingskade by pruime was nie. Lokvaltellings dien dus slegs as 'n aanduiding van die aan- of afwesigheid van WBB in 'n boord. Om WBB eierleggingskade effektief te verminder, moet monitering begin sodra blomknoppe begin swel, taai lokvalle behoort aan die begin van die seisoen nader aan die grond gehang te word en, indien WBB teenwoordig, moet die eersre bespuiting aangewend te word sodra die bloeisels ballonstadium bereik.

Om die afweringselement van 'n afweer-aanlok stelsel te voorsien, is die potensiaal van drie essensiële plantolies om die eierleggingstempo van WBB op pruimbloeisels te verlaag, ondersoek. Hierdie studie was die eerste om te wys dat suspensies van timol (10%), metiel salisilaat (1% en 10%) en karvakrol (1% en 5%) WBB eierleggingstempo betekenisvol verlaag het in laboratoriumstudies met enkel pruimbloeisels. Betekenisvolle resultate kon nie verkry word in semi-veldroewe met pruimbome in potte nie, hoofsaaklik weens die onvermoë van die suspensies om volgehoue vrylating van vlugtige essensiële plantolies teen biologies effektiewe konsentrasies te voorsien. Hoër konsentrasies van die essensiële olies het fitotoksiese skade aan bloeisels veroorsaak. Alhoewel daar bewys is dat timol, metiel salisilaat en karvakrol potensiaal het om eierlegging deur WBB op pruimbloeisels te verhinder, kan hierdie verbindings slegs vir kommersiële gebruik oorweeg word indien stabiele suspensies ontwikkel kan word wat volgehoue, eweredige vrystelling biologies effektiewe konsentrasies sonder enige fitotoksiese effek verseker.

'n Doeltreffende vangoes wat die aanlokkingselement van 'n afweer-aanlok stelsel voorsien, behoort ewe of meer aanloklik vir WBB te wees as pruimbloeisels. Wit klawer, *Trifolium repens* L., is gekies vir die ondersoek na 'n potensiële vangoes. Die vlugtige verbindings van klawerblomme en pruimbloeisels is versamel en die aanloklikheid daarvan is met behulp van 'n Y-buis olfaktometer bepaal. Resultate het gewys dat die vlugtige verbindings van klawerblomme en pruimbloeisels beide hoogs aanloklik is vir WBB. Wit klawer het potensiaal as 'n vangoes vir WBB, maar om doeltreffende bestuiwing te verseker, sal WBB op die klawer vroegtydig beheer en die klawerblomme afgesny moet word voordat bye in die boorde ingebring word.

Hierdie studie het uiters noodsaaklike inligting verskaf om die doeltreffendheid van chemiese beheer van blaaspootjies vroeg in die seisoen te verbeter. Drie essensiële olies is as potensiële afweermiddels vir eierlegging deur WBB op pruimbloeisels geïdentifiseer en wit klawer is geïdentifiseer as 'n potensiële vangoes. Ontwikkeling van geskikte formulasies van die essensiële plantolies is 'n voorvereiste vir implementering van 'n afweer-aanlok stelsel om WBB meer volhoubaar te bestuur.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1. INTRODUCTION AND LITERATURE OVERVIEW

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is a flower-inhabiting thrips native to the west coast of California, United States of America (USA). In 1987 it was first recorded on chrysanthemums (Asterales: Asteraceae) near Krugersdorp in South Africa (Giliomee 1989). As elsewhere in the world (Kirk and Terry 2003), this polyphagous thrips spread rapidly. In 1990 WFT were first collected from apple (Rosales: Rosaceae) orchards near Grabouw in the Western Cape Province (Badenhorst 1993) and by 2001 it was the dominant thrips species in apple and nectarine (Rosales: Rosaceae) orchards in the Western Cape (Van der Merwe 2001).

Western flower thrips is a serious economic pest on a wide range of crop and ornamental plants in fields and greenhouses, causing damage through feeding, oviposition and transmission of tospoviruses (Bunyaviridae) (Kirk 1997). This insect has been studied extensively because of its status as a major pest worldwide (Reitz 2009). However, comparatively little research has been done on WFT in South Africa because it only appeared in the country in the late 1980's and because outbreaks resulting in economic damage tend to be sporadic occurrences in most deciduous fruit growing areas. An overview of literature dealing specifically with WFT on deciduous fruit is presented here.

1.1 Western flower thrips damage to deciduous fruit

1.1.1 Feeding damage
Like all thrips, WFT has piercing-sucking mouthparts and feeds by sucking up the cell contents of leaves, petals, fruit and pollen grains (Kirk 1997). Bailey (1933) described feeding damage by WFT resulting in "silver-spotting" on peaches (Rosales: Rosaceae), apricots (Rosales: Rosaceae), oranges (Rutales: Rutaceae) and apples. In nectarines feeding by WFT adults and larvae on developing fruitlets results in scarring that appears russet-like (Fig. 1.1, left), while silvering (Fig. 1.1, right) occurs when adults and larvae feed on the surface of ripening fruit (Felland et al. 1995; Childers 1997; Pearsall 2000a).

1.1.2 Oviposition damage
Female WFT insert eggs singly just under the plant epidermis with about a third of the egg protruding (Terry 1997). On fruit the spot where the egg was inserted develops a central russet area surrounded by a white halo (Fig. 1.2), hence the name pansy spot (Childers 1997). In table grapes these lesions are referred to as halo spots (Jensen 1973). Childs (1927) reported that oviposition by *Aeolothrips fasciatus* (L.) (Thysanoptera: Aeolothripidae) and WFT cause

1

pansy spot lesions on apples in the Pacific Northwest of the USA and Swift and Madsen (1956) reported pansy spot damage on Golden Delicious and Red Delicious apples in California (USA) caused by oviposition of *Thrips madroni* Moulton (Thysanoptera: Thripidae).

<div align="center">Photo: ARC Infruitec-Nietvoorbij   Photo: ARC Infruitec-Nietvoorbij</div>

Figure 1.1. *Frankliniella occidentalis* feeding damage on nectarines: russetting (left), silvering (right).

<div align="center">Photo: ARC Infruitec-Nietvoorbij   Photo: ARC Infruitec-Nietvoorbij</div>

Figure 1.2. Pansy spot oviposition damage by *Frankliniella occidentalis* on apple (left) and plum (right).

Oviposition damage by WFT does not only cause pansy spot damage. Kasimatis et al. (1954) described pitting damage on Santa Rosa plums in California (USA) which they attributed to WFT. Swift and Madsen (1956) also described pitting damage on Golden Delicious and Red Delicious apples in California caused by oviposition of *T. madroni*, whilst McMullen (1972) reported that oviposition by *Taeniothrips orionis* Treherne (Thysanoptera: Thripidae) in the ovaries of cherry (Rosales: Rosaceae) flowers in British Columbia (Canada) resulted in

dimpling damage. This happens when the injured tissue around the oviposition site does not develop as rapidly as the healthy tissue, which causes a shallow pit or dimple to form, sometimes surrounded by a pansy spot.

1.2 Biology and seasonal occurrence on deciduous fruit

Yonce et al. (1990) found that scarring and silvering of nectarines in Georgia (USA) were associated with high densities of WFT. Population density varied greatly from year to year and was lowest in years when most rain occurred prior to harvest in May and June. Felland et al. (1993) showed that adult WFT females overwinter in the humus layer of soil and in leaf litter in orchards in Pennsylvania, USA. In nectarine orchards where silvering damage occurred, WFT was the dominant thrips species (Felland et al. 1995). It also occurred widely on white clover, *Trifolium repens* L. (Fabales: Fabaceae). In these orchards WFT emerged later from overwintering sites due to lower temperatures early in the season and therefore there was no scarring damage from early season larval feeding, only silvering damage during fruit ripening.

The biology and seasonal occurrence of WFT in nectarine orchards were also extensively studied in British Columbia, Canada (Pearsall 2000a & b, 2002; Pearsall and Myers 2000, 2001). Pearsall and Myers (2000) showed that WFT overwintered as mated adult females in soil, leaf litter and protected places under the bark of nectarine trees, and that emergence is triggered when daily maximum temperatures reached 10 °C. It was not possible to time insecticide applications to coincide with WFT emergence, because adult emergence occurred over an extended period. During spring WFT moved into the orchards from adjacent wild vegetation, and it was found that orchards bordered by wild areas had higher densities of WFT adults and larvae in flower buds than orchards surrounded by other orchards (Pearsall and Myers 2000). In early spring, WFT tended to move into orchards, keeping close to ground level, but as the ground cover grew taller and temperatures increased, they flew higher into the trees (Pearsall and Myers 2000). According to Pearsall and Myers (2001) and Pearsall (2002), WFT only flew during the day when wind speed was below 15 km/h and temperatures between 15 and 30 °C. In windy conditions, WFT moved close to the ground by hopping, rather than by flying. Peaks in WFT abundance coincided with pink balloon (flower buds fully swollen, just before petals open) and full bloom of the nectarines. Pearsall (2000a) found that most WFT eggs were laid early in the season on the sepals of flower buds at the white swell to pink colour stage, therefore fruit damage was almost entirely due to WFT larval feeding rather than to oviposition. This was similar to the findings of Terry (1991), who found that WFT eggs were mostly laid during early-pink to early-bloom stage on the sepals and stems of apple buds. Pearsall (2000a) also concluded that counts of adult WFT on yellow sticky traps cannot be used to predict larval densities or damage levels on nectarines, consequently no threshold

density could be calculated for WFT on nectarines. Pearsall (2000b) demonstrated that WFT preferred highly scented flowers to unscented flowers, and that adults and larvae of WFT were found on all ground cover species, particularly clover, dandelion (*Taraxacum officinale* Weber, Asterales: Asteraceae) and alfalfa (*Medicago sativa* L.*,* Fabales: Fabaceae), throughout summer and autumn. The presence of highly attractive ground cover flowers did not reduce the number of WFT on nectarine blooms significantly, which led to the conclusion that a ground cover will only be successful as a trap crop if used in conjunction with a powerful deterrent on the nectarine blooms (Pearsall 2000b).

In South Africa the seasonal variation in overall numbers of various thrips species in nectarine orchards were monitored (Jacobs 1995 a & b), but individual species were not identified or recorded. These studies showed that thrips numbers in nectarine orchards began to increase from August/September, during flowering, and peaked in December/January. Van der Merwe (2001) showed that WFT numbers in apple and nectarine orchards began to increase from September/October, which coincided with flowering, and peaked from November to January. Thrips population levels were higher in seasons with higher mean monthly temperatures (exceeding 20 °C) and lower rainfall during spring. The seasonal occurrence of WFT was also studied on table grapes (*Vitis vinifera* L., Vitales: Vitaceae) in the Hex River Valley (Western Cape Province) in South Africa (De Villiers and Pringle 2007; Allsopp 2010). These studies showed that WFT numbers, monitored by means of blue sticky traps, began to increase from spring (September/October) and peaked from November (which coincided with grapevine flowering) until January. Smaller peaks occurred towards the end of the growing season in March/April.

1.3 Identification and monitoring methods

Correct identification of pest thrips in orchards is crucial for effective control (Broughton and Harrison 2012). Thrips are very small and agile, and generally need to be examined under a microscope for accurate identification of the species. Besides WFT, a variety of phytophagous thrips have been identified in local deciduous fruit orchards. Most prevalent are onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), and blossom thrips, *Frankliniella schultzei* (Trybom) (Thysanoptera: Thripidae), although they are generally not considered to be of economic importance. Predatory thrips, including *Aeolothrips brevicornis* (Thysanoptera: Aeolothripidae) and *Scolothrips* spp. (Thysanoptera: Thripidae) have also been recorded (Jacobs 1995 a & b). Distinguishing between adult females of WFT, *T. tabaci* and *F. schultzei* is not easy, because size and colouration is variable and the main morphological characteristics used to distinguish between these species are difficult to observe due to the minute size of these insects. In mature WFT females the head and thorax are usually orange-

yellow and the abdomen straw-coloured to grey-brown (Fig. 1.3), which easily distinguishes them from *T. tabaci* which is uniformly yellow to straw-coloured. However, newly emerged WFT females also appear yellow to straw-coloured all over. The defining characteristics used to separate WFT from *T. tabaci* are the four pairs of long setae at each of the anterior and posterior corners of the pronotum (Fig. 1.4) and the two complete rows of setae (Fig. 1.5) along the length of the main and secondary veins on the forewings (Mound and Kibby 1998). Onion thrips, *T. tabaci*, have an interrupted row of setae along the main vein of the forewing (Fig. 1.5). Blossom thrips, *F. schultzei*, share the above characteristics with WFT, but are generally stockier and darker brown. They are distinguished from WFT by the position of their ocellar setae III between the hind ocelli (Fig.1.6) and the longer major postocular setae of WFT (Fig.1. 7).
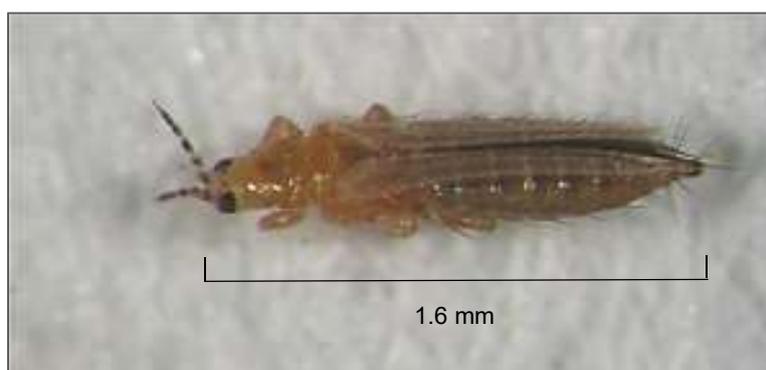
Photo: ARC Infruitec-Nietvoorbij

Figure 1.3. *Frankliniella occidentalis*. Female.
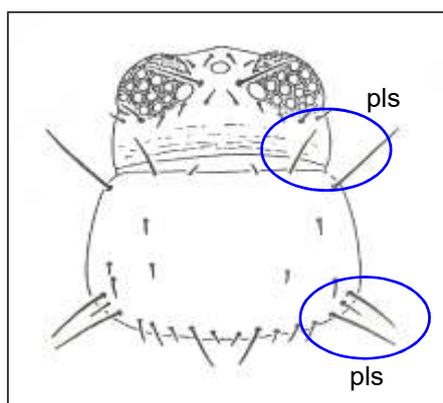
Figure 1.4. Pronotum of *Frankliniella occidentalis* with paired long setae (pls) anteriorly and posteriorly (Mound and Kibby 1998).
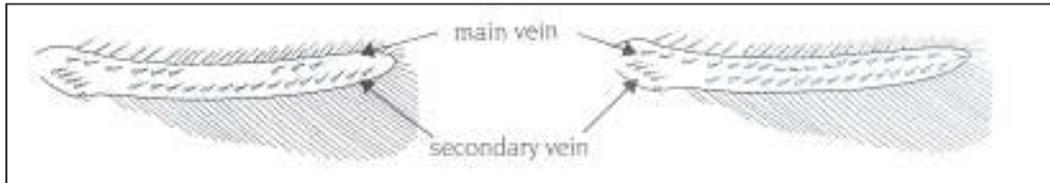
Figure 1.5. Forewings with interrupted setae along main vein (left), as found in *Thrips tabaci,* and with uninterrupted rows of setae (right), as found in *Frankliniella occidentalis* (Mound and Kibby 1998).

Figure 1.6. Position of ocellar setae III (OS) in *Frankliniella schultzei* (Mound and Kibby 1998).
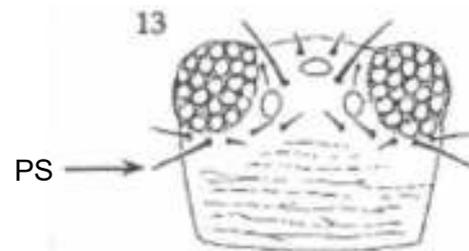
Figure 1.7. Major postocular setae (PS) of *Frankliniella occidentalis* (Mound and Kibby 1998).

Molecular identification keys have been developed for thrips (Timm et al. 2008). These are very useful for research and phytosanitary purposes to confirm species identity, particularly when superficial damage to specimens or the lack of adult thrips make morphological identification impossible. However, cost and the care required for handling specimens to prevent degradation of their DNA signature mean that it is not a practicable option for routine identification of thrips for pest management services.

Lewis (1997a) summarises a variety of techniques and methods used to assess thrips numbers in field and on greenhouse crops. Some methods are useful for research purposes, but not practicable for use by producers and pest management advisors to carry out routine monitoring of thrips pests. These include collecting clusters of apple blossoms and washing them in ethanol to dislodge the thrips for counting under a binocular microscope, or simply flicking the flower clusters against the side of a container with ethanol to capture dislodged thrips (Terry and De Grandi-Hoffman 1988).

Coloured sticky traps are most commonly used for monitoring thrips, as they are practicable and affordable for research purposes, as well as for commercial use. An added advantage is

6

that catches on them can be preserved indefinitely in a plastic cover and easily examined under a binocular microscope to identify the thrips material. Yellow traps are most widely used, as they attract a wide variety of insects, but research has shown that blue traps are more effective for monitoring WFT than yellow traps (Gillespie and Vernon 1990; Vernon and Gillespie 1990; Gaum and Giliomee 1994). Research conducted on table grapes in South Africa showed that blue sticky traps hung outside the vine canopy in full sun were most effective for monitoring WFT in vineyards and trapped fewer beneficial insects than yellow traps (De Villiers and Pringle 2007; Allsopp 2010). These studies also showed that monitoring should start early in the season as soon as grape inflorescences appear. Both studies concluded that WFT numbers on sticky traps in spring do not provide a reliable estimate of oviposition (halo spot) damage on grape berries at harvest. No treatment threshold could be established either and blue sticky traps merely served to indicate the presence or absence of WFT in vineyards.

1.4 Plant volatiles for western flower thrips monitoring and control

Research has shown that WFT uses both visual (colour, shape, size) and olfactory (plant volatiles) cues to find suitable plant hosts (Terry 1997). Whilst some plant volatiles attract WFT, others have been shown to deter or repel them (Koschier 2008). In some cases, the same compound can be either attractive or repellent, depending on the concentration (Terry 1997; Manjunatha et al. 1998; Koschier et al. 2000). Koschier (2008) reviewed the use of attractant and deterrent plant essential oils for thrips control. Research with particular reference to WFT is discussed below.

1.4.1 Attractants

It has been shown as far back as 1914 that anisaldehyde attracts thrips, although the species were not identified (Howlett 1914). Since then many plant essential oils and volatile compounds have been demonstrated to attract WFT, as summarised in Table 1.1. These compounds do not necessarily attract WFT exclusively, and some of them have already been found to attract other thrips, such as *T. tabaci* and *T. obscuratus* (Thysanoptera: Thripidae).

Howlett (1914) first suggested using attractants in the form of benzaldehyde, cinnamyl-aldehyde and anisaldehyde to improve the efficacy of thrips traps. Currently there are two thrips attractant commercial products containing semiochemicals, namely Thripline[TM] ams (Syngenta Bioline LTD) which contains the synthetic aggregation pheromone neryl (S)-2-methylbutanoate (Hamilton et al. 2005) released by WFT males, and Lurem-TR (Koppert) which contains the host plant-derived attractant methyl isonicotinate (Davidson et al. 2008). These were shown to improve monitoring efficacy of various thrips species, including WFT, in

glasshouse, field and orchard trials (Teulon et al. 2008; Nielsen et al. 2010; Broughton and Harrison 2012; Muvea et al. 2014).

Table 1.1. Plant volatile compounds that specifically, but not exclusively, attract *Frankliniella occidentalis*. Adapted from Koschier (2008).

Compound	References
p-anisaldehyde	Teulon et al. 1993; Frey et al. 1994; Roditakis and Lykouressis 1996; Manjunatha et al. 1998; Koschier et al. 2000;
o- anisaldehyde	Koschier et al. 2000
benzaldehyde	Teulon et al. 1993; Koschier et al. 2000
salicylaldehyde	Roditakis and Lykouressis 1996; Katerinopoulos et al. 2005
eugenol	Frey et al. 1994; Koschier et al. 2000
3-phenylpropion-aldehyde	Koschier et al. 2000
(+)-citronellol	Koschier et al. 2000
1,8 cineole (eucalyptol)	Chermenskaya et al. 2001; Katerinopoulos et al. 2005
geraniol	Frey et al. 1994; Koschier et al. 2000
linalool	Koschier et al. 2000; Katerinopoulos et al. 2005
linalool oxide pyran	Pow et al. 1998
nerol	Koschier et al. 2000
(E)- β-Farnesene	Manjunatha et al. 1998; Pow et al. 1998; Bennison et al. 2003; Koschier et al. 2000
ethyl nicotinate	Teulon et al. 1993; Frey et al. 1994; Koschier et al. 2000

1.4.2 Deterrents

Koschier (2008) defines olfactory repellents as plant volatile chemicals that cause insects to avoid contact with the plant, whilst deterrents inhibit feeding or oviposition after the insect has landed on the plant. Sedy & Koschier (2003) found that the plant essential oils thymol and carvacrol, derived from *Thymus vulgaris* L. and *Origanum vulgare* L. (Lamiales: Lamiaceae), reduced WFT oviposition on leaf discs of bean cotyledons. Chermenskaya et al. (2001) demonstrated that the plant essential oil methyl salicylate, derived from *Gaultheria procumbens* L. (Ericales: Ericaceae), was repellent to WFT in olfactometer bioassays, while a deterrent effect was demonstrated by Koschier et al. (2007), who found that it reduced the oviposition rate of WFT when applied to cucumber, *Cucumis sativus* L. (Cucurbitales:

Cucurbitaceae) and bean, *Phaseolus vulgaris* L. (Fabales: Fabaceae), leaf discs. Methyl jasmonate, an essential oil constituent of various plant species including *Jasminum officinale* L. (Laminales: Oleaceae), was also shown to deter WFT from settling and feeding on treated chrysanthemums, consequently also reducing oviposition on the plants (Bruhin 2009, as cited by Egger et al. 2014).

Recently Egger et al. (2014) identified allylanisole, a compound in the essential oil of *Pimpinella anisum* L. (Apiales: Apiaceae) as a feeding and oviposition deterrent for WFT. However, they also demonstrated that WFT can become habituated to deterrent compounds if these are applied at concentrations below the FDC_{15}, which is the concentration required to reduce feeding damage on treated leaf discs by 15% relative to the untreated control leaf disc.

1.4.3 Push-pull strategies

Stimulo-deterrent diversionary strategies (SDDS), also known as push-pull strategies, use interplanting of repellent cultivars, application of plant-derived antifeedants or oviposition deterrents, and semiochemicals from non-host plants which interfere with host plant location by the pest to protect the harvestable crop (= 'push'), whilst luring it towards an attractive alternative (= 'pull') from where it can be removed (Pickett et al. 1997; Agelopoulos et al. 1999; Cook et al. 2007). This concept was first proposed by Pyke et al. (1987) for control of *Heliothis* (species not specified) (Lepidoptera: Noctuidae) on cotton, *Gossypium hirsutum* L. (Malvales: Malvaceae).

Some push-pull systems use interplanting of repellent plants in combination with planting of attractive trap crops adjacent to the crop. Khan et al. (2000) developed such a system to control the stem borers *Busseola fusca* (Full) (Lepidoptera: Noctuidae) and *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) in subsistence cereal plantings in Kenya. Napier grass, *Pennisetum purpureum* Schumach (Poales: Poaceae), and Sudan grass, *Sorghum sudanensis* Stapf (Poales: Poaceae), were identified as the most attractive trap crops, while molasses grass, *Melinis minutiflora* Beauv (Poales: Poaceae) and the legumes *Desmodium unicatum* DC (Fabales: Fabaceae) and *D. intortum* (Mill) Urb (Fabales: Fabaceae) were found to be the most effective repellent plants for intercropping with maize *Zea mayz* L. (Poales: Poaceae). All of the trap crops and repellent intercrops also provide forage for cattle (Khan et al. 2000). Other systems propose the use of plant-derived antifeedants, oviposition deterrents or semiochemicals from non-host plants, which interfere with host plant location by the pest, in combination with trap crops or attractants to protect the harvestable crop (Bennison et al. 2003; Koschier 2008 and references therein).

9

The use of highly attractive lure or trap plants to enhance integrated management of WFT on ornamentals in greenhouses has been proposed by Bennison et al. (1999) and Bennison et al. (2003). Bennison et al. (1999) identified two *Verbena* (Lamiales: Verbenaceae) cultivars ('Sissinghurst Pink' and 'Tapien Pink') that were effective lure plants for WFT in ivy-leaf geraniums, *Pelargonium peltatum* (Geraniales: Geraniaceae)) and pot chrysanthemums (*Dendranthema* spp), while Bennison et al. (2003) found that the chrysanthemum cultivar 'Swingtime', together with additional lures of *E-β*-Farnesene effectively attracted WFT away from the crop chrysanthemum cultivar ('Charm') until it came into flower.

Only one study looked at possible trap crops for WFT in orchards. Pearsall (2000b) investigated the possibility of using naturally occurring ground covers, mainly dandelion as trap crops under nectarine trees in British Columbia, Canada, but came to the conclusion that none of the ground covers would be effective as a trap crop. The absence of literature on trap crops for WFT indicates that no effective trap crop for WFT has thus far been identified for use in open fields or orchards.

1.5 Insecticide resistance

The ability of WFT to develop resistance to insecticides poses a particular challenge to chemical control of this pest in crops (Lewis 1997b). According to Reitz (2009) and references therein, a number of inherent characteristics of WFT contribute to its ability to develop and retain insecticide resistance: as a pest of many crops, populations are constantly exposed to insecticides which increases selection for resistance; the haploid sex determination system in WFT allows resistance alleles to become fixed in the population more rapidly; resistance can persist over many generations in the absence of selection pressure; resistance to some insecticides does not entail a fitness cost to WFT; and because it is polyphagous, it has evolved many metabolic detoxification pathways to deal with a diversity of plant allelochemicals which predisposes WFT to be able to metabolize many insecticides and often confer cross-resistance to other insecticides. Insecticide failure against WFT was recorded as early as 1956 (Lewis 1997b), and since then resistance to most classes of insecticides have been documented all over the world (Jensen 2000). In a review of WFT insecticide resistance, Gao et al. (2012) concluded that metabolic detoxification is the main mechanism involved in WFT insecticide resistance and that the cytochrome-P450-dependent monooxygenases appears to be the most important enzyme system that imparts metabolic resistance in WFT.

In South Africa no scientifically verified cases of insecticide resistance in WFT have been published. Fruit growers are advised to alternate products with different modes of action and in cases where an insecticide no longer appears to provide satisfactory control, producers will

10

switch to a different product. In view of the fact that WFT has become so widely established in South Africa and the many documented cases of insecticide resistance in other parts of the world, specifically to the products currently registered for WFT control on stone fruit in South Africa (Gao et al. 2012), the absence of scientifically verified data on insecticide resistance is a grave concern.

1.6 Problem identification and research objectives

After WFT had become established as the dominant thrips species in Western Cape stone and pome fruit orchards, researchers at ARC Infruitec-Nietvoorbij increasingly received reports were from technical advisors and pest management consultants in the stone fruit industry of typical pansy spot damage, attributed to WFT, and also of small pits of varying depth, particularly on late blooming plum cultivars, that rendered fruit unfit for export. This was in spite of insecticides that were routinely applied from 20% bloom until petal fall to control WFT. This raised the questions of why insecticides did not succeed in preventing serious pansy spot damage and what was the causal agent of the pitting damage.

Currently control of WFT on pome and stone fruit to reduce feeding damage (russetting and silvering) and oviposition damage (pansy spot) relies on the application of a variety of contact insecticides registered for thrips control, as shown in Table 1.2. Insecticide applications during flowering are detrimental to pollinators, and timing insecticide applications to be completed before bees are brought in and after they have been removed from orchards, and still achieve effective thrips control often proves to be difficult to manage at farm level. Contact insecticides are also not effective against WFT already inside partially opened flowers where the insecticides cannot penetrate (Lewis 1997b). When feeding damage occurs close to harvest, insecticides can no longer be applied, due to the risk of residues on fruit and food safety concerns, as indicated by the withholding periods on the insecticide labels. Western flower thrips is known for its ability to develop resistance to pesticides very rapidly (Reitz 2009) and consumer demands for residue-free fruit are also increasing, with some markets even demanding pesticide-free products. These issues further limit the chemical control options available for producers.

The use of plant essential oils to modify pest behaviour, and thereby reduce or eliminate crop damage, offers an environmentally sustainable alternative to toxic insecticides. The feasibility of developing a push-pull system to minimize economic WFT damage by using semiochemicals and trap crops depends on finding a deterrent that can significantly reduce WFT oviposition on plum blossoms and a trap crop attractive enough to lure WFT away from plum blossoms.

The ultimate aim of this research is to develop an effective, environmentally sustainable integrated management strategy for WFT on deciduous fruit crops. The objectives of this study were:

(1) to determine why current management practices, based on monitoring for WFT presence and application of insecticides, failed to prevent pansy spot and pitting damage in some orchards (Chapter 2),

(2) to determine whether WFT is responsible for pitting damage on plums (Chapter 2),

(3) to determine if the plant volatiles thymol, methyl salicylate and carvacrol can reduce the oviposition rate of WFT on plum blossoms (Chapter 3), and

(4) to determine if white clover has potential as a trap crop for WFT in plum orchards (Chapter 4).

Two of the four chapters have been published in peer reviewed journals. For this reason, each chapter is written as a separate article, and some repetition may therefore occur.

Table 1.2. Contact insecticides registered for control of various thrips species, including *Frankliniella occidentalis,* on pome and stone fruit in South Africa.[*]

	Product	Instructions	Withholding period (days)
Pome fruit	Chlorfenapyr[a] Pyrrole[b] IRAC group 13[C]	Apply at early blossom (before bees are introduced) and repeat at 75% petal drop (after bees have been removed).	30
	Formetanate Carbamate IRAC group 1A	Apply at early blossom, if thrips are present, and repeat at 75 % petal fall. If thrips are still active after blossoming, apply a third application 14 days after petal fall.	100
	Spinosad Naturalyte IRAC group 5A	Apply during flowering when scouting indicates infestation. Depending on the duration of the flowering period and the infestation level of thrips, apply a follow-up application within 7 – 10 days.	30 apple 14 pear
Stone fruit	Chlorfenapyr Pyrrole IRAC group 13	Nectarines: For prevention of russetting: Apply at 50% blossom and repeat 14 days later.	30
		For prevention of silvering: Apply a single spray as close to harvest as possible but not within 30 days before harvest.	
		Plums: Apply at early blossom (before bees are introduced into the orchard) and repeat at 75% petal drop (after bees have been removed).	57
	Formetanate Carbamate IRAC group 1A	First application at 10 – 20 % blossom, followed by a second application at 75 – 100 % petal fall.	100
	Spinetoram Spinosyn IRAC group 5A	Nectarines and plums: Apply during full blossom up to 75% petal fall when scouting/monitoring indicates infestation of pest. Depending on the duration of the flowering period and the infestation level of thrips, apply a follow-up application within 7 – 10 days.	7
	Spinosad Naturalyte IRAC group 5A	Nectarines and plums: Apply during flowering when scouting indicates infestation. Depending on the duration of the flowering period and the infestation level of thrips, a follow-up application within 7 – 10 days	7 nectarine 21 plums

a = active ingredient, b = insecticide group, c = Insecticide Resistance Action Committee mode of action class

* Retrieved from www.agri-intel.co.za accessed on 17 November 2015

1.6 REFERENCES

Agelopoulos N, Birkett MA, Hick AJ, Hooper AM, Pickett JA, Pow EM, Smart LE, Smiley DWM, Wadhams LJ, Woodcock CM (1999) Exploiting semiochemicals in insect control. Pestic Sci 55, 225-235

Allsopp E (2010) Seasonal occurrence of western flower thrips, *Frankliniella occidentalis* (Pergande), on table grapes in the Hex River Valley, South Africa. S Afr J Enol Vitic 31, 49-57

Badenhorst J (1993) Californian thrips, *Frankliniella occidentalis*, on apples. Decid Fruit Grow 43, 204-205

Bailey SF (1933) A contribution to the knowledge of the western flower thrips, *Frankliniella californica* (Moulton). J Econ Entomol 26, 836-840

Bennison JA, Maulden K, Dewhirst S, Pow E, Slatter P, Wadhams LJ (2003) Towards the development of a push–pull strategy for improving biological control of western flower thrips on chrysanthemum. In: Marullo R, Mound L (Eds). 7th International Symposium on Thysanoptera: ''Thrips, Plants, Tospoviruses: the Millenial Review'', 2–7 July 2001, Reggio Calabria, Italy. CSIRO Entomology, Canberra, Australia, 199–206

Bennison JA, Pow EM, Wadhams LJ, Maulden KA, Wardlow LR, Buxton JH (1999) Improving biological control of western flower thrips, *Frankliniella occidentalis*, on greenhouse ornamentals. Proceedings Sixth International Symposium on Thysanoptera, Antalya, Turkey, April 27 to May 1. Akdeniz University, Antalya, Turkey. 19-24

Broughton S, Harrison J (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 Prot 42:156-163

Bruhin D (2009) Direct and Indirect Effects of Methyl Salicylate and Methyl Jasmonate on *Frankliniella occidentalis* Pergande on Pot Chrysanthemum. MSc Thesis, Universität für Bodenkultur, Vienna, Austria

Chermenskaya TD, Burov VN, Maniar SP, Pow EM, Roditakis N, Selytskaya OG, Shamshev IV, Wadhams LJ, Woodcock CM (2001) Behavioural responses of western flower thrips, *Frankliniella occidentalis* (Pergande), to volatiles from three aromatic plants. Insect Sci Applic 21, 67-72

Childs L (1927) Two species of thrips injurious to apples in the Pacific Northwest. J Econ Entomol 20, 805-808

Childers CC (1997) Feeding and Oviposition Injuries to Plants. In *Thrips as crop pests*. Lewis T (Ed). CAB International, Oxon, UK, 505-537

Cook SM, Khan ZR, Pickett JA (2007) The use of push-pull strategies in integrated pest management. Annu Rev Entomol 52, 375-400

Davidson MM, Perry NB, Larsen L, Green VC, Butler RC, Teulon DAJ (2008) 4-Pyridyl carbonyl compounds as thrips lures: effectiveness for western flower thrips in Y-tube bioassays. J Agr Food Chem 56, 6554-6561

De Villiers M, Pringle KL (2007) Seasonal occurrence of vine pests in commercially treated vineyards in the Hex River Valley in the Western Cape Province, South Africa. Afr Ent 15, 241-260

Egger B, Spangl B, Koschier EH (2014) Habituation in *Frankliniella occidentalis* to deterrent plant compounds and their blends. Entomol Exp Appl 151, 231-238

Felland CM, Hull LA, Teulon DAJ, Cameron EA (1993) Overwintering of western flower thrips (Thysanoptera: Thripidae) in Pennsylvania. Can Entomol 125, 971-973

Felland CM, Teulon DAJ, Hull LA, Polk DF (1995) Distribution and management of thrips (Thysanoptera: Thripidae) on nectarine in the Mid-Atlantic region. J Econ Entomol 88, 1004-1011

Frey JE, Cortada RV, Helbling H (1994) The potential of flower odours for use in population monitoring of western flower thrips *Frankliniella occidentalis* Perg. (Thysanoptera: Thripidae). Biocontrol Sci Tech 4, 177-186

Gao Y, Lei Z, Reitz SR (2012) Western flower thrips resistance to insecticides: detection, mechanisms and management strategies. Pest Manag Sci wileyonlinelibrary.com DOI 10.1002/ps.3305

Gaum WG, Giliomee JH (1994) Preference of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), and greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), for differently coloured sticky traps. J S Afr Soc Hort Sci 4, 39-41

Giliomee JH (1989) First record of Western Flower Thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) from South Africa. J ent Soc sth Afr 52, 179-180

Gillespie DR and Vernon RS (1990) Trap catch of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), as affected by colour and height of sticky traps in greenhouses. J Econ Entomol 83, 971-975

Hamilton JGC, Hall DR, Kirk WDJ (2005) Identification of a male-produced aggregation pheromone in the western flower thrips *Frankliniella occidentalis*. J Chem Ecol 31, 1369-1379

Howlett FM (1914) A trap for thrips. Jour Econ Biol 9, 21-23

Jacobs S (1995a) Thrips damage and control in nectarine orchards. Decid Fruit Grow 45, 274-280

Jacobs S (1995b) Thrips damage and control in apple orchards. Decid Fruit Grow 45, 323-331

Jensen F (1973) Timing of halo spotting by western flower thrips on table grapes. Calif Agric 27, 6-8

Jensen SE (2000) Insecticide resistance in the western flower thrips, *Frankliniella occidentalis*. Integrated Pest Mgt Rev 5, 131-146

Kasimatis AN, Swift JE, Bailey SF (1954) Thrips on Santa Rosa plums. Calif Agric 8, 9-10

Katerinopoulos HE, Pagona GG, Afratis A; Stratigakis N, Roditakis N (2005) Composition and insect attracting activity of the essential oil of *Rosmarinus officinalis.* J Chem Ecol 31, 111-122

Khan ZR, Pickett JA, van den Berg J, Wadhams LJ, Woodcock CM (2000) Exploiting chemical ecology and species diversity: stem borer and striga control for maize and sorghum in Africa. Pest Manag Sci 56:957-962

Kirk WDJ (1997) Feeding. In *Thrips as crop pests*. Lewis T (Ed). CAB International, Oxon, UK, 119-174

Kirk WDJ, Terry LI (2003) The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). Agr Forest Entomol 301-310

Koschier EH (2008) Essential oil compounds for thrips control – A review. Nat Prod Commun 3, 1171-1182

Koschier EH, De Kogel WJ, Visser JH. 2000. Assessing the attractiveness of volatile plant compounds to western flower thrips *Frankliniella occidentalis*. J Chem Ecol 26, 2643-2655

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

Lewis T (1997a) Field and laboratory techniques. In *Thrips as crop pests*. Lewis T (Ed). CAB International, Oxon, UK, 435-475

Lewis T (1997b) Chemical control. In *Thrips as crop pests*. Lewis T (Ed). CAB International, Oxon, UK, 567-593

Manjunatha M, Pickett JA, Wadhams LJ, Nazzi F (1998) Response of western flower thrips, *Frankliniella occidentalis* and its predator *Amblyseius cucumeris* to chrysanthemum volatiles in olfactometer and greenhouse trials. Insect Sci Applic 18, 139-144

McMullen RD (1972) *Taeniothrips orionis* oviposition and feeding injury on Cherries. J Entomol Soc Brit Columbia 69, 3-5

Mound LA, Kibby G (1998) Thysanoptera: an identification guide. 2nd Ed. CAB International, Oxon, New York

Muvea AM, Waigani MM, Kutima HL, Osiemo Z, Nyasani JO, Subramanian S (2014) Attraction of pest thrips (Thysanoptera: Thripidae) infesting French beans to coloured sticky traps with Lurem-TR and its utility for monitoring thrips populations. Int J Trop Insect Sci 34, 197-206

Nielsen M-C, Worner SP, Chapman RB, de Kogel WJ, Perry NB, Sansom CM, Murai T, Muvea AM, Subramanian S, Teulon DAJ (2010) Optimising the use of allelochemicals for thrips pest management. International Society of Chemical Ecology Conference 31 July-4 Aug 2010, Tours, France

Pearsall IA (2000a) Damage to nectarines by the western flower thrips (Thysanoptera: Thripidae) in the interior of British Columbia, Canada. J Econ Entomol 93, 1207-1215

Pearsall IA (2000b) Flower preference behaviour of western flower thrips in the Similkameen Valley, British Columbia, Canada. Entomol Exp Appl 95, 303-313

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

Pearsall IA, Myers JH (2000) Population dynamics of western flower thrips (Thysanoptera: Thripidae) in nectarine orchards in British Columbia. J Econ Entomol 93, 264-275

Pearsall IA, Myers JH (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

Pickett JA, Wadhams LJ, Woodcock CM. 1997. Developing sustainable pest control from chemical ecology. Agric Ecosyst Environ 64, 149-156

Pow EM, Hooper AM, Luzniak MC, Pickett JA, Wadhams LJ, Bennison JA (1998) Novel strategies for improving biological control of western flower thrips on protected ornamentals – attraction of western flower thrips to verbena plants. Proceedings Brighton

Crop Protection Conference Pests and Diseases. Vol. 2. British Crop Protection Council, Farnham, UK. 417-422

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

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

Roditakis NE, Lykouressis DP (1996) Prospects for the use of volatile chemicals and a new pyrrole in integrated pest management of western flower thrips. Acta Hort 431, 513-520

Sedy KA, Koschier EH (2003) Bioactivity of carvacrol and thymol against *Frankliniella occidentalis* and *Thrips tabaci.* J Appl Ent 127, 313-316

Swift JE, Madsen HF (1956) Thrips damage to apple. J Econ Entomol 49, 398-399

Terry LI (1991) *Frankliniella occidentalis* (Thysanoptera: Thripidae) oviposition in apple buds: role of bloom state, blossom phenology, and population density. Environ Entomol 29, 1568-1576

Terry LI (1997) Host selection, Communication and Reproductive Behaviour. In *Thrips as crop pests*. Lewis T (Ed). CAB International, Oxon, UK, 65-118

Terry LI, DeGrandi-Hoffman G (1988) Monitoring western flower thrips (Thysanoptera: Thripidae) in 'Granny Smith' apple blossom clusters. Can Entomol 120, 1003-1016

Teulon, DAJ, Davidson MM, Nielsen M-C, Perry NB, van Tol RWHM, De Kogel W-J (2008) The potential use of lures for thrips biological control in greenhouses: Practice and theory. In Proceedings Third International Symposium on Biological Control of Arthropods, Christchurch, New Zealand, 8-13 February (PG Mason, DR Gillespie and R Vincent Eds). pp 301-308

Teulon DAJ, Penman DR, Ramakers PMJ (1993) Volatile chemicals for thrips (Thysanoptera: Thripidae) host-finding and applications for thrips management. J. Econ. Entomol. 86, 1405-1415

Timm AE, Stiller M, Frey JE (2008) A molecular identification key for economically important thrips species (Thysanoptera: Thripidae) in southern Africa. Afr Ent 16, 68-75

Van der Merwe FS (2001) Bionomics of thrips on apples and nectarines. Final research report, project 230014, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa

Vernon RS and Gillespie DR (1990) Spectral responsiveness of *Frankliniella occidentalis* (Thysanoptera: Thripidae) determined by trap catches in greenhouses. Environ. Entomol. 19, 1229-1241

Yonce CE, Beshear RJ, Payne JA, Horton DL (1990) Population distribution of flower thrips and the western flower thrips (Thysanoptera: Thripidae) in nectarines and their relative association with injury to fruit in the southeastern United States. J Entomol Sci 25, 427-438

CHAPTER 2. THE APPARENT FAILURE OF CHEMICAL CONTROL FOR MANAGEMENT OF WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* (PERGANDE), ON PLUMS IN THE WESTERN CAPE PROVINCE OF SOUTH AFRICA[1]

2.1 INTRODUCTION

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), originates from the west coast of California (Bryan and Smith 1956). It was first identified in South Africa on chrysanthemums near Krugersdorp in 1987, and on roses and chrysanthemums in greenhouses near Cape Town in 1988 (Giliomee 1989). In 1990 the first specimens were collected from apple orchards near Grabouw in the Western Cape Province (Badenhorst 1993). Prior to the introduction of WFT into South Africa, thrips damage to pome and stone fruit was sporadic and seldom resulted in economic loss. Consequently, thrips in pome and stone fruit were poorly studied. Pansy spot damage was reported on apples in South Africa in 1975 (Rust et al. 1975), but was simply attributed to "thrips". Based on findings by Childs (1927) that oviposition by *Aeolothrips fasciatus* (L.) and *F. occidentalis* cause pansy spot lesions on apples in the Pacific Northwest of the USA, it was assumed that a local *Aeolothrips* species was responsible for similar damage to apples in South Africa (Jacobs 1995b). Myburgh (1986) also reported russetting, silvering and oviposition damage (pansy spot) on apples, grapes and plums, but did not identify the thrips species responsible. According to Jacobs (1995a), russetting and silvering damage to nectarines were associated with high thrips numbers during flowering and just prior to harvest, but it was not known whether only one or several species were responsible. In addition to WFT, other thrips species occur on apples and nectarines, including predatory species (Jacobs 1995a, 1995b). Van der Merwe (2001) confirmed that WFT had become the dominant thrips species in apple and nectarine orchards in the Western Cape and that it caused pansy spot on apples and silvering on nectarines. He also reported that WFT had become a problem on plums in the Western Cape. By 2002 several insecticides were registered in South Africa for control of WFT on stone and pome fruit (Nel et al. 2002).

Despite routine insecticide applications for thrips control, some plum producers still reported economic losses due to thrips damage. Typical pansy spot damage attributed to WFT, and small pits of varying depth, particularly to late blooming cultivars, render fruit unfit for export. Myburgh (1986) mentioned that pitting damage sporadically occurred on plums. These pits

---

[1] Published as Allsopp E (2010) Investigation into the apparent failure of chemical control for management of western flower thrips, *Frankliniella occidentalis* (Pergande), on plums in the Western Cape Province of South Africa. Crop Prot. 29, 824-31

21

appeared similar to damage on Santa Rosa plums in the USA (Kasimatis et al. 1954), which is attributed to WFT. Also in the USA, Swift and Madsen (1956) described pitting damage on Golden Delicious and Red Delicious apples caused by oviposition of *Thrips madroni* Moulton, whilst McMullen (1972) reported that oviposition by *Taeniothrips orionis* Treherne in the ovaries of cherry flowers resulted in dimpling damage. These dimples form when the injured tissue surrounding the oviposition site does not grow at the same rate as the uninjured tissue, developing into a depression.

This study was initiated to determine (1) why current management practices, based on monitoring for WFT presence and application of insecticides, failed to prevent damage in some orchards, and (2) whether WFT is responsible for pitting damage.

2.2 MATERIAL AND METHODS

2.2.1 Trial sites

Six commercial plum orchards, planted with two early ripening cultivars and four mid to late season cultivars, were selected on farms in the Western Cape Province where pansy spot and pitting damage had been reported. Orchard 1 (var. Pioneer, early ripening, 1.1 ha), orchard 2 (var. Larry Ann, mid-season to late ripening, 2.1 ha) and orchard 3 (var. Larry Ann & Songold, late ripening, 2.1 ha) were situated on a farm (33.59316 S, 18.92915 E) located between Wellington and Malmesbury on the coastal plain. These orchards were all surrounded by other orchards and ground cover consisted primarily of volunteer grasses. Orchard 4 (var. Sapphire, early ripening, 1.23 ha), orchard 5 (var. Laetitia & Songold, late ripening, 4 ha) and orchard 6 (var. Santa Rosa & Angelino & Larry Ann, mid-season ripening, 1.99 ha) were located on three different farms (33.93958 S, 19.62250 E; 33.83735 S, 20.01881 E; 33.85988 S, 19.98805 E) around the towns of Robertson and Ashton in the Breede River Valley. This valley lies between mountain ranges that run parallel to the south-eastern coast and annual rainfall averages 200-300 mm (http://gis.elsenburg.com/apps/cfm, accessed 14 July 2016). Orchard 4 was adjacent to natural veld with numerous flowering plants. Orchard 5 had a mixed ground cover of volunteer weeds, including several flowering species, and it was also adjacent to a large area of natural vegetation (veld). Orchard 6 was surrounded by other orchards and there was virtually no ground cover. Producers applied their normal cultivation and horticultural practices, including contact insecticide applications specifically targeting WFT (Table 2.1). Registration of tau-fluvalinate, a pyrethroid, for thrips control has been retracted since these applications were made.

22

Table 2.1 Dates and types of insecticides applied for control of *Frankliniella occidentalis* during flowering in 2006/07 and 2007/08 in the study orchards.

Orchard & Cultivar	2006/07		2007/08	
Orchard 1	20 Aug	Tau-fluvalinate	15 Aug	Tau-fluvalinate
Pioneer	3 Sept	Tau-fluvalinate	22 Aug	Tau-fluvalinate
Orchard 2	7 Sept	Tau-fluvalinate	17 Sept	Tau-fluvalinate
Larry Ann	14 Sept	Tau-fluvalinate		
	29 Sept	Tau-fluvalinate		
Orchard 3	7 Sept	Tau-fluvalinate	17 Sept	Tau-fluvalinate
Larry Ann & Songold	14 Sept	Tau-fluvalinate		
	29 Sept	Tau-fluvalinate		
Orchard 4	20 Aug	Tau-fluvalinate	2 Sept	Chlorfenapyr
Sapphire	27 Aug	Spinosad	13 Sept	Tau-fluvalinate
			8 Oct	Spinosad
Orchard 5	20 Aug	Chlorfenapyr	4 Sept	Chlorfenapyr
Laetitia & Songold	6 Sept	Spinosad	20 Sept	Tau-fluvalinate
			30 Sept	Spinosad
			12 Oct	Alpha-cypermethrin
Orchard 6	20 Aug	Tau-fluvalinate	3 Sept	Tau-fluvalinate
Santa Rosa, Angelino & Larry Ann	27 Aug	Spinosad	8 Sept	Tau-fluvalinate
			15 Sept	Spinosad
			29 Sept	Spinosad
			7 Oct	Lambda-cyhalothrin

Insecticides: Tau-fluvalinate = Klartan®, Chlorfenapyr = Hunter®, Spinosad = Tracer®, Alpha-cypermethrin = Fastac® and Lambda-cyhalothrin = Karate®

2.2.2 Monitoring seasonal occurrence of western flower thrips

Pest management advisors in the fruit industry use commercially available blue or yellow sticky traps to monitor occurrence of WFT in orchards and insecticide applications are recommended accordingly. For practical reasons, two sticky traps are used per orchard in orchards larger than 1 ha, with one trap/ha being the preferred norm. Since the aim of this study was to determine why WFT management practices appeared to be failing in some plum orchards, monitoring in the trial orchards was done according to industry practice (J. Lerm, personal communication). During the 2006/07 growing season one blue and one yellow commercially

available sticky trap, each measuring 100 mm x 290 mm (Bug trap from Agribiol®, Box 16388, Vlaeberg 8018, South Africa) was suspended at a height of approximately 1.6 m from a branch on the outside of the tree canopy in each orchard as soon as the swelling flower buds reached the white tip stage. Prior to balloon stage, the bare plum trees hold no attraction for WFT. Some advisors and producers already use yellow traps to monitor a variety of pests and are loath to buy different traps just for monitoring WFT. Yellow traps were therefore included to determine their reliability as indicators of WFT presence. During the 2007/08 season only blue traps (2 traps/orchard) were used. Sticky traps were examined under a stereo microscope to identify and quantify thrips. Thrips were identified with the aid of a key (Mound & Kibby 1998) and samples were sent to Dr. L.A. Mound at CSIRO in Canberra, Australia for identification verification.

Meteorological data (daily maximum and minimum temperature, average daily temperature, average daily windspeed, daily rainfall) from the closest weather stations to each of the trial sites was obtained from ARC Institute for Soil, Climate & Water as follows: orchards 1 to 3 from Diemerskraal (33,5680 S, 18,9083 E), orchard 4 from Rabiesdal (33,9198 S, 19,6379 E), orchard 5 from Zandvliet (33,8458 S, 20,0611 E) and orchard 6 from Goudmyn (33,8789 S, 20,1012 E).

2.2.3 Blossom and fruit samples

During the 2006/07 season, blossom samples (60 blossoms/orchard) were collected at weekly intervals during the flowering periods of the various cultivars as indicated in Table 2.2 and placed in cooler boxes. Samples were kept in a cold room at 5 °C until examined under a stereo microscope to determine which thrips and other insects occurred in the blossoms. Sampling commenced as soon as the first flower buds approached balloon stage, i.e. before petals opened. Fruit samples (60 fruit/orchard) were collected two to three weeks before harvest of the different cultivars for thrips damage assessments. Blossom and fruit samples were collected randomly throughout each orchard, following a zig-zag pattern to include trees at the row ends and in the middle of rows. One blossom/fruit was picked per tree at a height of 1 – 1.5 m.

During the 2007/08 season, blossoms (70/orchard) were collected at weekly intervals from each orchard and examined under a stereo microscope to record thrips feeding and oviposition damage to ovaries and other flower parts, as well as thrips presence. Based on the results of the previous season, it was decided to increase the number of samples to 70 per orchard. From fruit set until harvest, 70 fruitlets and later 50 fruits (producers were loath to sacrifice more fruit) were collected from each trial orchard at regular intervals, as indicated in Table 2.3,

to record thrips damage. On 31 October 2007 a total of 88 fruits were collected from the producers' thinning sample in orchard 4 and thrips damage recorded. This was done to verify whether damage ascribed to thrips by the producer in his own assessment concurred with the researchers' assessment.

For the 2006/2007 season the producers supplied data of their own WFT damage assessments at harvest. During the 2007/2008 season damage assessments were done in conjunction with the producers at harvest to determine the percentage of fruit from each orchard culled because of WFT oviposition damage. A total of 100 plums were taken from the culling bins in the pack house to determine how many plums were discarded because of WFT pitting and pansy spot damage.

2.2.4 Statistical analysis

A Pearson Product Moment Correlation (SAS, 2008) was performed to determine the relationship between WFT trap catches and oviposition damage to plum ovaries during flowering and fruit set. The null hypothesis was that there is a significant linear relationship between trap counts and damage.

2.3 RESULTS

2.3.1 Monitoring and seasonal occurrence

The numbers of thrips collected on sticky traps over two seasons are presented in Figs 2.1 and 2.3. The dominant thrips species were *F. occidentalis* and *F. schultzei* Trybom. Low numbers of other species were also recorded on the traps, including *Tenothrips frici* (Uzel), *Thrips tabaci* Lindeman*,* various predatory thrips (*Scolothrips* and *Aeolothrips* spp.) and several species of Tubulifera (data not shown). Because of the low numbers and sporadic occurrence of the other species, they were not considered to be significant as far as fruit damage was concerned.

Yellow and blue traps began to register thrips catches at the same time (September/October) early in the season. The yellow sticky trap counts for the 2006/07 season followed the same seasonal trend as blue traps, but the number of thrips per trap was consistently lower, particularly later during the season when thrips numbers began to peak. Blue trap catches peaked at 385, 298, 294, 1640, 746 and 270 in orchards 1 to 6, respectively, while yellow trap catches peaked at 265, 135, 145, 400, 346 and 145, respectively (data not shown).

The earlier ripening cultivar (Pioneer) in orchard 1 flowered approximately four weeks earlier during both seasons than the later ripening cultivars (Larry Anne and Songold) in orchards 2 and 3 on the same farm between Wellington and Malmesbury (Figs 2.1a-c and 2.3a-c). Monitoring data for the 2006/07 season (Fig. 2.1a-c) showed that the number of WFT recorded in orchard 1 during September 2006 was higher than in orchards 2 and 3. This trend persisted throughout the season, and WFT numbers in orchard 1 also peaked at a higher level of 385 WFT/blue trap compared to 298 and 294 WFT/blue trap in orchards 2 and 3, respectively. The number of *F. schultzei* recorded in all three orchards was very low throughout the season, although numbers were slightly higher towards the end of the season, particularly in orchard 3 (Fig. 2.1a-c).

According to Pearsall (2002), temperature and wind speed are the main environmental factors determining WFT flight, therefore average daily temperature and average daily windspeed exceeding 15 km/h for each farm were plotted from August to November for the 2006/07 and 2007/08 seasons (Figs 2.2. & 2.4). Average daily temperatures below 15 °C were recorded frequently during the month of August in both seasons, and also on several occasions during September and October of both seasons. Average daily wind speed in excess of 15 km/h were recorded less frequently, except for the month of October 2006 in orchards 1, 2 and 3 (Fig. 2.2 A).

In the 2007/08 season, the number of WFT recorded on blue sticky traps during flowering and fruit set (August and September 2007) in orchard 1 was lower than for the previous season, but numbers peaked at approximately the same level of 412 and 385 WFT/blue trap, with a pronounced peak of 397 and 419 thrips/trap in the number of *F. schultzei* recorded towards the end of January and beginning of February 2007, respectively (Fig. 2.3a). This was more than two months after harvest and inspection of plum tree shoot tips showed that no thrips adults or larvae were present. The number of WFT in the two later cultivars in orchards 2 and 3 peaked at higher levels of 359 and 789 WFT/blue trap, respectively, compared to peaks of 298 and 294 WFT/trap during the previous season, but the number of *F. schultzei* recorded towards the end of the season did not reach the same peak as in orchard 1 (Fig. 2.3 b and c).

Results for the orchards in the Breede River Valley show that during the 2006/07 season the highest numbers of WFT were recorded in orchard 4, peaking at 1640 WFT/blue trap, but during September 2006 WFT numbers were higher in orchard 6 than in orchards 4 and 5 (Fig. 2.1d-f). The number of *F. schultzei* recorded in all three orchards remained low throughout the season.

During the 2007/08 season, the highest numbers of WFT were recorded in orchard 4 again, with peaks of 1546 and 1677 WFT/blue trap compared to 320 and 530 WFT/blue trap in orchards 5 and 6, respectively (Fig. 2.3d-f).

The number of WFT recorded during September 2007 in orchards 4 and 5 was higher than in the previous season and populations also peaked earlier. The number of *F. schultzei* recorded in orchards 5 and 6 remained very low throughout the season, but in orchard 4 very high numbers, namely 1253 and 2015 thrips/blue trap were recorded during the end of January and beginning of February 2008, respectively, more than a month after the fruit had been harvested (Fig. 2.3f). Shoot tip inspections at that time showed that no *F. schultzei* was present in the growth tips. Weeds in the orchard were also examined, but no *F. schultzei* was found.

2.3.2 Blossom and fruit samples

Few adult thrips were collected from plum blossoms sampled during the 2006/07 season. In orchards 1, 3 and 5 only one WFT was found in the blossom samples from each orchard. Orchard 4 yielded two WFT and in orchard 6 three adult WFT and three immature thrips, presumed to be WFT, were found. During the 2007/08 season, thrips larvae were found in many blossoms from orchards 4, 5 and 6 in the Breede River Valley (up to 60 larvae in a sample of 70 blossoms – data not shown), but larvae could not be reared through to adulthood successfully for positive species identification. Death of larvae before reaching adulthood was probably caused by injuries sustained during flower dissection and capturing. Since WFT was the dominant thrips sampled on sticky traps, it was assumed that these larvae were predominantly WFT.

Results of blossom examinations (n = 70/orchard) during 2007/08 to record thrips feeding and oviposition damage to ovaries and other flower parts (Table 2.2) clearly indicate that thrips damage to plum blossoms on the cooler coastal plain was less severe than in the Breede River Valley orchards. With the exception of orchard 6, there was noticeably more oviposition damage than feeding damage in all orchards. The petals of some of the blossoms in which thrips eggs were found in the styles and ovaries, were not yet fully open, suggesting that adult thrips entered the blossoms very early when the blossoms were in the balloon stage and the petals not tightly closed.
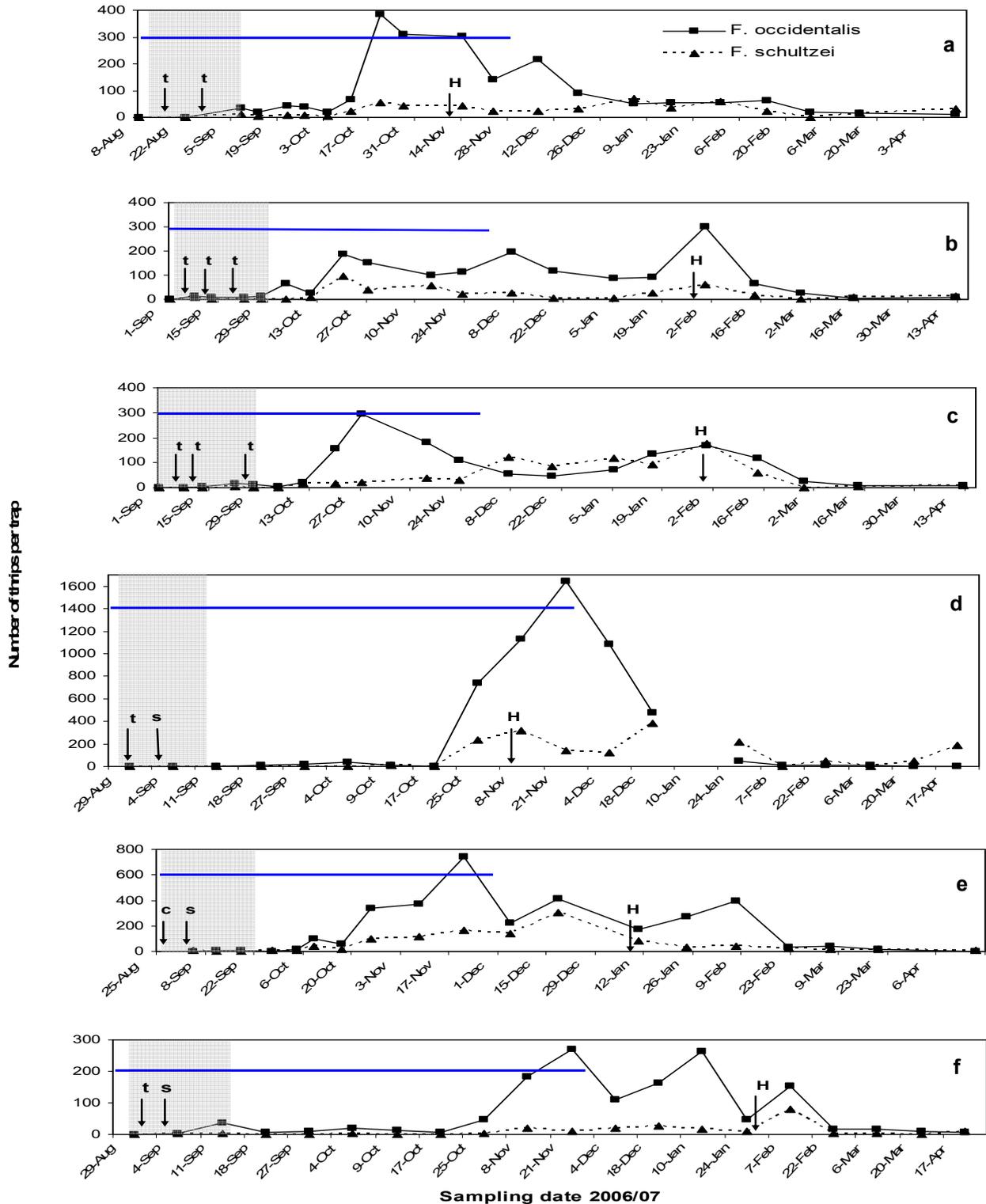
Figure 2.1. Seasonal occurrence of *Frankliniella occidentalis* and *Frankliniella schultzei* on plums sampled with blue sticky traps in orchard 1 (a), orchard 2 (b), orchard 3 (c), orchard 4 (d), orchard 5 (e) and orchard 6 (f) during the 2006/07 season. The shaded area indicates the flowering period and H indicates the beginning of harvest, t = application of tau-fluvalinate, s = application of spinosad, c = application of chlorfenapyr. Blue line indicates period for weather data presented in Fig. 2.2.

28
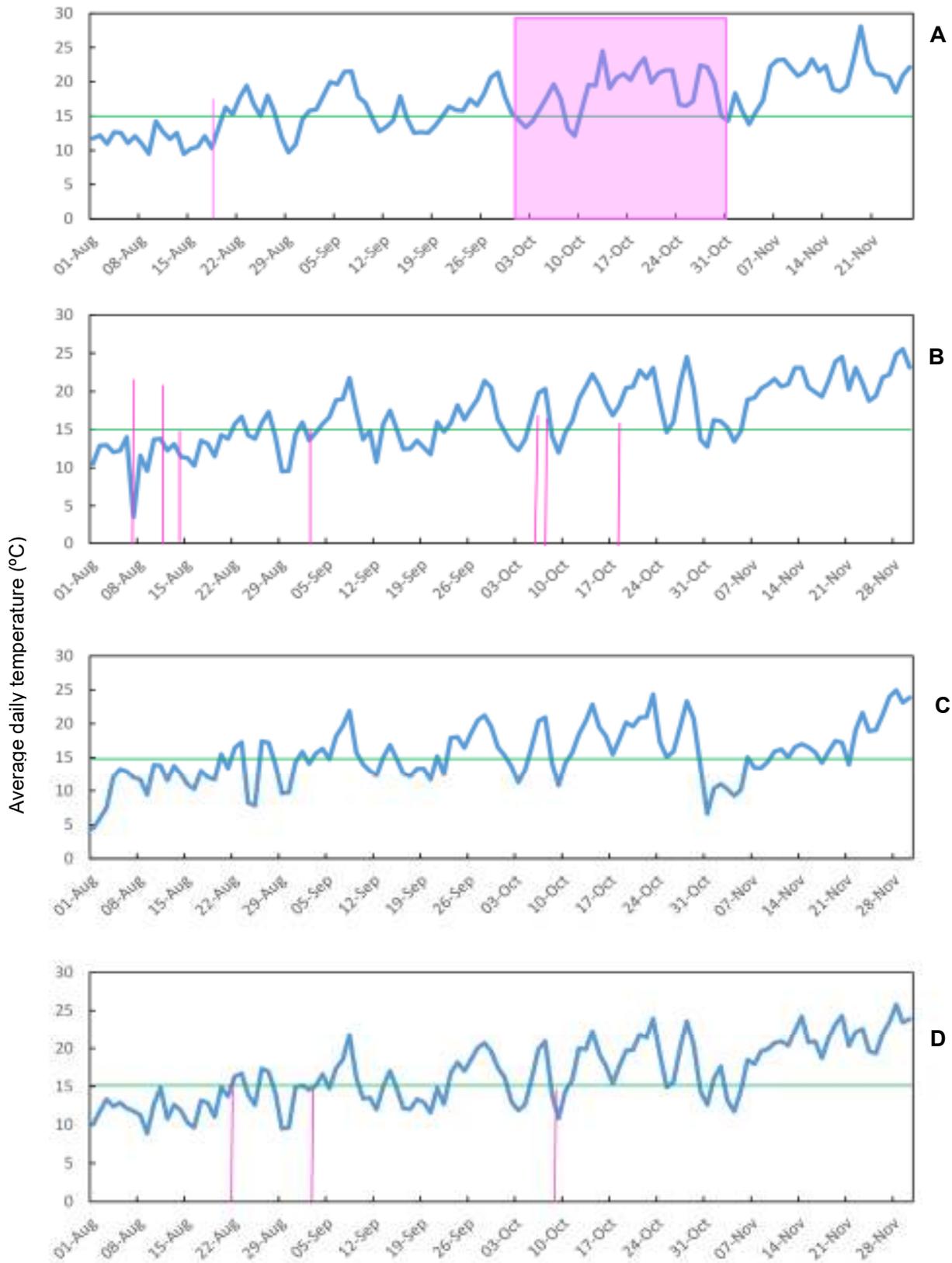
Figure 2.2. Average daily temperature (°C) during 2006/2007 for orchard 1, 2 & 3 (A), orchard 4 (B), orchard 5 (C) and orchard 6 (D). Vertical lines and coloured block indicate average daily windspeed of 15 km/h and higher.

29
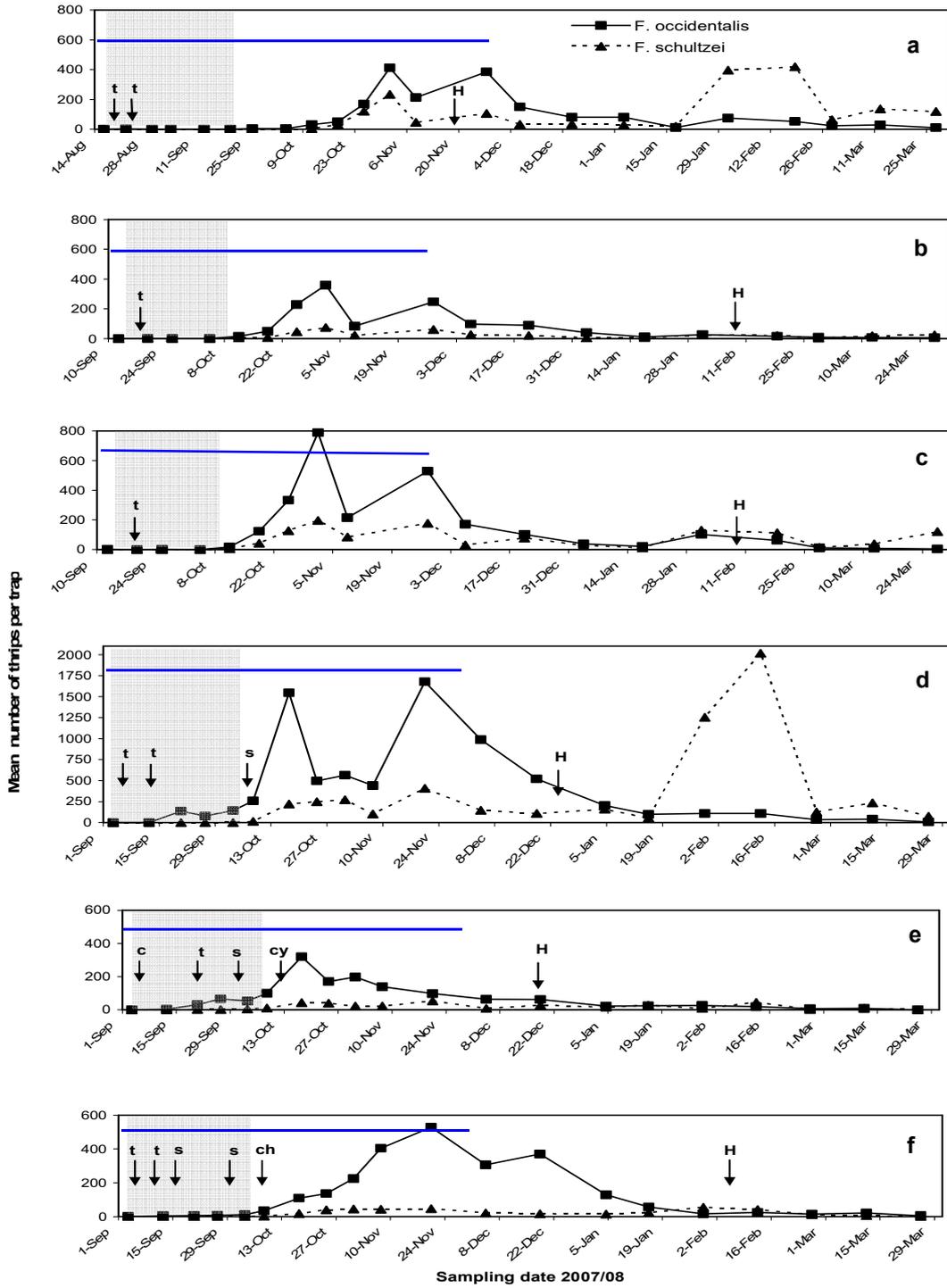
Figure 2.3. Seasonal occurrence of *Frankliniella occidentalis* and *Frankliniella schultzei* on plums sampled with blue sticky traps in orchard 1 (a), orchard 2 (b), orchard 3 (c), orchard 4 (d), orchard 5 (e) and orchard 6 (f) during the 2007/08 season. The shaded area indicates the flowering period and H indicates the beginning of harvest, t = application of tau-fluvalinate, s = application of spinosad, c = application of chlorfenapyr, cy = application of α-cypermethrin, ch = application of λ-cyhalothrin. Blue line indicates period for weather data presented in Fig. 2.4.

30
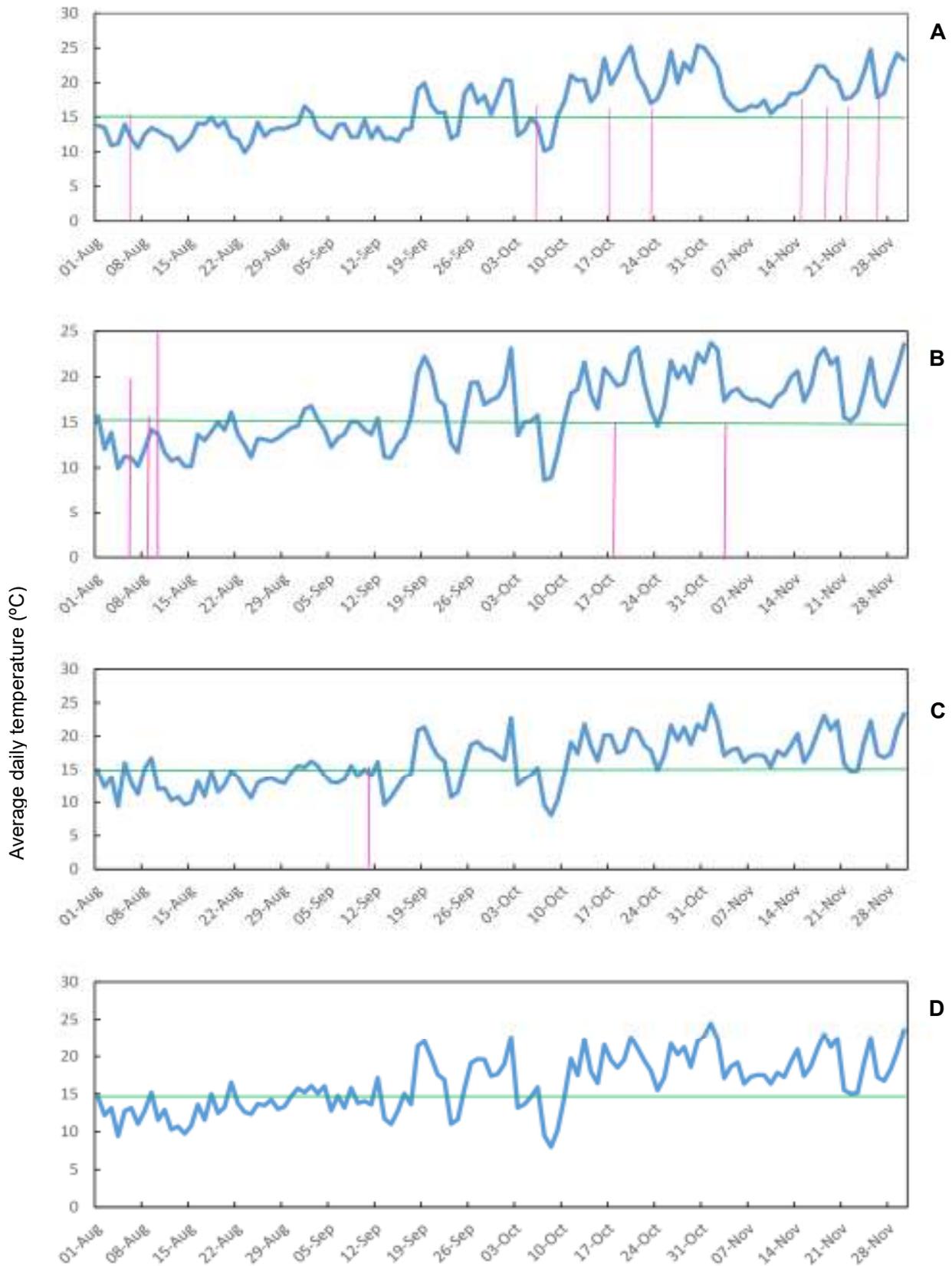
Figure 2.4. Average daily temperature (ºC) during 2007/2008 for orchard 1, 2 & 3 (A), orchard 4 (B), orchard 5 (C) and orchard 6 (D). Vertical lines indicate average daily windspeed of 15 km/h and higher.

31

Examination of WFT oviposition sites on ovaries and developing fruitlets showed that some oviposition sites developed into small, pinprick-sized pits surrounded by lighter coloured pansy spots. Other sites showed a small, raised, corky lesion or pimple surrounded by a depression or dimple. Producers generally describe this as dimpling damage. In some cases, the small pits were not surrounded by pansy spots, but the pits grew larger as the fruit developed (Fig. 2.5). Many fruits had multiple oviposition sites, resulting in mature fruit with multiple pansy spots and/or pits of varying depth.
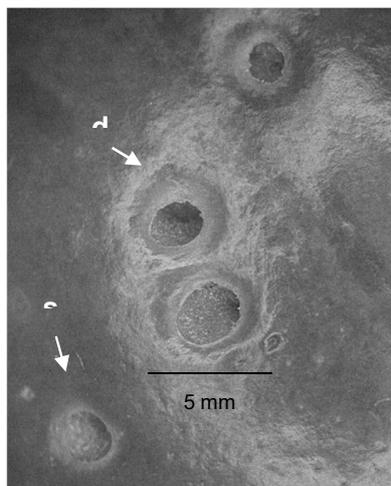
Photo: ARC Infruitec-Nietvoorbij

Figure 2.5. Oviposition damage on mature plums by *Frankliniella occidentalis*. Shallow pits, depth 1-1.5 mm (s); and deep pits, depth 2-4 mm (d).

Fruit damage assessments done by the ARC research team prior to harvest in 2006/07 yielded only one fruit out of 60 with WFT oviposition damage in each of orchards 1 and 2, while no oviposition damage was found in the samples from the other orchards. However, damage assessments done by producers at harvest showed that a sizeable percentage of fruit from each orchard was culled in the pack house because of WFT oviposition damage (pitting and pansy spot): Orchard 1, 2%; Orchard 2, 1.5%; Orchard 3, 1.5%; Orchard 4, 15%; Orchard 5, 5%; and Orchard 6, 5%.

Results of fruit damage assessments during fruit development and ripening for 2007/08 are presented in Table 2.3. With a few exceptions, conspicuously more oviposition damage than feeding damage occurred in all orchards. In orchards 1, 2, 3 and 6 most damaged fruit could be culled during the fruit thinning process. The number of fruit with WFT oviposition damage at

32

harvest was negligible and therefore recorded as zero. Although fruit thinning does not specifically target fruit damaged by thrips and other pests, it is often possible to get rid of such damaged fruit during thinning, provided the level of damage is not too high. For orchards 4 and 5, damage levels were too high for all damaged fruit to be removed during thinning, resulting in 15% and 5% fruit with thrips damage at harvest, respectively.

Statistical analysis showed that there was no consistent significant linear relationship between trap catches and oviposition damage to fruit. Pearson correlation coefficients were as follows: Orchard 1, $r = 0.93139$ ($p = 0.0069$); Orchard 2, $r = 0.62272$ ($p = 0.1353$); Orchard 3, $r = 0.90609$ ($p = 0.0019$); Orchard 4, $r = 0.39297$ ($p = 0.2954$); Orchard 5, $r = 0.57548$ ($p = 0.1049$); Orchard 6, $r = 0.65465$ ($r = 0.0557$).

2.4 DISCUSSION

Western flower thrips was shown to be the dominant thrips species in plum orchards, particularly during the first half of the season, and most adult thrips recovered from blossom samples were WFT. This species was assumed to be the chief causal agent of oviposition and feeding damage to plum flowers and fruit in the Western Cape. In the few instances that *F. schultzei* outnumbered WFT (Fig. 2.1c and d; Fig. 2.3a, c and d), these peaks occurred towards the end of the season, well after harvest. Sampling of shoot tips and weeds at these times failed to find any *F. schultzei.* It is possible that most *F. schultzei* were intercepted by the sticky traps while simply moving through the orchards in search of more suitable hosts.

Superior attractiveness of blue traps for *F. occidentalis* compared to yellow traps has been reported in literature (Vernon and Gillespie 1990; Gillespie and Vernon 1990; Gaum and Giliomee 1994). The results of this study showed that yellow traps may result in underestimation of WFT numbers, therefore the use of blue sticky traps is recommended for monitoring WFT.

In general, the numbers of WFT and other thrips on sticky traps in all the trial orchards were very low during flowering for both seasons (Figs 2.1 and 2.3). In all orchards the period in both seasons where no or very few WFT were caught on blue sticky traps coincided with periods where average daily temperatures often were below 15 °C and average daily wind speed exceeded 15 km/h (Figs 2.2 and 2.4).

Table 2.2. Incidence of feeding and oviposition damage by *Frankliniella occidentalis* in plum blossoms sampled during the 2007/08 season. Sampling dates for orchards 1 – 3 differed from those of orchards 4 – 6 and a dash (-) indicates no blossoms present on the sampling date.

Sampling date	Description of damage	Oviposition sites	No. blossoms with damage (n = 70/orchard)					
			Orchard 1	Orchard 2	Orchard 3	Orchard 4	Orchard 5	Orchard 6
29 Aug. 2007	Feeding & oviposition		0	-	-			
3 Sep. 2007	Feeding & oviposition		0	-	-	0	0	0
12 Sep. 2007	Feeding & oviposition					0	0	0
14 & 19 Sep. 2007	Feeding & oviposition		0	0	0			
20 Sep. 2007	Feeding					0	0	0
	Oviposition					4	12	0
26 Sep. 2007	Feeding					1	2	0
	Oviposition	Style				20	13	1
		Ovary				4	9	1
		Style + ovary				5	7	0
		Flower stem				21	12	4
27 Sep. 2007	Feeding		-	0	0			
	Oviposition	Style	-	2	0			
		Ovary	-	3	0			
		Flower stem	-	1	0			
3 Oct. 2007	Feeding					5	2	8
	Oviposition	Style				0	1	1
		Ovary				10	10	6
		Style + ovary				0	1	0
		Flower stem				2	10	2
4 Oct. 2007	Feeding		-	0	0			
	Oviposition	Style	-	3	0			
		Ovary	-	3	6			

Table 2.3. Incidence of feeding and oviposition damage by *Frankliniella occidentalis* on plum fruit sampled during the 2007/08 season. A dash (-) indicates that fruit had been harvested.

Sampling date	Description of damage	Oviposition sites		No. fruitlets/fruit with damage					
				Orchard 1	Orchard 2	Orchard 3	Orchard 4	Orchard 5	Orchard 6
8 Oct. 2007	Feeding		*n* = 70				3	2	7
	Oviposition	Fruit					20	14	1
		Fruit stem					11	5	0
11 Oct. 2007	Feeding		*n* = 70	9	8	3			
	Oviposition			0	13	1			
17 Oct. 2007	Feeding		*n* = 50				8	4	5
	Oviposition	Fruit					10	19	1
18 Oct. 2007	Feeding		*n* = 50	8	0	3			
	Oviposition	Fruit		0	8	5			
24 Oct. 2007	Feeding		*n* = 50				4	1	5
	Oviposition	Single site/fruit					13	12	5
		Multiple sites/fruit					12	17	0
25 Oct. 2007	Feeding		*n* = 50	-	3	2			
	Oviposition	Single site/fruit		-	9	9			
		Multiple sites/fruit		-	2	3			
31 Oct. 2007	Feeding		*n* = 88, 50				1	4	-
	Oviposition	Single site/fruit, pansy spot					33	3	-
		Single site/fruit					13	6	-
		Multiple sites/fruit, pansy spot					19	0	-
		Multiple sites/fruit					13	8	-
18 Dec. 2007	Feeding		*n* = 50				2	2	-
	Oviposition						4	6	-
17 Jan. 2008	Feeding		*n* = 50	-	0	2			
	Oviposition	Multiple sites/fruit		-	5	4			
29 Jan. 2008	Feeding		*n* = 50				-	-	1
	Oviposition	Multiple sites/fruit					-	-	2
	Feeding and oviposition damage: Whole orchard at harvest			0%	0%	0%	15%	5%	0%

35

According to Pearsall (2002), WFT is most likely to fly at temperatures between 15 ºC and 30 ºC and a wind speed of less than 15 km/h. She also found that when conditions were not conducive for flight (e.g. too windy), WFT could still move around close to the ground in a series of short flights or hops. In view of the fact that sticky traps primarily catch thrips that fly or are blown onto them during flight, the weather conditions during August and September would largely explain the low catches recorded during flowering.

Insecticide applications for thrips control during and after bloom would have reduced the numbers of WFT on the trees and even on the orchard floor vegetation, thereby contributing to low WFT numbers on sticky traps. Once insecticide applications ceased and temperatures increased during October and November (Figs 2.2 and 2.4), WFT numbers on the sticky traps began to increase (Figs 2.1 and 2.3).

Conditions not favourable for flight would not necessarily preclude WFT movement within and over short distances between flowers, nor would it prevent WFT egg laying and development inside unopened blossoms where the microclimate may be more favourable than outside. Examination of unopened plum blossoms during the 2007/2008 season revealed the presence of WFT eggs at times when no or very few WFT adults were caught on sticky traps. It has been reported that WFT development can occur when temperatures exceed a minimum threshold of 8 – 10 °C (Reitz 2009). These results show that when conditions are not conducive to thrips flight, trap counts may not accurately reflect actual thrips infestation in an orchard. Research on WFT in nectarines in British Columbia, Canada (Pearsall and Myers 2000) and apples in Washington State, USA (Cockfield and Beers 2008) found that WFT was active in cover crops and native vegetation adjacent to orchards early in the season and moved into the fruit trees when blossoms developed. Similarly, this study showed WFT movement into plum trees is primarily triggered by the emergence of plum blossoms, since no WFT were trapped in orchards before blossoms emerged.

Pearsall and Myers (2000) showed that orchards adjacent to areas of wild vegetation where WFT can overwinter had higher densities of buds infested by WFT and higher levels of fruit damage than orchards surrounded entirely by other orchards. Orchard 4 and 5 bordered on extensive areas of wild vegetation and these orchards had the highest levels of WFT oviposition damage to fruit in both seasons. Orchard 4 also had the highest trap catches of all the orchards over both seasons (Fig. 2.1d, Fig. 2.3d). Orchard 1, 2 and 3 were surrounded by other orchards and had the lowest levels of fruit damage over both seasons (Figs 2.1a to c, Figs 2.3 a to c). Trap catches in these orchards were relatively low over both seasons, although orchard 3 did show a sharp, high peak in November 2007. Orchard 6 was also

surrounded by other orchards. Although trap catches in this orchard peaked higher in 2007/08 than in 2006/07, WFT numbers during flowering were higher in 2006/07 (Fig. 2.1f, Fig. 2.3f). This may explain why oviposition damage at harvest was 5% in 2006/07 compared to 0% in 2007/08.

The higher population levels recorded in orchards that bordered on wild vegetation, compared to those surrounded by other orchards in this study, concur with the findings of Pearsall and Myers (2000), since wild vegetation provides for a continuous influx of WFT during the season, including after insecticide applications. Furthermore, fluctuations in trap catches from week to week during the season often reflect short periods of weather conditions unfavourable for WFT flight rather than actual WFT activity in the orchard, or short term effects of insecticide applications. Based on these findings, sticky trap counts should be used rather as indicators of WFT presence than as indicators of actual population levels.

In 2006/07 pre-harvest damage assessments done by technical staff of ARC Infruitec-Nietvoorbij indicated almost zero fruit damage, whereas damage assessments at harvest received from the producers indicated that between 1.5% and 15% of fruit was culled because of WFT oviposition damage. This discrepancy may be due to sampling error, given that pre-harvest assessments were based on 60 fruit per orchard, or because of inaccuracies in the damage assessments conducted by the producers. Fruit damage may also have been concentrated in certain areas, such as edge rows, that were under-represented in the pre-harvest samples.

The levels of thrips oviposition damage recorded from ovaries and other flower parts in 2007/08 (Table 2.2) appear to be at odds with the low trap catches during flowering. Examination of unopened blossoms showed that adult WFT enter flowers and begin laying eggs in various flower structures before the petals fully open. Other researchers have shown that WFT enter apple blossoms (Terry 1991) and nectarine blossoms (Pearsall and Myers 2000) at a similar phenological stage. The contact insecticides applied for thrips control during bloom in the trial orchards would not have affected WFT already present in unopened flowers, and would therefore not have prevented oviposition damage to developing fruit at this time.

Since no consistent significant relationship between sticky trap counts and WFT oviposition damage to plums were found, no treatment threshold could be determined. This concurs with findings by Pearsall (2000), who concluded that a threshold density for WFT on nectarines could not be calculated as sticky trap counts and the number of adult WFT per bud did not

give a reliable indication of damage. In the absence of a threshold level, sticky trap counts serve mainly to indicate the presence or absence of WFT in an orchard.

Pearsall and Myers (2000) found that wild vegetation adjacent to nectarine orchards provided a source of continuous WFT infestation throughout the season. Pearsall and Myers (2001) further showed that WFT movement from wild vegetation into orchards in spring mostly occurs in a series of low jumps at ground level. Lower temperatures prevented the formation of thermal updraughts required to assist in flight at higher elevations, and WFT moved closer to ground level if the ground cover consisted of a favourable, flowering host. In the present study sticky traps were suspended at head height on the outside of the tree canopy. In view of the low numbers of WFT on sticky traps early in the season, and the above-mentioned findings, it is suggested that the placement of additional sticky traps closer to the ground cover in orchards during the early part of the season (until petal fall) should be investigated. This may provide a more accurate indication of WFT movement into and their presence in orchards and could aid in the more strategic application of pesticides against thrips.

Damage assessments (Tables 2.2 and 2.3) indicate that most of the observed damage was due to WFT oviposition and not feeding. The low incidence of feeding damage to fruitlets and mature fruit observed in this study is ascribed to the insecticide applications at petal fall and shortly thereafter (Table 2.1) which targeted WFT larvae hatching from eggs laid during bloom and WFT moving in from adjacent vegetation. Fruit with WFT feeding and oviposition damage could also be culled during fruit thinning, which is done by hand, resulting in lower assessments of thrips damage at harvest.

In conclusion, this study showed that insecticide applications failed to prevent oviposition damage to plums because WFT entered flowers and began laying eggs in various flower structures before petals had opened and the first insecticides had been applied. Pansy spotting, dimpling and pitting damage were shown to be the result of WFT eggs laid in developing ovaries. Insecticide applications from 20% bloom until after petal fall did, however, appear to suppress WFT populations during the early part of the growing season, as indicated by the drastically reduced incidence of thrips feeding damage to plums. Weeds and wild vegetation in and around orchards provide a thrips refugium and as such a continuous source of potential infestation for as long as the flowers and fruit remain attractive and vulnerable to WFT, whilst repeated insecticide applications during bloom may pose unacceptable risks to pollinators, particularly with increasing consumer demands for environmentally sustainable fruit production. A control strategy aimed at eliminating WFT from orchards therefore does not

appear feasible. Further research will focus on the possible use of semiochemicals in a "push-pull" strategy to protect flowers and fruit from WFT oviposition and feeding damage.

2.5 REFERENCES

Badenhorst J (1993) Californian thrips, *Frankliniella occidentalis*, on apples. Decid Fruit Grow 43, 204-205

Bryan DE, Smtih RF (1956) The *Frankliniella occidentalis* (Pergande) complex in California (Thysanoptera: Thripidae). University of California Publications in Entomology 10, 359-410

Childs L (1927) Two species of thrips injurious to apples in the Pacific Northwest. J Econ Entomol 20, 805-808

Cockfield SD, Beers EH (2008) Management of dandelion to supplement control of western flower thrips (Thysanoptera: Thripidae) in apple orchards. J Entomol Soc Brit Columbia 105, 89-96

Gaum WG, Giliomee JH (1994) Preference of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), and greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae), for differently coloured sticky traps. J S Afr Soc Hort Sci. 4 39-41

Giliomee JH (1989) First record of Western Flower Thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) from South Africa. J ent Soc sth Afr 52, 179-180

Gillespie DR, Vernon RS (1990) Trap catch of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), as affected by colour and height of sticky traps in greenhouses. J Econ Entomol 83, 971-975

Jacobs S (1995a) Thrips damage and control in nectarine orchards. Decid Fruit Grow 45, 274-280

Jacobs S (1995b) Thrips damage and control in apple orchards. Decid Fruit Grow 45, 323-331

Kasimatis AN, Swift JE, Bailey SF (1954) Thrips on Santa Rosa Plums. Calif Agric 8, 9-10

McMullen RD (1972) *Taeniothrips orionis* oviposition and feeding injury on Cherries. J Entomol Soc Brit Columbia 69, 3-5

Mound LA, Kibby G (1998) Thysanoptera : an identification guide. 2nd ed., Oxon; New York : CAB International

Myburgh AC (1986) Crop Pests in southern Africa: Vol. 1 Deciduous Fruit, Grapes and Berries. Directorate of Agricultural Information Bulletin 407, p 85

Nel A, Krause M, Khelawanlall N (2002) A guide for the control of plant pests,.39th ed. National Department of Agriculture, Pretoria, South Africa

Pearsall IA (2000) Damage to nectarines by the western flower thrips (Thysanoptera: Thripidae) in the interior of British Columbia, Canada. J Econ Entomol 93, 1207-1215

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

Pearsall IA, Myers JH (2000) Population dynamics of western flower thrips (Thysanoptera: Thripidae) in nectarine orchards in British Columbia. J Econ Entomol 93, 264-275

Pearsall IA, Myers JH (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

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

Rust DJ, Bosman IP, De Kock AE (1975) Identification of pests on apples. Apple Series, Diseases and pests in apples, No. 62, Farming in South Africa. Government Printer, Pretoria

SAS Institute, Inc. (2008) SAS Version 9.2. SAS Institute Inc, SAS Campus Drive, Cary, North Carolina 27513, USA

Swift JE, Madsen HF (1956) Thrips damage to apple. J Econ Entomol 49, 398-399

Terry LI (1991) *Frankliniella occidentalis* (Thysanoptera: Thripidae) oviposition in apple buds: Role of bloom state, blossom phenology, and population density. Environ Entomol 20, 1568-1576

Van der Merwe FS (2001) Bionomics of thrips on apples and nectarines. Final research report, project 230014, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch 7599, South Africa

Vernon RS, Gillespie DR (1990) Spectral responsiveness of *Frankliniella occidentalis* (Thysanoptera: Thripidae) determined by trap catches in greenhouses. Environ Entomol 19, 1229-1241

CHAPTER 3. METHYL SALICYLATE, THYMOL AND CARVACROL AS OVIPOSITION DETERRENTS FOR *FRANKLINIELLA OCCIDENTALIS* (PERGANDE) ON PLUM BLOSSOMS[2]

3.1 INTRODUCTION

Female western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), were shown to enter plum (*Prunus salicina* Lindl.) blossoms, even before the petals have opened, to lay eggs in the ovaries (Chapter 2). After the larvae hatch, these oviposition sites either develop into shallow pits surrounded by lighter coloured tissue, known as pansy spots (Childers 1997), or the shallow pits increase in size as the fruit expands, giving the mature fruit a pitted appearance. These lesions can render fruit unfit for marketing and export (Childers 1997). Insecticide applications during flowering are detrimental to pollinators (Desneux et al. 2007) and contact insecticides are not effective against WFT already inside partially opened flowers. The use of plant essential oils to modify pest behaviour, and thereby reduce or eliminate crop damage, offers an environmentally sustainable alternative to the use of toxic insecticides (Agelopoulos et al. 1999; Cook et al. 2007), particularly in view of increasing market demands for residue-free fruit (Kumar 2012).

Koschier (2008) reviewed the use of plant essential oils, that is oils containing compounds responsible for the characteristic fragrance of the plant from which they are derived, for thrips control. Considerable research has been done on the use of semiochemicals (behaviour-modifying chemicals) that attract thrips to increase trapping efficiency for monitoring purposes in greenhouses, as well as on their use in push-pull strategies for thrips control (Koschier 2008 and references therein). Products containing semiochemicals are now also available commercially. Thripline™ ams (Syngenta Bioline LTD) contains the synthetic aggregation pheromone released by WFT males, whilst Lurem-TR (Koppert) contains a host plant derived attractant (Broughton and Harrison 2012). These were shown to improve monitoring efficacy of various thrips species in glasshouse, field and orchard trials (Teulon et al. 2008; Nielsen et al. 2010; Broughton and Harrison 2012).

Push-pull systems typically use non-toxic behavioural stimuli to make the crop less attractive to the pest (push), while luring it towards an attractive alternative (pull) from where it can be

---

[2] This chapter (excluding potted trials) was published as Allsopp E, Prinsloo GJ, Smart LE, Dewhirst SY (2014) Methyl salicylate, thymol and carvacrol as oviposition deterrents for *Frankliniella occidentalis* (Pergande) on plum blossoms. Arthropod-Plant Interactions 8, 421-427

removed (Cook et al. 2007). Some push-pull systems use interplanting of repellent plants in combination with planting of attractive trap crops adjacent to the crop (Khan et al. 2000), whilst others propose the use of plant-derived antifeedants, oviposition deterrents or semiochemicals from non-host plants which interfere with host plant location by the pest, in combination with trap crops or attractants to protect the harvestable crop (Bennison et al. 2003; Koschier 2008 and references therein).

Interplanting with repellent plants to deter WFT is not practical in plum orchards, because WFT flying in from outside to land on the plum blossoms are unlikely to be deterred by repellent plants at ground level. Furthermore, many producers do not want plants growing in orchards during the growing season because they compete with the trees for water and nutrients. However, using a plant-derived essential oil as the "push" element in a push-pull strategy to reduce or prevent egg-laying in plum blossoms could offer a sustainable management option. According to the classification of Dethier et al. (1960), a repellent would cause insects to move away from the source and may prevent them from alighting, whereas a deterrent acts after landing and inhibits feeding and/or oivposition. Sedy and Koschier (2003) showed that the essential oils thymol and carvacrol reduced WFT oviposition on leaf discs of bean cotyledons. Koschier et al. (2007) demonstrated that the essential oil methyl salicylate reduced the oviposition rate of WFT when applied to cucumber and bean leaf discs. Electroantennography with excised WFT antennae showed that methyl salicylate elicited a response (Pow et al. 1999), which indicates that it is an olfactory response originating in the antennae. However, WFT is a flower specialist and Pearsall (2000) found that WFT were more attracted to highly scented tree fruit blossoms e.g. apple and crab apple, *Malus baccata* (L.) (Rosales: Rosaceae), than to less scented blossoms e.g. peach and nectarine. This raised the question whether these essential oils would be as effective in reducing WFT egg-laying when applied to fragrant flowers.

Reduction in genetic diversity of laboratory-reared insect populations compared to wild populations may lead to subtle behavioural changes (Schutze et al. 2015). Mirnezhad et al. (2012) demonstrated the evolution of a WFT lab biotype specialized in a particular host which showed variation in genetics and reproductive behaviour. The ultimate aim of this study is to identify deterrents able to significantly reduce WFT oviposition in plum blossoms in open orchard conditions where WFT populations consist of females of all ages and reproductive stages. It was therefore to decided to use field-collected WFT females rather than laboratory reared thrips to ensure a robust test that better reflects the potential impact of the deterrents on wild WFT populations.

The aim of this study was to determine if thymol, methyl salicylate and carvacrol can reduce the oviposition rate of WFT on plum blossoms. The possibility that the addition of Citrex® medium mineral oil could provide a more sustained release of the essential oils compared to a water/ethanol/Triton X-100 suspension (pers. comm. G Prinsloo, ARC Small Grains Institute, Bethlehem, South Africa) was also investigated.

3.2 MATERIAL AND METHODS

3.2.1 Preparation of essential oil suspensions

Thymol crystals (min. 99.5%), methyl salicylate oil (≥ 99%), carvacrol oil (98%) and ethanol (98%) were sourced from Sigma-Aldrich Pty. Ltd. (Johannesburg, South Africa). Triton X-100 (0.05% in distilled water), also sourced from Sigma-Aldrich, was used as a wetting agent in all treatments, according to the method of Sedy and Koschier (2003). Citrex® medium mineral oil (1% in distilled water), obtained from BASF South Africa (Pty) Ltd, was also used in some treatments as an additional wetting agent in an attempt to slow down the release of the essential oils over 24 hours.

Each of the essential oils was tested in three consecutive experiments (Table 3.1). Essential oil concentrations of 0.1% and 1%, as used by Sedy & Koschier (2003) and Koschier et al. (2007), were tested. Since the ultimate aim was to determine if applications of these essential oils could reduce WFT egg-laying in an orchard situation, it was decided to include a higher concentration (10% or 5%) as well. Based on levels of phytotoxicity observed in the first experiment whilst investigating Thymol, essential oil concentrations were adjusted in subsequent experiments.

Thymol crystals were dissolved in ethanol (50% weight/volume solution), and then either diluted with distilled water containing 0.05% Triton X-100 to obtain 0.1%, 1% and 10% EWT (ethanol/water/Triton) suspensions or with distilled water containing 0.05% Triton X-100 and 1% Citrex® to obtain 0.1%, 1% and 10% EWT plus Citrex® suspensions. A control EWT and a control EWT plus Citrex® suspension both containing 10% ethanol, the same as the 10% thymol suspensions but with no thymol, were also prepared.

Methyl salicylate oil was dissolved in ethanol (50% volume/volume solution), and then diluted with distilled water containing 0.05% Triton X-100 to obtain 0.05%, 0.5% and 5% EWT suspensions. A control EWT treatment which contained 5% ethanol, but no methyl salicylate was also prepared. In addition, methyl salicylate was added directly to distilled water containing 0.05% Triton X-100 and 1% Citrex® medium mineral oil to obtain 0.1%, 1% and

10% WT (water/Triton) plus Citrex® suspensions. A control WT plus Citrex® suspension with no methyl salicylate was also prepared.

Carvacrol oil was dissolved in ethanol (50% volume/volume solution) and then diluted either with distilled water containing 0.05% Triton X-100 to obtain 0.1%, 1% and 5% EWT suspensions or with distilled water containing 0.05% Triton X-100 and 1% Citrex® to obtain 0.1%, 1% and 5% EWT plus Citrex® suspensions. A control EWT and a control EWT plus Citrex® suspension, both containing 5% ethanol, the same as the 5% carvacrol suspensions, but with no carvacrol, were also prepared.

Table 3.1. Experimental design of three separate experiments with plum blossoms treated with thymol (n=23), methyl salicylate (n=20) or carvacrol (n=23) in two different suspensions. One block, equalling one replicate, is shown. EWT = ethanol/water/Triton suspension, WT = water/Triton suspension, control = suspensions containing no essential oil, untreated = untreated plum blossom.

Thymol	Untreated	Suspension	
		EWT	EWT plus Citrex®
Untreated	✓		
Control		✓	✓
0.1%		✓	✓
1%		✓	✓
10%		✓	✓

Methyl salicylate	Untreated	Suspension	
		EWT	WT plus Citrex®
Untreated	✓		
Control		✓	✓
0.05%		✓	
0.1%			✓
0.5%		✓	
1%			✓
5%		✓	
10%			✓

Carvacrol	Untreated	Suspension	
		EWT	EWT plus Citrex®
Untreated	✓		
Control		✓	✓
0.1%		✓	✓
1%		✓	✓
5%		✓	✓

3.2.2 Western flower thrips

Clover flowers were collected in a field 7 km south of Stellenbosch, South Africa (33.92330º S, 18.87331º E) and taken to the laboratory in a cooler box, where a small manual aspirator was used to collect individual WFT females in separate glass vials. Females of approximately similar age were selected, based on the degree of sclerotization and colour development. The vials with WFT females were placed a fridge at 5° C for 10-15 min to immobilize the thrips. Each female was examined under a binocular microscope to confirm species identity (according to Mound and Kibby 1998) and used immediately in a bioassay. Randomly selected specimens that were used in the laboratory bioassays were taken to the Central DNA Sequencing Facility at the University of Stellenbosch for PCR and sequencing to verify the morphological identifications of WFT. Primers C1-J-1751 and C1-N-2191 (Simon et al. 1994) were used to amplify a fragment of the WFT cytochrome oxidase subunit I gene.

3.2.3 Laboratory bioassay method

Dormant plum (*Prunus salicina* Lindl. cv. Laetitia) shoots were collected from an orchard on the ARC Bien Donnè research farm (Groot Drakenstein, South Africa; 33.84303 S, 18.98039 E) and from a farm near Ashton (33.83735 S, 20.01881 E) during winter (July). The plum cultivar Laetitia was previously shown to be vulnerable to WFT oviposition damage (Chapter 2) and it was used for the bioassay because the blossoms have longer stems than the other cultivars susceptible to WFT, e.g. Sapphire. Stem ends of the shoots were dipped in melted paraffin wax, shoots were wrapped in newspaper and sealed plastic bags to prevent desiccation, and kept in a cold room at 3 ºC from July until used (September and October). From September, when WFT became available, 3-5 shoots were removed at a time and induced to flower by placing them in water in a breeding room at room temperature with a north-facing glass wall to let in sunlight. This extended the availability of blossoms for some months beyond the normal flowering period. The flower stems of individual plum blossoms were embedded in small cubes (1 cm^3) of water-saturated florist's sponge covered with aluminium foil to prevent desiccation (Fig. 3.1). To obtain an even distribution of the essential oil suspensions, a sprayer system, based on a Potter tower sprayer, was used (Potter 1952). Four plum blossoms, each embedded in a cube of florist's sponge as described above, were placed on the tray of the sprayer in a square formation. The essential oil suspension was stirred constantly by means of a magnetic stirrer, while 2 ml were extracted with a micro-pipette and injected into the sprayer with constant air pressure at 150 kPa supplied by a compressor and regulated by a Camozzi air flow meter (range 0 – 400 kPa) obtained from Airpower Pty Ltd in Bellville, South Africa.

Photo: ARC Infruitec-Nietvoorbij

Figure 3.1. Plum blossom with stem embedded in florist's sponge covered with aluminium foil.

After application of the essential oil, each blossom was placed in a separate plastic container (170 ml capacity) and allowed to dry before being covered with a lid. A hole (4 cm x 4 cm) was cut into each lid and a square of thrips-proof gauze was glued over the opening to prevent build-up of volatile compounds in the container. A single female WFT was added to each container. A strip (5 mm x 15 mm) of sterile filter paper with a smear of pollen and a smear of honey as additional food sources was placed next to the blossom embedded in florist's sponge in the bottom of each container. After addition of the WFT, the containers (i.e. experimental units) each with a treated blossom and a WFT female, were placed in Perspex chambers (40 cm x 40 cm x 25 cm) attached to a ventilation system in an insect rearing room to prevent build-up of the volatile essential oils and ethanol in the containers and cross-contamination between experimental units of different concentrations (Fig. 3.2). This room had one north-facing glass wall to provide natural light and was kept at 25 ºC and 60% relative humidity.

After 24 hours the WFT were removed from each container and after a further 24 hours each flower was examined under a binocular microscope and all WFT eggs counted to determine the oviposition rate, that is the number of eggs laid per WFT female in 24 hours (Koschier et al. 2007). Phytotoxic effects, manifesting as browning and cellular collapse of plant tissue, of the treatments on the blossoms were recorded.

Photo: ARC Infruitec-Nietvoorbij

Figure 3.2. Perspex cages attached to ventilation system which extracted air from each chamber.

3.2.4 Experimental design of laboratory bioassay

Due to the volatile nature of the essential oils, each essential oil was tested in a separate experiment to avoid cross contamination between the oils (Table 3.1). Each experiment with an essential oil adopted a randomised block design where one block consisted of one replicate of each of the eight treatments (four concentrations, two suspensions) randomly assigned to individual plum blossoms, plus an untreated plum blossom. Application of the essential oil suspensions was described under 3.2.3 and four replicates were conducted simultaneously. Three of the Perspex containers each held the containers with four replicates of the same concentration of essential oil, applied in two suspensions; totalling eight small containers per cage. The fourth Perspex container held containers with four replicates of the two controls, plus four untreated plum blossoms; totalling 12 small containers. The treatments consisting of each of the two control suspensions were included to account for the possibility that the suspensions themselves may affect WFT behaviour. Twenty-three replicates were conducted with thymol and carvacrol, whereas 20 replicates were conducted with methyl salicylate.

3.2.5 Experimental design of trials with potted plum trees

Screen house trials were conducted during September 2013, using shoots of two year old plum trees (cv. Laetitia) in 25 L ceramic pots. The screen house consisted of a wooden framework covered by 50 mesh nylon anti-virus netting (Econoheat, Blackheath, South Africa). Six essential oil suspensions, namely 0.5% methyl salicylate EWT, 1% methyl salicylate WT

48

+ Citrex®, 0.1 % thymol EWT, 1 % thymol EWT + Citrex®, 0.1 % carvacrol EWT and 1 % carvacrol EWT + Citrex®, were tested in separate trials, with a treatment and an untreated control in each trial. Since only three screen house compartments (3 m x 3 m) were available and the flowering period of plums only lasts for two to three weeks, the two trials with different formulations of the same essential oil were conducted concurrently in the same screen house compartment. As the number of potted trees was limited, individual shoots were used as replicates, with a minimum of eight required for statistical analysis (M Booyse, ARC Biometry Unit, Stellenbosch). Each tree was used only once. For each trial suitable shoots with flower buds at white tip stage were selected on two potted trees and treatments (essential oil suspension and untreated control) assigned randomly to shoots on both trees. The number of replicates for trials one to six were 11, 10, 8, 8, 10 and 10, respectively. Where more than the minimum of 8 shoots were available on the trees, the maximum number available were used. All selected shoots were covered with plastic bags and the remaining shoots stripped of flower buds. After 24 hours the plastic bags were removed one at a time to apply the essential oil suspensions by means of a handheld, 1.5 L pressurised spray pump. The contents was constantly shaken and swirled to keep the essential oils in suspension. Depending on shoot length, five to ten seconds were required to obtain approximately even wetting of the balloon stage blossoms with the fine spray mist. Once the spray deposit had dried, the bags were removed from the control shoots and a total of 45 WFT females released at a central point between the trees in each trial.

During September 2014 two essential oil suspensions, namely 1% methyl salicylate WT plus Citrex® and 1% carvacrol EWT plus Citrex®, were tested on six potted plum trees in a single trial, using individual shoots as replicates. A total of 24 shoots with blossoms at white tip stage were selected and treatments, including an untreated control, assigned randomly. Treatments were applied according to the same procedure used in the screen house trials. After application of the essential oil suspensions, all the trees were taken into an insectary room with a north-facing glass wall to provide natural light. A total of 70 WFT females were released at a central point between the trees. Shoots were removed after 3 days to count WFT eggs laid in the flower structures.

3.2.6 Statistical Analysis

The experimental design for the laboratory bioassays and the trials with potted trees were completely randomized. The laboratory bioassay data for each essential oil were analyzed separately. The Shapiro Wilk's test was performed to test for normality on the standardized residuals (Shapiro & Wilk 1965). Square root transformation was performed on the bioassay raw data. The data of the laboratory bioassay and potted trials were subjected to an analysis

of variance (ANOVA) using General Linear Models Procedure (PROC GLM) of SAS software (Version 9.2; SAS Institute Inc, Cary, USA). Fischer's least significant difference was used at the 5% probability level to facilitate comparison between treatment means.

3.3 RESULTS

Morphological identifications were corroborated by sequence results of the cytochrome oxidase subunit I gene which showed 100% identity to *F. occidentalis* (GenBank: AF378686.1).

3.3.1 Thymol

Table 3.2 shows the number of eggs laid per WFT female in 24 hours, i.e. oviposition rate, on plum blossoms treated with different concentrations and formulations of thymol. The mean oviposition rate did not differ significantly between the untreated blossoms and control treatments, indicating that oviposition rate was not significantly affected by suspension. The 10% thymol in EWT suspension effected a significant reduction of 83% in mean oviposition rate compared to the untreated blossoms. Although not statistically significant, the 10% thymol in EWT plus Citrex® suspension reduced the mean oviposition rate by 59% compared to the untreated blossoms. Browning of petals was recorded for 91% of flowers treated with the 10% EWT suspension and 61% of flowers treated with the 10% EWT suspension, but not for blossoms treated with the 0.1% and 1% suspensions.

3.3.2 Methyl salicylate

Table 3.3 shows the number of eggs laid per WFT female in 24 hours, i.e. oviposition rate, on plum blossoms treated with different concentrations and formulations of methyl salicylate. The mean oviposition rate did not differ significantly between the untreated blossoms and control treatments, indicating that oviposition rate was not significantly affected by suspension. The 1% methyl salicylate in WT plus Citrex® suspension reduced the mean oviposition rate significantly by 71% compared to the untreated blossoms and the 10% methyl salicylate in WT plus Citrex® suspension reduced the mean oviposition rate significantly by 69% compared to the untreated blossoms. Phytotoxicity (browning) was observed in 20% of blossoms treated with 5% methyl salicylate in EWT and 25% treated with 10% methyl salicylate in WT plus Citrex®, but not in blossoms treated with the lower concentrations.

3.3.3 Carvacrol

Table 3.4 shows the number of eggs laid per WFT female in 24 hours, i.e. oviposition rate, on plum blossoms treated with different concentrations and formulations of carvacrol. The mean

oviposition rate did not differ significantly between the untreated blossoms and control treatments, indicating that oviposition rate was not significantly affected by suspension. Both the 5% carvacrol in EWT suspension and the 5% carvacrol in EWT plus Citrex® suspension reduced the mean oviposition rate significantly by 71% compared to the untreated blossoms. The 1% carvacrol in EWT plus Citrex® suspension also reduced the mean oviposition rate significantly compared to the untreated blossoms. Browning of petals and some browning of sepals and anthers were recorded on 50% of flowers treated with 5% carvacrol in EWT and on 38% treated with 5% carvacrol in EWT plus Citrex® suspensions, but not in blossoms treated with the 0.1% and 1% carvacrol suspensions.

3.3.4 Trials with potted plum trees

No meaningful results were obtained in the screen house trials (Table 3.5). Only seven eggs were laid in one of the 11 replicates in trial 1 where blossoms were treated with 0.1% methyl salicylate EWT and only three eggs were laid in a single replicate on the untreated blossoms. A single egg was laid in one replicate of trial 4 where the blossoms were treated with 1% thymol EWT plus Citrex®, but none in the untreated blossoms. No eggs were laid in trials 2, 3, 5 and 6 where blossoms were treated with 1% methyl salicylate WT plus Citrex®, 0.1% thymol EWT, 0.1% carvacrol EWT or 1% carvacrol EWT plus Citrex®, respectively, or in the untreated blossoms.

In the trial conducted in the insectary during September 2014, a total of 21 eggs were laid in 192 blossoms treated with 1% methyl salicylate WT plus Citrex®, 14 eggs were laid in 234 blossoms treated with 1% carvacrol EWT plus Citrex® and 33 eggs were laid in the 322 untreated blossoms. These differences were not statistically significant ($F_{(2; 21)} = 0.44$, $P = 0.648$), mainly due to the huge variation between replicates. Slight to severe phytotoxicity was observed in all essential oil treatments in both sets of trials.

Table 3.2   Number of eggs laid per *Frankliniella occidentalis* female in 24 hours on plum blossoms treated with different concentrations and formulations of thymol. EWT = ethanol/water/Triton suspension, control = suspensions containing no essential oil, untreated = untreated plum blossom.

	No eggs/female/24 h (*n* = 23)																							Total	Mean[*]
Untreated	7	6	0	8	3	0	0	0	5	0	0	1	0	9	8	0	5	0	5	0	0	7	5	69	3.0[abc]
<u>Control</u>																									
EWT	3	5	6	0	6	4	0	0	9	0	0	4	3	0	0	1	2	5	2	2	5	5	0	62	2.70[ab]
EWT + Citrex®	2	5	0	2	0	0	0	7	5	0	0	5	1	0	5	3	3	1	0	4	7	0	4	54	2.35[abc]
<u>EWT</u>																									
0.1% thymol	0	0	3	0	1	0	0	3	0	0	0	0	3	3	2	5	5	2	4	0	0	2	1	34	1.48 [abcd]
1% thymol	8	-	6	0	7	0	0	9	9	3	0	7	5	5	0	5	0	3	4	0	0	0	0	71	3.23[a]
10% thymol	0	0	1	0	3	-	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	-	3	11	0.52[d]
<u>EWT + Citrex®</u>																									
0.1% thymol	9	0	0	0	0	2	0	6	3	0	0	2	9	1	0	6	4	7	-	0	2	3	0	54	2.45 [abc]
1% thymol	-	0	2	0	0	0	0	7	5	0	0	6	0	0	4	3	4	1	0	0	0	0	0	32	1.45[bcd]
10% thymol	0	0	0	-	0	0	0	4	0	1	0	7	3	2	4	0	0	0	3	4	0	0	0	28	1.22[cd]

LSD $_{(p = 0.05)}$ = 0.5873

[*] Means followed by the same letter do not differ significantly.

Table 3.3   Number of eggs laid per *Frankliniella occidentalis* female in 24 hours on plum blossoms treated with different concentrations and formulations of methyl salicylate. EWT = ethanol/water/Triton suspension, WT = water/Triton suspension, control = suspensions containing no essential oil, untreated = untreated plum blossom.

	No eggs/female/24 h (n = 20)																				Total	Mean*
Untreated	4	0	0	3	6	0	0	0	0	0	1	5	6	0	0	3	6	11	6	4	55	2.75[ab]
Control																						
EWT	0	0	1	0	2	3	2	0	2	0	3	6	0	6	5	2	6	0	6	0	44	2.20[ab]
WT + Citrex®	-	1	4	4	1	4	0	3	0	2	2	3	5	7	5	6	1	0	0	5	53	2.79[a]
EWT																						
0.05% methyl salicylate	2	0	1	4	4	3	0	0	0	0	3	0	5	2	0	8	0	0	10	0	42	2.10[abc]
0.5% methyl salicylate	2	0	0	1	0	1	3	0	0	0	1	5	0	0	4	0	5	0	2	0	24	1.20[bc]
5% methyl salicylate	7	0	0	0	0	1	3	0	0	0	0	0	2	6	5	0	8	0	6	2	40	2.00[abc]
WT + Citrex®																						
0.1% methyl salicylate	9	1	0	0	1	0	1	0	1	0	3	0	1	0	5	2	3	2	2	0	31	1.55[abc]
1% methyl salicylate	0	0	0	1	0	0	0	0	0	0	0	1	3	0	6	0	0	5	0	0	16	0.80[c]
10% methyl salicylate	4	0	0	0	0	0	0	-	0	0	0	0	4	1	4	1	0	0	0	2	16	0.84[c]

LSD $_{(p = 0.05)}$ = 0.5625

 * Means followed by the same letter do not differ significantly.

Table 3.4 Number of eggs laid per *Frankliniella occidentalis* female in 24 hours on plum blossoms treated with different concentrations and formulations of carvacrol. EWT = ethanol/water/Triton suspension, control = suspensions containing no essential oil, untreated = untreated plum blossom.

	No eggs/female/24 h (*n* = 24)																								Total	Mean
Untreated	0	3	6	1	8	8	5	10	5	11	0	0	0	13	1	0	5	0	9	8	11	11	17	17	149	6.21[a]
Control																										
EWT	2	3	6	1	7	5	11	9	5	0	0	0	2	4	11	1	8	0	7	4	0	8	0	0	94	3.92[ab]
EWT + Citrex®	0	3	5	6	2	0	0	7	0	4	1	0	4	4	8	7	0	2	0	0	6	2	8	7	76	3.17[abcd]
EWT																										
0.1% carvacrol	7	0	11	0	0	0	4	9	2	0	5	5	13	0	0	0	6	5	3	0	0	0	9	3	82	3.42[bcd]
1% carvacrol	6	0	0	0	6	7	8	0	5	0	0	0	3	8	13	0	4	5	7	0	6	0	8	13	99	4.13[abc]
5% carvacrol	1	0	0	5	6	0	5	0	3	0	0	3	0	0	7	1	0	6	0	2	0	4	0	0	43	1.79[cd]
EWT + Citrex®																										
0.1% carvacrol	5	2	0	3	0	0	0	9	0	0	1	4	7	1	8	5	4	0	3	7	8	0	12	0	79	3.29[abcd]
1% carvacrol	0	0	1	5	15	3	0	3	0	0	7	0	5	14	1	8	4	0	0	3	4	6	0	0	79	3.29[bcd]
5% carvacrol	0	0	0	0	5	9	1	5	0	0	6	2	5	5	0	0	0	0	5	0	0	0	0	0	43	1.79[d]

LSD $_{(p = 0.05)}$ = 0.7069

* Means followed by the same letter do not differ significantly.

Table 3.5. Oviposition of *Frankliniella occidentalis* on potted plum tree blossoms treated with methyl salicylate, thymol and carvacrol in two different suspensions. EWT = ethanol/water/Triton suspension, WT = water/Triton suspension.

	Treatment	No eggs laid by *F. occidentalis* (number of blossoms per shoot inspected) per replicate											Mean no eggs/rep**
	Screen house trial												
Trial 1	0.5% methyl salicylate EWT	0 (23)	0 (30)	0 (29)	0 (15)	0 (35)	0 (30)	7 (30)	0 (30)	0 (30)	0 (30)	0 (30)	0.64
	Untreated control	0 (20)	0 (20)	0 (15)	0 (15)	0 (30)	0 (30)	0 (30)	0 (30)	3 (30)	0 (30)	0 (30)	0.27
Trial 2	1% methyl salicylate WT + Citrex®	0 (14)	0 (35)	0 (27)	0 (35)	0 (35)	0 (30)	0 (30)	0 (30)	0 (30)	0 (29)		0
	Untreated control	0 (20)	0 (40)	0 (30)	0 (30)	0 (30)	0 (35)	0 (35)	0 (30)	0 (40)	0 (30)		0
Trial 3	0.1 % thymol EWT	0 (6)	0 (35)	0 (22)	--*	0 (4)	0 (18)	0 (17)	0 (30)				0
	Untreated control	0 (10)	0 (6)	0 (10)	0 (19)	0 (45)	0 (17)	–	–				0
Trial 4	1 % thymol EWT + Citrex®	0 (29)	0 (18)	0 (25)	1 (29)	0 (25)	0 (15)	0 (25)	0 (20)				0.13
	Untreated control	0 (11)	0 (35)	0 (20)	0 (35)	0 (25)	0 (20)	0 (20)	0 (20)				0
Trial 5	0.1 % carvacrol EWT	0 (10)	0 (20)	0 (13)	0 (29)	0 (35)	0 (20)	0 (25)	0 (30)	0 (25)	0 (15)		0
	Untreated control	0 (30)	0 (20)	0 (20)	0 (25)	0 (15)	0 (20)	0 (20)	0 (30)	0 (24)	0 (22)		0
Trial 6	1 % carvacrol EWT + Citrex®	0 (25)	0 (10)	0 (15)	0 (25)	0 (20)	–	0 (15)	0 (15)	0 (15)	0 (20)		0
	Untreated control	0 (30)	0 (28)	0 (15)	0 (20	0 (17)	0 (10)	0 (18)	–	0 (25)	0 (15)		0
	Insectary trial												
Trial 1	1% methyl salicylate WT + Citrex®	9 (14)	0 (23)	2 (50)	0 (19)	0 (14)	10 (20)	0 (28)	0 (24)				2.63 [a]
	1 % carvacrol EWT + Citrex®	4 (37)	0 (55)	7 (29)	0 (15)	0 (20)	1 (35)	2 (23)	0 (20)				1.75 [a]
	Untreated control	0 (15)	19 (18)	0 (22)	1 (46)	12 (70)	1 (85)	0 (36)	0 (30)				4.13 [a]

* Blossoms did not develop, many showed phytotoxic damage.
** Values followed by the same letter do not differ significantly (p ≤ 0.05)

55

3.4 DISCUSSION

Methyl salicylate is an essential oil in plants such as wintergreen (*Gaultheria procumbens* L.), but it is also a herbivore-induced plant volatile (HIPV) that can either attract, repel or deter target organisms, depending on the concentration (Koschier et al. 2007). Synthetic methyl salicylate attracts natural enemies under laboratory and field conditions and commercial products have been developed for this purpose (Woods et al. 2011 and references therein). Thymol and carvacrol are monoterpenoid phenols that occur in various plants in the Lamiaceae family and are major compounds in thyme (*Thymus vulgaris* L.) and oregano (*Origanum vulgare* L.). Their repellent and deterrent effects on WFT and *T. tabaci* were demonstrated by Sedy and Koschier (2003). These compounds have also been shown to have insecticidal activity against other insects, such as tobacco cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) (Isman et al. 2001), as well as house fly, *Musca domestica* Linnaeus (Diptera: Muscidae) and rice weevil, *Sitophilus oryzae* Linnaeus (Coleoptera: Curculionidae) (Saleh 1986).

In the laboratory bioassay, thymol (10% in EWT), methyl salicylate (1% and 10% WT plus Citrex®) and carvacrol (5% in EWT, 1% and 5% EWT plus Citrex®) significantly reduced the oviposition rate of WFT when applied to individual plum blossoms. These results compare favourably with Sedy and Koschier (2003) and Koschier et al. (2007), who found 1% thymol, methyl salicylate, and carvacrol, in EWT suspensions, resulted in a reduction in the oviposition rate of WFT on bean leaf discs (92%, 26% and 42% respectively). This study is the first to show that these essential oils are also effective in reducing WFT oviposition when applied to fragrant plum flowers.

Plum trees blossom for a short period only, therefore the trials using potted plum trees could only be conducted once in a season. The fact that no eggs were laid in four of the six screen house trials conducted during September 2013 is attributed mainly to inclement weather with cold wind, rain and even snow on the mountains that set in shortly after the trails commenced. To avoid the effect of weather, the trees were moved into an insectary room during the trial in September 2014. More eggs were laid in the untreated blossoms than in blossoms treated with 1% methyl salicylate WT plus Citrex® and 1% carvacrol EWT plus Citrex®. Although not statistically significant these results showed the same trend as the laboratory assay where 1% methyl salicylate and 1% carvacrol reduced WFT oviposition significantly.

For this study, WFT females of different ages were collected from field populations rather than from an insectary-adapted colony, because the reaction of wild females to the treatments were expected to better reflect real orchard situations. Variation in the number of eggs laid over 24

56

hours within treatments is ascribed mainly to the natural variation in fecundity expected in a wild population and with females of different ages.

In the laboratory bioassays the 5% and 10% essential oil concentrations of thymol, methyl salicylate and carvacrol resulted in varying degrees of phytotoxic damage to the blossoms. This is attributed to the inadequacy of the wetting agents used to maintain these higher concentrations in suspension. The role of phytotoxicity in the reduction in mean oviposition rate in these treatments is unclear, but since eggs were found in some flowers with phytotoxic damage, it is possible that visual cues did not play a significant role in host selection within the confines of the small containers with the flowers and thrips. According to Terry (1997), olfactory, gustatory and tactile stimuli are used to identify suitable host plants at short range. It is also possible that eggs were laid before phytotoxic damage developed. Phytotoxicity of any degree is unacceptable, therefore 5% and 10% concentrations of thymol, methyl salicylate and carvacrol can only be considered for use in a push-pull system if a formulation can be developed to prevent phyotoxicity.

However, the 1% concentration of methyl salicylate, as well as the 1% and 5% concentrations of carvacrol reduced oviposition significantly in the laboratory bioassays without any observable phytotoxic effects and these oils seem to offer the best prospects for use in a push-pull system. Sedy and Koschier (2003) similarly did not report phytotoxicity with 0.01%, 0.1% and 1% thymol and carvacrol on bean leaf discs, while Koschier et al. (2007) also found no phytotoxicity with 0.1% and 1% methyl salicylate on bean and cucumber leaf discs. Phytotoxicity was also observed in some of the trials with potted plum trees. The insolubility of the essential oils in water and the inadequacy of the wetting agents used to maintain them in suspension, combined with difficulties to keep the spray mixture sufficiently agitated in the handheld spray pump may have resulted in uneven deposits on the blossoms.

Determining the true potential of thymol, methyl salicylate and carvacrol as oviposition deterrents for WFT in a push-pull system in trials with potted plum trees was limited by the inability of the wetting agents, Triton X-100 and Citrex® medium mineral oil, to maintain stable suspensions of the essential oils. Similar difficulties in finding suitable formulations for WFT deterrents were identified by Reitz et al. (2008) in trials with geraniol, lemon grass (*Cymbopogon flexuosus,* Poales: Poaceae) oil and tea tree (*Melaleuca alternifolia,* Myrtales: Myrtaceae) oil on field grown tomatoes in Florida. Isman (2006) also pointed out that the essential oils most effective against pests are often phytotoxic. Micro-encapsulation of active ingredients of pesticides and pheromones used for mating disruption is a technology that provides for sustained release of active ingredients in an environmentally sustainable way

(Masuda 2011) and could hold the solution to the problem of delivering sustained, effective doses of essential oils without phytotoxic effects.

This study showed that thymol, methyl salicylate and carvacrol can reduce WFT oviposition and may have potential for use as part of a pest management strategy for WFT on plums. Further research under semi-field and field conditions are recommended to verify these results. However, further research and commercial use of these essential oils require the development of stable suspensions or formulations to deliver behaviourally effective concentrations with improved deposition, sustained release and without phytotoxic effects..

3.5 REFERENCES

Agelopoulos N, Birkett MA, Hick AJ, Hooper AM, Pickett JA, Pow EM, Smart LE, Smiley DWM, Wadhams LJ Woodcock CM (1999) Exploiting semiochemicals in insect control. Pestic Sci 55, 225-235

Bennison JA, Maulden K, Dewhirst S, Pow E, Slatter P, Wadhams LJ (2003) Towards the development of a push-pull strategy for improving biological control of western flower thrips on chrysanthemum. In 7th International Symposium on Thysanoptera: "Thrips, Plants, Tospoviruses: the Millenial Review", 2-7 July 2001, Reggio Calabria, Italy. Marullo R, Mound L (Eds). CSIRO Entomology, Canberra, Australia, 199-206

Broughton S, Harrison J (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 Prot 42, 156-163

Childers CC (1997) Feeding and Oviposition Injuries to Plants. In *Thrips as crop pests*. Lewis T (Ed). CAB International, Oxon, UK, 505-537

Cook SM, Khan ZR, Pickett JA (2007) The use of push-pull strategies in integrated pest management. Annu Rev Entomol 52, 375-400

Desneux N, Decourtye A, Delpuech J-M (2007) The Sublethal Effects of Pesticides on Beneficial Arthropods. Annu Rev Entomol 52, 81-106

Dethier VG, Browne LB, Smith CN (1960) The designation of chemicals in terms of the responses they elicit from insects. J Econ Entomol 53, 134-136

Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 51, 45-66

Isman MB, Wan AJ, Passreiter CM (2001) Insecticidal activity of essential oils to the tobacco cutworm, *Spodoptera litura*. Fitoterapia 72, 65-68

Khan ZR, Pickett JA, van den Berg J, Wadhams LJ, Woodcock CM (2000) Exploiting chemical ecology and species diversity: stem borer and striga control for maize and sorghum in Africa. Pest Manag Sci 56, 957-962

Koschier EH (2008) Essential oil compounds for thrips control – A review. Nat Prod Commun 3, 1171-1182

Koschier EH, Hoffmann D, Riefler J (2007) Influence of salicylaldehyde and methyl salicylate on post-landing behaviour of *Frankliniella occidentalis* (Pergande). J Appl Entomol 131(5), 362-367

Kumar S (2012) Biopesticides: A Need for Food and Environmental Safety. J Biofertil Biopestici 3: e107. doi:10.4172/2155-6202.1000e107

Masuda M (2011) Microencapsulation of Pesticides for Controlling Release from Coatings. Unpublished PhD thesis, Chalmers University of Technology, SE-412 96 Göteborg, Sweden

Mirnezhad M, Schidlo N, Klinkhamer PGL, Leiss KA (2012) Variation in genetics and performance of Dutch western flower thrips populations. Econ Entomol 105, 1816-1824

Mound LA, Kibby G (1998) Thysanoptera: an identification guide. 2nd Ed. CAB International, Oxon, New York

Nielsen M-C, Worner SP, Chapman RB, de Kogel WJ, Perry NB, Sansom CM, Murai T, Muvea AM, Subramanian S, Teulon DAJ (2010) Optimising the use of allelochemicals for thrips pest management. In International Society of Chemical Ecology Conference Vol 31, 324

Pearsall IA (2000) Flower preference behaviour of western flower thrips in the Similkameen Valley, British Columbia, Canada. Entomol Exp Appl 95, 303-313

Potter C (1952) An improved laboratory apparatus for applying direct sprays and surface films, with data on the electrostatic charge on atomised spray fluids. Ann Appl Biol 39, 1-29

Pow EM, Bennison JA, Birkett MA, Luzniak MJ, Manjunatha M, Pickett JA, Segers IS, Wadhams LJ, Wardlow LR, Woodcock CM (1999) Behavioural responses of western flower thrips (*Frankliniella occidentalis*) to host plant volatiles. Proceedings sixth International Symposium on Thysanoptera, Antalya, Turkey, April 27 to May 1. Akdeniz University, Antalaya, Turkey. 121-128

Reitz SR, Maiorino G, Olson S, Sprenkel R, Crescenzi A, Momol MT (2008) Integrating plant essential oils and kaolin for the sustainable management of thrips and tomato spotted wilt on tomato. Plant Dis 92, 878-886

Saleh MA (1986) A desert plant from Egypt, *Anabasis setifera*: An efficient natural factory of carvacrol and thymol. J Agr Food Chem 34, 192-194

Schutze MK, Dammalage T, Jessup A, Vreysen MJB, Wornoayporn V, Clarke AR (2015) Effects of laboratory colonization on *Bactrocera dorsalis* (Diptera: Tephritidae) mating behaviour: 'what a difference a year makes'. Zookeys 540, 369-383

Sedy KA, Koschier EH (2003) Bioactivity of carvacrol and thymol against *Frankliniella occidentalis* and *Thrips tabaci*. J Appl Entomol 127, 313-316

Shapiro SS, Wilk MB (1965) An Analysis of Variance Test for Normality (complete samples). Biometrika 52, 591-611

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

Terry LI (1997) Host selection, communication and reproductive behaviour, pp 65-118 in T Lewis (ed) Thrips as Crop Pests. CAB International, Oxon, UK

Teulon DAJ, Nielsen M-C, de Kogel WJ, van Tol RWHM, Davidson MM (2008) A new lure for *Thrips major*. N Z Plant Protect-SE. 61, 386

Woods JL, James DG, Lee JC, Gent DH (2011) Evaluation of airborne methyl salicylate for improved conservation biological control of two-spotted spider mite and hop aphid in Oregon hop yards. Exp Appl Acarol 55, 401-416

CHAPTER 4. ATTRACTIVENESS OF WHITE CLOVER (*Trifolium repens* L.) AND PLUM (*Prunus salicina* LINDL. CV. SAPPHIRE) FLOWER VOLATILES TO FEMALE WESTERN FLOWER THRIPS, *Frankliniella occidentalis* (PERGANDE)

4.1 INTRODUCTION

Push-pull strategies that entail the use of plant-derived antifeedants, oviposition deterrents or semiochemicals from non-host plants, which interfere with host plant location by the pest, in combination with trap crops or attractants to protect the harvestable crop have been proposed for various crops grown under protection by Bennison et al. (2003), van Tol et al. (2007) and Koschier (2008). The semiochemicals thymol, methyl salicylate and carvacrol, known to affect WFT behaviour (Koschier 2008), were shown to deter WFT ovipositioning in plum flowers significantly (Chapter 3) and could be developed to provide the "push" in a push-pull strategy for WFT on deciduous fruit in South Africa. However, a "pull" element still has to be found and this chapter focuses on research to identify a suitable trap crop to provide this "pull"

In anthophilous thrips, such as WFT, flowers play a very important role in host selection and since thrips larvae are not able to move very far to find host plants, selection of a suitable host for larval feeding and development by ovipositing females is crucial (Terry 1997). Volatiles emitted from flowers and flower colour are the main factors that determine the attractiveness of a plant to WFT (Blumthal et al. 2005 and references therein). Both these host plant cues act over distance and do not require physical contact. Like all thrips, WFT are weak flyers that can easily be transported by wind (Lewis 1997) and blown onto plants. Once a WFT has landed on a potential host plant, odour, tactile and gustatory stimuli are used to identify whether it is a suitable host or not (Terry 1997).

Insect antennae contain sensilla which house olfactory receptor neurons (Abdullah and Butt 2015). When activated by chemical ligands from the environment, they produce signals that travel to the brain's processing regions and ultimately result in behavioural responses. Evidence from research conducted on the role of plant volatiles in insect host location seems to overwhelmingly support the hypothesis that phytophagous insects recognise their host plants by identifying the correct blends and ratios of ubiquitous plant volatiles, rather than by detecting the presence or absence of compounds specific to that host plant (Bruce et al. 2005 and references therein). The location of olfactory receptor neurons that each detect a specific plant volatile on the same olfactory sensillum on the antenna makes it possible for high-resolution spatio-temporal information to be sent to the central nervous system for processing.

This enables the insects to recognise host plant volatiles within a complex background of non-host volatiles (Bruce et al. 2005). Physiological changes related to different plant growth stages can result in changes in the ratio of volatiles emitted by a plant over time (Johnson et al. 2004). These changes often indicate changes in host suitability, which is very important for insects that only utilise a specific growth stage of the host plant. According to Bruce et al. (2005), recognizing host odour by detecting different ratios of common plant volatiles means that processing of peripheral signals from the olfactory receptor neurons by the central nervous system is of paramount importance. This means that insects with different host preferences can have similar peripheral plant volatile receptors, but their behavioural responses can vary. It also enables polyphagous insects to recognise many different host plants with different volatile bouquets. Instead of being constrained by peripheral inputs, using the central nervous system to process and identify the desired volatile blends and ratios affords insects the flexibility that could explain how host switching occurs, as no 'new' olfactory receptor neurons are required (Bruce et al. 2005).

The findings by Abdullah and Butt (2015), using electroantennography, that the olfactory system of WFT is very sensitive to green leaf volatiles produced by many plants and that their olfactory receptor neurons can even distinguish between geometric isomers of some compounds, are consistent with the hypothesis of Bruce et al. (2005) and would indicate that WFT recognises its many host plants by detecting the correct blends of common plant volatiles. Findings by Koschier et al. (2000) that various common plant and flower volatiles like *E-β*-Farnesene, *p-* and *0*-anisaldehyde, linalool and geraniol attract WFT further support this hypothesis.

Extensive research has been conducted on the identification of semiochemicals that attract WFT and their application in practice to enhance the efficacy of traps for monitoring WFT (Roditakis and Lykouressis 1996; Manjunatha et al. 1998; Koschier 2008; Teulon et al. 2008; Broughton and Harrison 2012; Muvea et al. 2014). Research has also identified highly attractive lure or trap plants that can be used to enhance integrated management of WFT on ornamentals in greenhouses (Bennison et al. 1999; Pow et al. 1998; Bennison et al. 2003). The plants identified in these studies, namely a *Verbena officinalis* L. (Lamiales: Verbenaceae) cultivar and a chrysanthemum cultivar, are, however, not suited for use as lure or pull plants in and around plum orchards in South Africa because they do not flower early in spring when grown outdoors here. To date reports of only two studies on possible trap crops for WFT in orchards worldwide could be traced (Pearsall 2000, Cockfield and Beers 2008). Pearsall (2000) investigated the possibility of using naturally occurring ground covers, mainly dandelion, as trap crops under nectarine trees in British Columbia, Canada, but came to the

conclusion that none of the ground covers would be effective as a trap crop because they did not consistently flower before the nectarines came into bloom and they were not attractive enough to lure WFT away from the nectarines. Cockfield and Beers (2008) concluded that dandelion cover crops did not significantly reduce WFT population density, particularly in apple blossoms, or fruit injury in apple orchards in central Washington, USA.

To be effective, the trap or lure plants that provide the "pull" in plum orchards should be equally as attractive, or more attractive to WFT than plum blossoms. Furthermore, bearing in mind that WFT enter blossoms even before the petals are fully open (Chapter 2), the trap crop should flower before the plums do. Pearsall (2000) found that WFT females moving into nectarine orchards during spring in British Columbia preferred flowers closer to the ground (up to 25 cm in height) and were attracted to highly scented flowers. White clover, *Trifolium repens* L. (Fabales: Fabaceae) grows close to the ground and has long been known as a favoured host plant for WFT (Felland et al. 1995; Pearsall & Myers 2000). Pobozniak and Wiech (2005) showed that intercropping white clover with cabbage reduced the number of *Thrips tabaci* on the cabbage, compared to cabbage cultivated in monoculture. Since it is widespread in and around orchards in the Western Cape Province of South Africa, white clover was selected for investigation as a possible trap crop.

Plum blossoms are only available for a short period in spring, which does not allow sufficient time to conduct bioassays to determine the attractiveness of plum blossoms and clover flowers to WFT using whole flowers. It was therefore decided to capture the volatile compounds of these flowers by means of air entrainment (Pow et al. 1999) so as to extend the time available for bioassays. The plum cultivars Laetitia and Sapphire were previously shown to be vulnerable to WFT oviposition damage (Chapter 2) and therefore deemed to be attractive to WFT. The potted Laetitia plum trees, used for the insectary trial in Chapter 3, produce both leaves and blossoms at bud burst, whereas Sapphire trees produce only flowers on the shoots and leaves develop after flowering (personal observation). In order to obtain only flower volatiles, potted Sapphire trees were used for air entrainment.

Olfactometers have been used to assess the short-distance attraction of volatile compounds for WFT. These include modified Pettersson four arm olfactometers, as well as Y-tube olfactometers (Manjunatha et al. 1998; Pow et al. 1999; Koschier et al. 2000; Bennison et al. 2003; Davidson et al. 2008). In this study a Y-shaped glass tube olfactometer was used for the bioassays because preliminary trials conducted at the beginning of this study indicated that WFT females tended to be inactive in the corners formed between the upper and side

panels of the Pettersson olfactometer without making any choice, whereas the Y-tube has no corners to hide in.

The aim of this study was to determine the attractiveness of the collected volatiles of white clover (*T. repens*) flowers and of unopened (balloon stage) and open plum (*Prunus salicina* Lindl. cv. Sapphire) blossoms to WFT females, using a Y-tube olfactometer.

4.2 MATERIAL AND METHODS

4.2.1 Preparation for air entrainment

A dynamic headspace or air entrainment technique was used to collect volatiles of clover and plum blossoms. This entails enclosing the clover or plum flowers in cellophane bags and using purified air to draw the volatiles onto the adsorbent Porapak Q (Waters Assoc. Inc, USA) in small glass tubes (Blight 1990, Pow et al 1999). Tubes containing 50 mg Porapak Q were prepared according to a protocol used at Rothamsted Research (Harpenden, UK) and described here. Porapak Q must be purified before use in air entrainment, as new samples contain many contaminants (Blight 1990). Approximately 200 ml glass wool (silane treated) was placed into a 500 ml Pyrex beaker and heated in an oven at 150 °C for 2-3 hours. Powder-free, nitrile examination gloves were worn when handling the glass wool to prevent contamination with oils and volatiles from the skin.

To construct glass ampoules, soda lime glass tubes (1 mm thickness, 7 mm diameter) were cut into 25-30 cm lengths and one end of each tube was sealed using a Bunsen burner. A small plug of glass wool was removed from the oven and pushed down into the sealed end of each tube to form a plug approximately 5 mm thick. The storage tubes were kept in an oven set at 150 °C until required.

Cotton glove liners were worn during preparation of the Porapak Q tubes to prevent contamination with volatiles. Soda lime glass tubes (1 mm thickness, 5 mm diameter) were cut into 10 cm lengths and one end of each tube was tapered by heating over a Bunsen burner. A small tuft of glass wool was inserted to plug the tapered end of the tube (plug about 5 mm thick), whereafter 50 mg of the porous polymer Porapak Q 50/80 (Supelco, Bellafonte, PA) was transferred into the tube using a Pasteur pipette fitted with a latex bulb, followed by another glass wool plug to hold the powder in place (Fig. 4.1a). To purify and condition the Porapak Q, the tubes were secured in a stand inside an extraction cabinet with tapered ends pointing downwards (Fig. 4.1b). Glass syringes were used to rinse the Porapak Q in each tube with 2 ml dichloro-methane, followed by 2 ml redistilled diethyl ether. After rinsing, the tubes were heated for two hours in a pre-heated, custom-built heating column set at 132 °C under a

65

stream of purified nitrogen supplied through PTFE Teflon tubing with 1.5 mm inner diameter (Fig. 4.2). This rinsing and heating process was repeated three times for each group of tubes, after which each conditioned Porapak Q tube was carefully slid, tapered end first, into a glass ampoule. The ampoules were filled with nitrogen and the open ends sealed using a Bunsen burner. Sealed ampoules were kept in a freezer at -18 °C until used.

Glass wool plug

Porapak Q

(a)

Photo: ARC Infruitec-Nietvoorbij

(b)

Photo: ARC Infruitec-Nietvoorbij
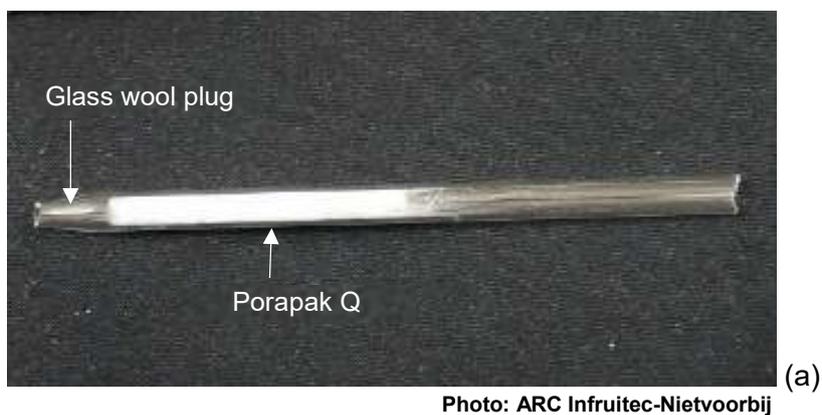
Figure 4.1. Glass tube containing Porapak Q (a) and tubes with Porapak Q in stand (b), ready for rinsing with dichloro-methane and redistilled diethyl ether.
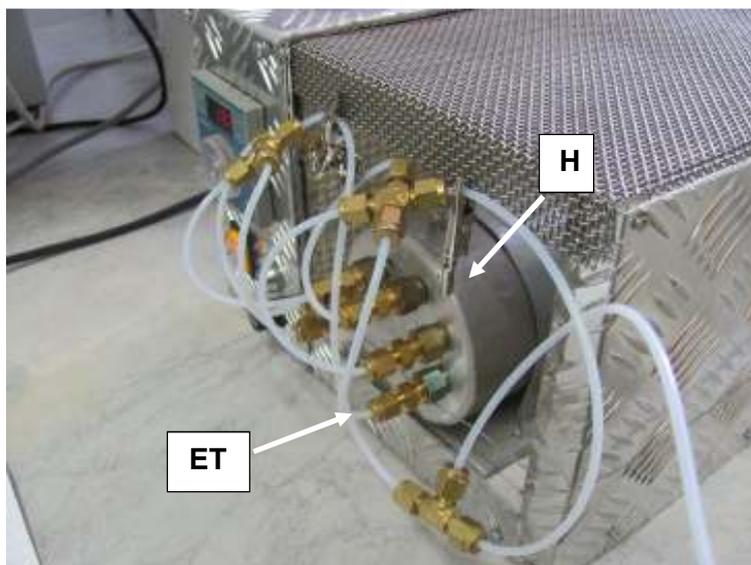
Figure 4.2. Heating block (H) with six entrainment tubes (ET) filled with Porapak Q inserted. Purified nitrogen supplied through PTFE Teflon tubing.

4.2.2 Volatile capture

A custom-made air entrainment kit (Fig. 4.3), obtained from Rothamsted Research, was used to capture the flower volatiles. Subjecting plants to stress, for example by wounding and cutting or by exposure to high temperatures, has been shown to cause changes in their volatile emissions (e.g. Loreto et al. 2006). It was therefore decided to use six month old whole white clover plants (*Trifolium repens* L.) in plastic pots and three year old plum trees (*Prunus salicina* Lindl. cv. Sapphire) in 25 L ceramic pots for volatile capture by means of air entrainment. Cellophane oven roasting bags (Glad®, 500 mm x 500 mm) were heated in an oven at 100 °C for an hour to eliminate possible volatile odours and left to cool in the oven, after which they were secured over a cluster of clover flowers (mixture of half and fully open) or over a shoot with unopened (balloon stage) or fully open plum blossoms with the ties provided with the oven bags. Air was purified by passing it through the charcoal filter of the custom-made air entrainment kit at a rate of 1.5 L/min. The filtered air was then pumped into one oven roasting bag covering the test flowers and one empty bag that served as a control. At the other end of each bag, air was drawn out at a rate of 550 ml/min through a glass tube (5 mm outer diameter) containing Porapak Q to collect the volatile compounds (Fig. 4.4). Volatiles were collected from flowers over a 48 hour period to ensure a sufficient concentration of volatiles. To elute the volatile compounds from the Porapak Q, the tubes were secured in a stand inside an extraction cabinet with tapered ends pointing downwards and rinsed with redistilled diethyl ether (750 µl), applied through a glass syringe, into tapered vials (1.1 ml capacity) with PTFE seals in the caps (Chromacol vials, Supelco, Sigma-Aldrich Corp.) and stored in a freezer at -18 °C.

Samples of clover and plum volatiles were sent to Rothamsted Research for preliminary analysis by gas chromatography and mass-spectrometry. Further analysis to quantify and identify volatiles could not be completed due to equipment failure at the ARC laboratory.

4.2.3 Sourcing of western flower thrips

As explained in Chapter 3, field-collected WFT females rather than laboratory reared WFT were used to gauge the reaction of wild WFT populations to plum and clover flower volatiles, since the aim in this study was to identify a trap crop with similar or greater attraction for WFT than plum blossoms under open orchard conditions. Clover flowers were collected in a field 7 km south of Stellenbosch, South Africa (33.92330º S, 18.87331º E) every morning before olfactometer trials began and taken to the laboratory in a cooler box where a small, manual aspirator was used to collect individual WFT females in separate glass vials. As described in Chapter 3, females of approximately similar age were selected, based on the degree of sclerotization and colour development.
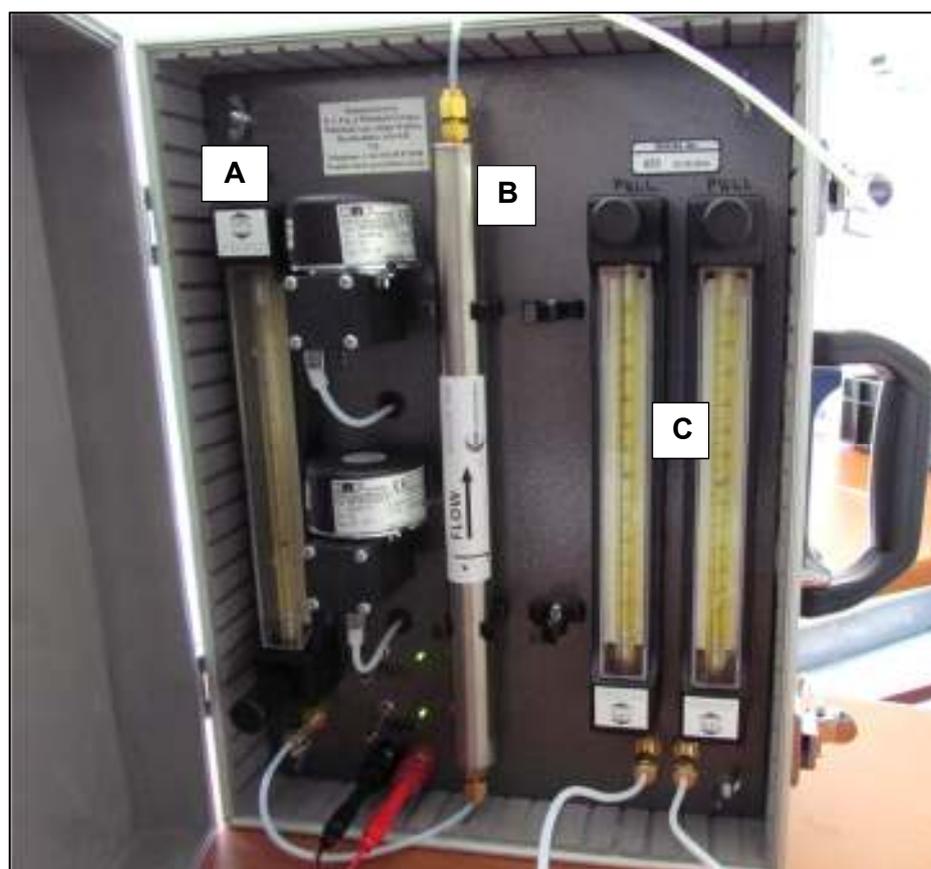
Figure 4.3. Air entrainment kit. A = regulator for air inlet; B = charcoal filter; C = regulators for air drawn out of oven bags through Porapak tubes.
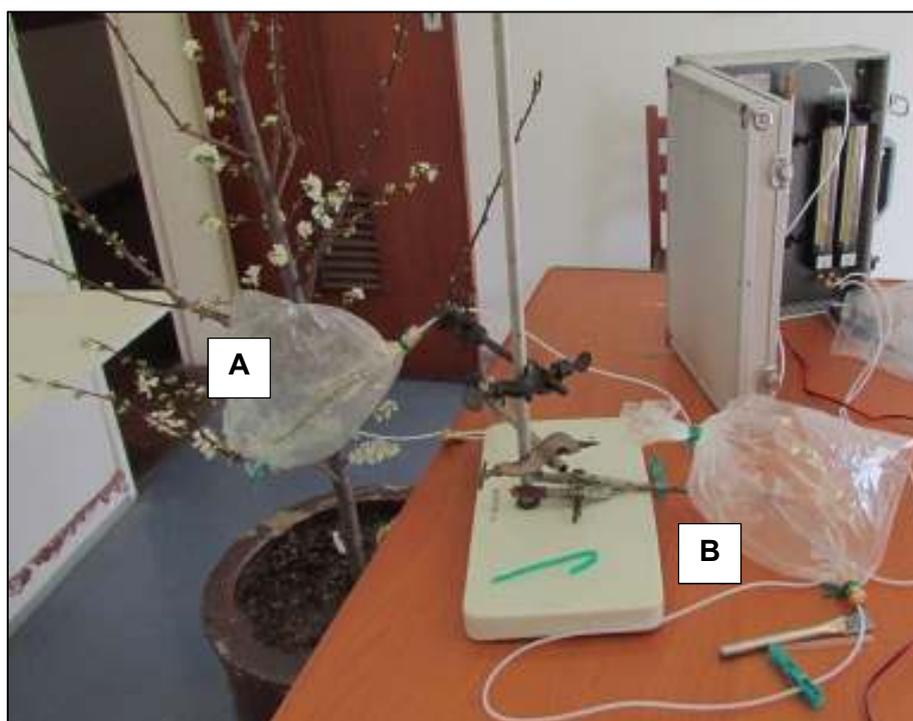
Figure 4.4. Air entrainment set-up. A = cellophane oven bag covering plum shoot with blossoms; B = control (empty bag).

The vials with WFT females were placed a fridge at 5° C for 10-15 minutes to immobilize the thrips and each female was examined under a binocular microscope to confirm species identity (according to Mound and Kibby 1998). Thrips were kept in the glass vials at room temperature for approximately 1 hour before the bioassay was conducted.

4.2.4 Olfactometer

For this study a Y-shaped glass tube olfactometer was used. The arms and stem were 15 cm long with an angle of 90 degrees between the arms, and the inner diameter of the glass tubing 14.5 mm. Before use the Y-tubes were washed with detergent and hot water, rinsed with 70% ethanol and dried overnight in an oven at 150 °C. The tubes were allowed to cool in the oven before use. The olfactometer was set up in a dark, windowless, air-conditioned room (5 m x 6 m) at 25 °C. Because WFT is attracted to light, the Y-tube was laid flat on a light table (50 cm x 30 cm) equipped with two fluorescent tubes (15 Watt) under a sheet of white opaque glass to provide even, diffuse light (Fig. 4.5). Before WFT were brought into the room, all lights except for those of the light table were switched off. Once the bioassay was completed, the light table was switched off and the main lights switched on.

A custom-made air entrainment kit, obtained from Rothamsted Research (Harpenden, UK), was used to draw air through a charcoal filter to clean the air at a rate of 800 ml/min and

bubble it through 80 ml distilled water to humidify the air, whereafter the air column was split and passed through two regulators to obtain a gentle air flow of 300 ml/min into each of the top arms of the Y-tube (Fig. 4.5). The air inlets in the Y-tubes were covered with thrips-proof nylon gauze (50 mesh). A smoke test, as described in Koschier et al. (2000), confirmed that the air of the odour arm did not mix with that of the control arm at the Y-junction. PTFE Teflon tubing (1.5 mm inner diameter) was used for all connections between elements of the olfactometer set-up.

4.2.5 Bioassay procedure

The attractiveness of flower volatiles from white clover, unopened (balloon stage) plum blossoms, fully open plum blossoms and of E-$β$-Farnesene to WFT females was tested. E-$β$-Farnesene was used as a positive control because it is a component of flower volatiles shown to be attractive to WFT (Manjunatha et al. 1998; Pow et al. 1999; Bennison et al. 2003). The E-$β$-Farnesene was synthesized at Rothamsted Research, as stated in Al Abassi et al. (1998), with hexane as solvent. A concentration of 100 ng E-$β$-Farnesene/μl hexane was used, as this was demonstrated to elicit peak attraction to WFT by Manjunatha et al. (1998).
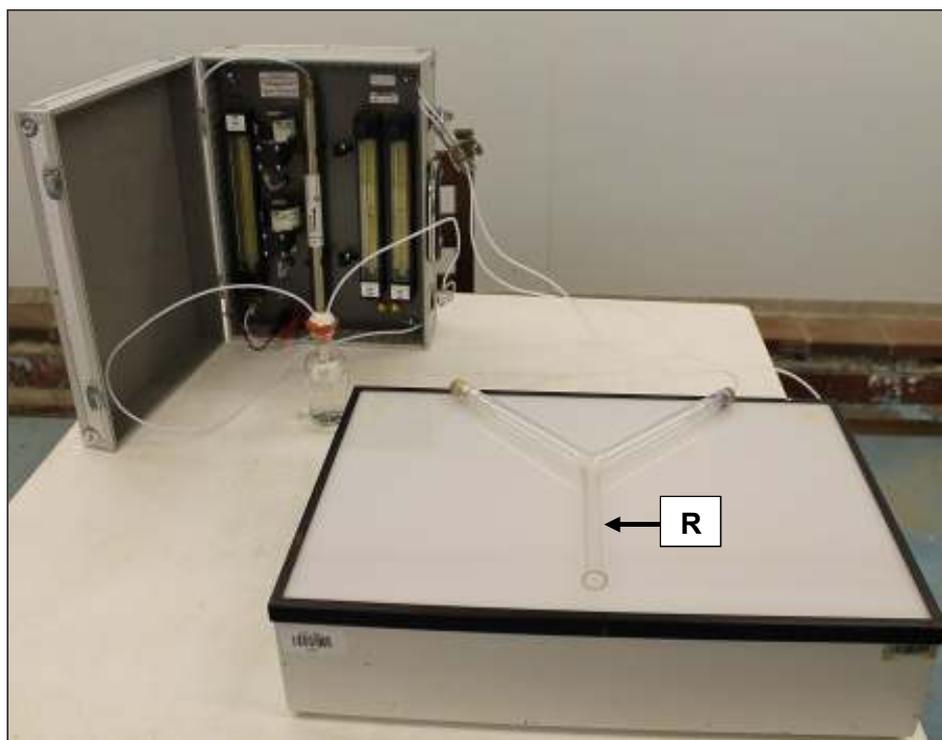
Figure 4.5. Y-tube olfactometer set-up showing release point (R).

To avoid contamination with volatile odours, filter paper (BIO-1 grade) squares (*ca* 1 cm^2) were prepared in an extraction cabinet. Forceps and scissors, cleaned with 70% ethanol, were used to handle the filter paper throughout the olfactometer study. Once cut, the filter paper squares

were kept in a glass Petri dish heated overnight in an oven at 150 °C and allowed to cool in the oven. For each test, 10 μl of the test odour was transferred to a sterile filter paper square with a micro pipette and 10 μl of solvent (hexane or diethyl ether) was transferred to another sterile filter paper square. Each of the treated filter paper squares was placed in a separate glass Petri dish heated overnight in an oven at 150 °C and allowed to cool in the oven. The solvents were allowed to evaporate for 1 min in the extraction cabinet before the filter paper squares were inserted into the two arms of the Y-tube olfactometer. In each test the filter paper with solvent was placed in the control arm of the olfactometer. After connecting the air flow, another 30 s was allowed for the system to stabilise before a WFT female was added halfway up the base tube (Fig. 4.5 R), using a small brush. Before being transferred to the olfactometer, WFT were chilled at 5 °C for 2 -3 min. to reduce mobility and avoid injury during the transfer process. After an adjustment period of 2 min. the behaviour of the WFT female was observed for a maximum of 5 min. and the final choice of arm (odour or control) recorded. Females that did not move or make a choice within 5 min. were discarded. When a WFT female walked into an arm beyond the 3 cm mark (made with a black felt-tip marker on the outside of the tube) from the split of the Y-tube and remained in that arm for at least 2 min. a choice was recorded and the bioassay terminated. Three females were tested in succession, whereafter the Y-tube was removed for cleaning as described under 4.2.4 and a clean tube inserted. The position of the odour and control arm (right or left) was alternated every time a clean Y-tube was used. A single female constituted a replicate. At least 35 replicates were done for each odour, the ultimate number of replicates being limited by the amount of volatiles available. The bioassays were done over a period of three weeks. Because the response of WFT females to volatile odours may differ from day to day, the odours were alternated after six replicates on each day that bioassays were conducted.

4.2.6 Statistical analysis

The data for each odour was compared separately to a ratio of 1:1 using Chi-square tests (PROC FREQ) with SAS software (2015).

4.3 RESULTS

The numbers of WFT females choosing the odour arm and control arm for each of the four odours are presented in Table 4.1. Sixty-three percent of WFT chose the arm containing E-β-Farnesene. The Chi-square analysis indicated that this was not statistically significant (X^2 = 2.31, p = 0.13).

The arm with volatile compounds of clover flowers was chosen by 69% of WFT females, which was statistically significant (X^2 = 4.81, p = 0.02). The volatile compounds of plum blossoms in

71

full bloom also produced statistically significant results (X^2 = 6.43, p = 0.01), with 71% of WFT choosing the odour arm. The volatiles of the balloon stage plum blossoms attracted 65% of WFT. This was not statistically significant according to the Chi-square analysis (X^2 = 3.60, p = 0.06).

Table 4.1. Bioassay results from a Y-tube glass olfactometer testing attractiveness of volatiles from clover (*Trifolium repens*) flowers, balloon stage and fully opened plum (*Prunus salicina* cv. Sapphire) blossoms and *E*-β-Farnesene for *Frankliniella occidentalis* females.

Odour/control (= solvent)	*n*	No. WFT choosing odour arm	No. WFT choosing control arm	% WFT choosing odour
E-β-Farnesene / hexane	35	22	13	63%
Clover flower volatiles / diethyl ether	35	24*	11	69%
Plum blossom full bloom volatiles / diethyl ether	35	25*	10	71%
Plum blossom balloon stage volatiles / diethyl ether	40	26	14	65%

* Significant at p ≤ 0.05

The preliminary GC-MS analysis revealed that the concentration of volatiles in the samples was low, since it required ten-fold concentration to enable peaks of clover and plum flower volatile compounds to be distinguished from the background reading. The chromatograms (Figs 4.6 & 4.7) indicate that there are differences between the volatile compound bouquets of the plum blossoms (balloon stage and open) and those of the clover flowers, and that there appear to be some differences in the volatile compounds of balloon stage plum blossoms compared to the open blossoms. However, the volatile bouquets of all three samples also appear to have many compounds in common. Further analysis to quantify and identify the compounds would require additional air entrainment geared to obtain greater volumes of samples with higher concentrations of volatiles.

4.4 DISCUSSION

Volatile odours of clover flowers and plum blossoms were successfully captured by air entrainment. The results of the olfactometer bioassays revealed that clover flowers and open plum blossoms are highly attractive to WFT females. Although attraction of *E*-β-Farnesene was not statistically significant in this study, the 65% of WFT females that chose this odour is comparable to results obtained by Koschier et al. (2000), where 64% of WFT chose *E*-β-Farnesene at 10% concentration and 65% chose the 1% concentration. The lower level of

attraction to balloon stage blossoms may simply be due to lower volatile concentrations captured from balloon stage blossoms with air entrainment. Although the concentration of bio-active volatiles in the clover flower and plum blossom bouquets was not quantified, the GC-MS analysis indicated that the concentration of volatiles in the plum blossom entrainment samples was low. Attraction to volatiles has been shown to depend on the optimal concentration of a volatile or combination of volatiles (Terry 1997). It could also reflect differences in volatile compounds produced by balloon stage and open blossoms, as indicated by the preliminary GC-MS analysis. Further air entrainment and olfactometer studies are recommended to elucidate these results, but would require optimizing the concentration of volatiles captured during air entrainment.

Pearsall and Myers (2000) found equal densities of WFT in balloon stage and open nectarine blossoms in British Columbia, Canada, while Terry and DeGrandi-Hoffman (1988) found more WFT in open apple blossoms than in balloon stage blossoms in Arizona, USA. During this study it was shown that WFT females move into orchards to lay eggs in balloon stage plum blossoms (Chapter 2). From these findings it could be concluded that while WFT may prefer open blossoms, this does not preclude WFT being attracted to unopened balloon stage blossoms at the beginning of the flowering period when there are no open blossoms available.

Observations during this study showed that the balloon stage in plum blossoms rarely lasts more than two days, open plum blossoms begin to lose petals after two to three days and clover flowers also begin to senesce after three days. This means that the entrainment period cannot be extended beyond 48 hours. Together with the fact that only one air entrainment kit was available, it limited the amount of volatiles collected. This placed a constraint on the number of replicates that could be included in the olfactometer bioassay and precluded a direct comparison of clover volatiles versus plum blossom volatiles. To increase the concentration of compounds in the flower volatile bouquets and the total volume of the samples, it is recommended that multiple entrainments be done for each flowering stage and the samples for each stage be pooled. This would enable more replicates and direct comparison of the different volatile bouquets in olfactometer studies.

Identification of a potential attractive trap crop for WFT and implementation of a push-pull system does not require identification and quantification of the volatile compounds of the two plum blossom stages and of clover flowers, but it would provide a better understanding of the chemical basis for the WFT responses to the volatile bouquets. Research by Koschier et al. (2000) and Abdullah and Butt (2015) indicate that WFT recognises its many host plants by detecting the correct blends of common plant volatiles, in accordance with the hypothesis of

Bruce et al. (2005). This would mean that the attraction of WFT to plum blossoms and clover flowers is most likely due to flower volatiles that they have in common, rather than to compounds unique to each species. No records of analyses of white clover flowers or plum blossoms have been found in the literature, although Louw and Theron (2012) identified aroma compounds of plum fruit. Among these are linalool and benzaldehyde, compounds that have been shown to be attractive to WFT (Koschier et al. 2000).

Koschier et al. (2000) noted that results regarding the attractiveness of volatiles to WFT are often inconsistent and even contradictory due to different methods and concentrations of chemicals used for testing. Davidson et al. (2006) studied the effects of starvation and age on the response of WFT females to visual and odour cues and concluded that hungry female WFT (starved for 4 h with access to water only) were more receptive to an odour cue than well-fed WFT, and that age also affects their response to olfactory cues. In this study WFT females were starved for *ca.* 1 h before the bioassay commenced and chilled to confirm their identity and to facilitate handling without injury. Thrips recovering from being chilled would possibly be more inclined to feed and therefore be receptive to a host plant odour. In view of the findings by Davidson et al. (2006), it is possible that field-collected WFT of different ages and reproductive stages would react differently to odour stimuli. A successful trap crop in a push-pull system for use in orchards would have to be attractive to as wide a range of WFT females in a population as possible, which is why field-collected WFT females were used to test the attractiveness of plum and clover flower volatiles.

White clover appears to fit the bill as a trap crop for WFT, since it flowers close to the ground (Pearsall 2000), provides complex flowers and is clearly attractive to WFT. Pobozniak and Wiech (2005) showed that intercropping white clover with cabbage reduced the number of *T. tabaci* on the cabbage, compared to cabbage monocultures. However, using it as a trap crop for WFT in plum and other deciduous fruit orchards will require careful management. Orchards in the Western Cape, South Africa, generally have volunteer weeds or cultivated cover crops growing in work and tree rows during winter. To avoid competition for water during the hot, dry summers, weeds and/or cover crops are either chemically controlled in spring or dry out naturally in drip-irrigated orchards as the summer progresses. To be effective as a trap crop, planting of the clover would have to be timed to ensure that the clover flowers before the plums blossom. It would not be advisable to plant clover in the orchard, as thrips will simply move into the plum trees when the trap crop begins to die off. This concern was also raised by Pearsall (2000) when considering ground covers in nectarine orchards as potential trap crops. The trap crop would have to be planted adjacent to the orchard where the WFT can be controlled during the flowering period.

74
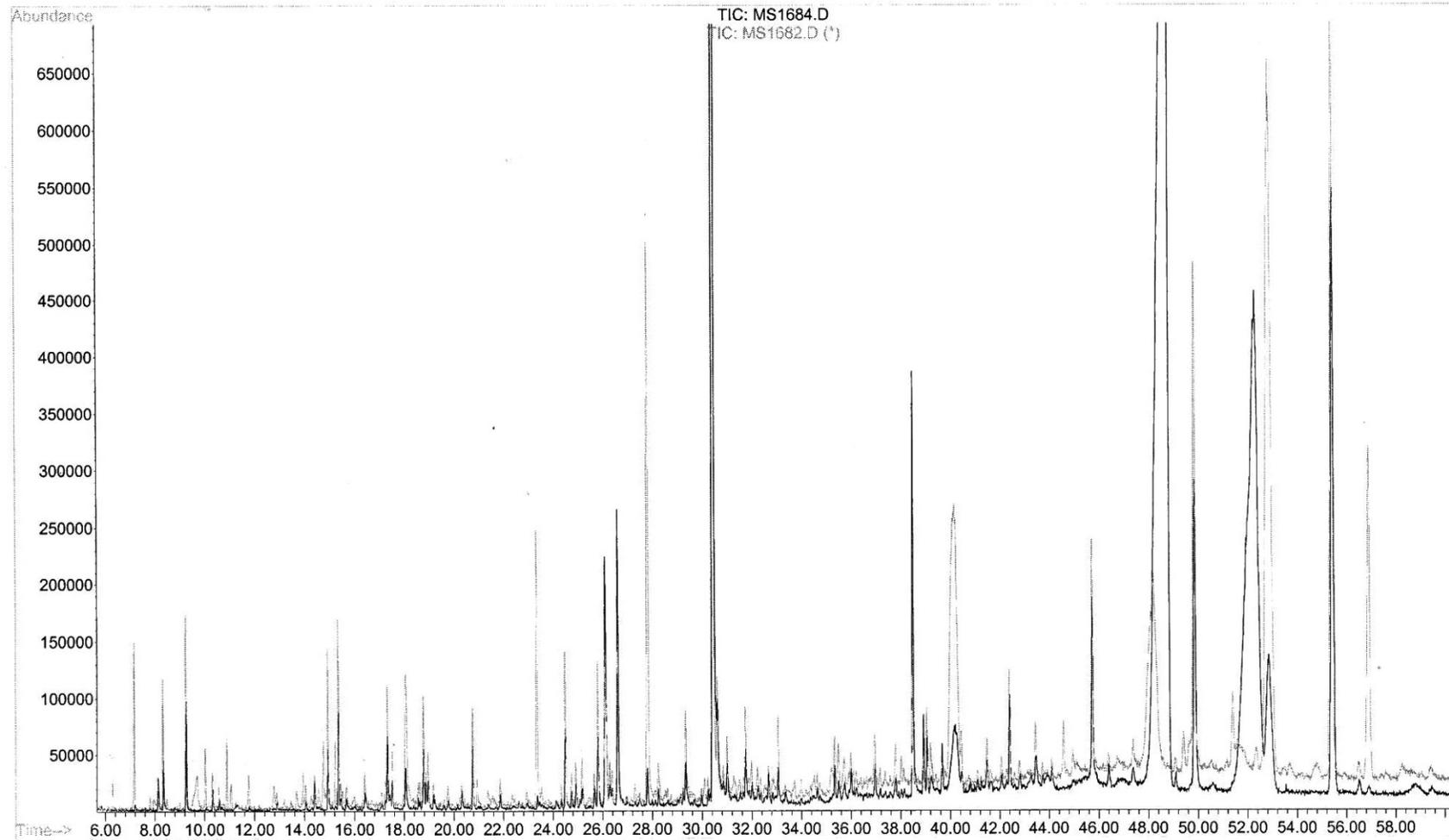
Figure 4.6. Chromatogram of volatiles extracted from clover (*Trifolium repens*) flowers (MS 1682 D, lighter grey peaks) compared to volatiles from the air entrainment control (MS 1684 D, dark grey peaks).
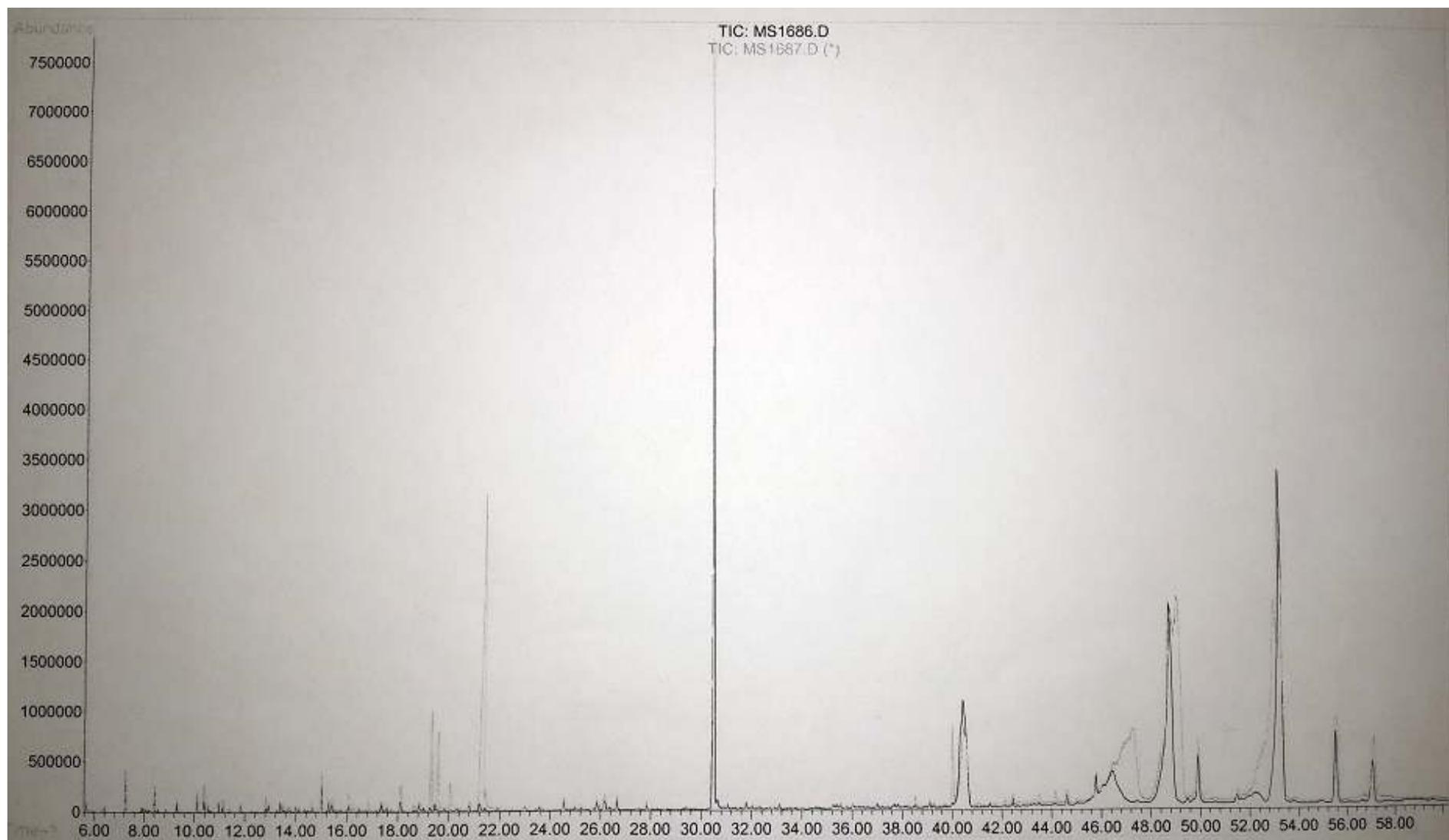
Figure 4.7. Chromatogram of volatiles extracted from balloon stage (MS 1686 D, darker peaks) and open (MS 1687 D, pale grey peaks) plum (*Prunus salicina)* blossoms.

Because plum blossoms and white clover flowers were both shown to be attractive to WFT, the use of a semiochemical to deter WFT from the plum blossoms is proposed to enhance the efficacy of the trap crop. This is in accordance with the conclusion by Pearsall (2000) that for a ground cover to be effective as a trap crop, it would have to be done in conjunction with the application of a powerful deterrent on the nectarine blooms.

A further complicating factor is the fact that farmers bring in honey bees for pollination during plum flowering. Since clover flowers are also highly attractive to honey bees (MacRae et al 2005), WFT on the trap crop would have to be controlled and the clover flowers cut before the bees are brought into the orchard to ensure effective pollination and to prevent WFT in the clover from moving into the plum orchards.

This study showed that white clover has potential as a trap crop for WFT, but planting it adjacent to plum orchards cannot be recommended until a suitable deterrent is available for application to plum blossoms to provide the "push" in a push-pull system. The potential of other flowering plants, including indigenous plants, as trap crops for WFT warrant further investigation. Possible deterrents, namely thymol, methyl salicylate and carvacrol, were identified (Chapter 3), but require development of a formulation to ensure effective, sustained release without phytotoxic effects.

4.5 REFERENCES

Abdullah ZS, Butt T (2015) Preferences of the peripheral olfactory system of western flower thrips, *Frankliniella occidentalis* towards stereoisomers of common plant volatiles. Chemoecology 25, 47-51

Al Abassi S, Birkett MA, Pettersson J, Pickett JA, Woodcock CM (1998) Ladybird beetle odour identified and found to be responsible for attraction between adults. Cell Mol Life Sci 54, 876-879

Bennison JA, Maulden K, Dewhirst S, Pow E, Slatter P, Wadhams LJ (2003) Towards the development of a push–pull strategy for improving biological control of western flower thrips on chrysanthemum. In: Marullo R, Mound L (Eds). 7th International Symposium on Thysanoptera: "Thrips, Plants, Tospoviruses: the Millenial Review", 2–7 July 2001, Reggio Calabria, Italy. CSIRO Entomology, Canberra, Australia. 199-206

Bennison JA, Pow EM, Wadhams LJ, Maulden KA, Wardlow LR, Buxton JH (1999) Improving biological control of western flower thrips, *Frankliniella occidentalis*, on greenhouse ornamentals. Proceedings sixth International Symposium on Thysanoptera, Antalya, Turkey, April 27 to May 1. Akdeniz University, Antalya, Turkey.19-24

Blight MM (1990) Techniques for isolation and characterization of volatile semiochemicals of phytophagous insects. In Chromatography and Isolation of Insect Hormones and Pheromones (McCaffrey ID and Wilson AR, eds), pp281-288, Plenum Press

Blumthal MR, Cloyd RA, Spomer LA, Warnock DF (2005) Flower color preference of western flower thrips. HortTechnology 15, 846-853

Broughton S, Harrison J (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 Prot 42:156-163

Bruce TJA, Wadhams LJ, Woodcock CM (2005) Insect host location: a volatile situation. Trends Plant Sci 6, 269-274

Cockfield SD, Beers EH (2008) Management of dandelion to supplement control of western flower thrips (Thysanoptera: Thripidae) in apple orchards. J Entomol Soc Brit Columbia 105, 89-96

Davidson MM, Butler RC, Teulon DAJ (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 MM, Perry NB, Larsen L, Green VC, Butler RC, Teulon DAJ (2008) 4-Pyridyl carbonyl compounds as thrips lures: Effectiveness for western flower thrips in Y-tube bioassays. J Agric Food Chem 56, 6554-6561

Felland CM, Teulon DAJ, Hull LA, Polk DK (1995) Distribution and management of thrips (Thysanoptera: Thripidae) on nectarine in the mid-Atlantic region. J Econ Entomol 88, 1004-1011

Johnson CB, Kazantzis A, Skoula M, Mitteregger U, Novak J (2004) Seasonal, populational and ontogenic variation in the volatile oil content and composition of individuals of

Origanum vulgare subsp. *hirtum*, assessed by GC headspace analysis and by SPME sampling of individual oil glands. Phytochem Analysis 15, 286-292

Koschier EH (2008) Essential oil compounds for thrips control – A review. Nat Prod Commun 3, 1171-1182

Koschier EH, De Kogel WJ, Visser JH. 2000. Assessing the attractiveness of volatile plant compounds to western flower thrips *Frankliniella occidentalis*. J Chem Ecol 26, 2643-2655

Lewis T (1997) Flight and dispersal, pp 175-196 in T Lewis (ed) Thrips as Crop Pests. CAB International, Oxon, UK

Loreto F, Barta C, Brilli F, Nogues I (2006) On the induction of volatile organic compound emissions by plants as consequence of wounding or fluctuations of light and temperature. Plant Cell Environ 29, 1820-1828

Louw ED, Theron KI (2012) The effects of ripening and cold storage on the volatile profiles of three Japanese plum cultivars (*Prunus salicina* Lindl.) and one interspecific plum-apricot cultivar. J Agr Sci 4, 257-275

MacRae AW, Mitchem WE, Monks DW, Parker ML (2005) White clover (*Trifolium repens*) control and flower head suppression in apple orchards. Weed Technol 19, 219-223

Manjunatha M, Pickett JA, Wadhams LJ, Nazzi F (1998) Response of western flower thrips, *Frankliniella occidentalis* and its predator *Amblyseius cucumeris* to chrysanthemum volatiles in olfactometer and greenhouse trials. Insect Sci Applic 18, 139-144

Mound LA, Kibby G (1998) Thysanoptera: an identification guide. 2nd Ed. CAB International, Oxon, New York

Muvea AM, Waigani MM, Kutima HL, Osiemo Z, Nyasani JO, Subramanian S (2014) Attraction of pest thrips (Thysanoptera: Thripidae) infesting French beans to coloured sticky traps with Lurem-TR and its utility for monitoring thrips populations. Int J Trop Insect Sci 34, 197-206

Pearsall IA (2000) Flower preference behaviour of western flower thrips in the Similkameen

Valley, British Columbia, Canada. Entomol Exp Appl 95:303–313

Pearsall IA, Myers JH (2000) Population dynamics of western flower thrips (Thysanoptera: Thripidae) in nectarine orchards in British Columbia. J Econ Entomol 93, 264-275

Pobozniak M, Wiech K (2005) The effect of undersowing cabbage with white clover on thrips infestation and flight activity. Commun Agric Appl Biol Sci 70, 517-526

Pow EM, Bennison JA, Birkett MA, Luzniak MJ, Manjunatha M, Pickett JA, Segers IS, Wadhams LJ, Wardlow LR, Woodcock CM (1999) Behavioural responses of western flower thrips (*Frankliniella occidentalis*) to host plant volatiles. Proceedings sixth International Symposium on Thysanoptera, Antalya, Turkey, April 27 to May 1. Akdeniz University, Antalaya, Turkey. 121-128

Pow EM, Hooper AM, Luzniak MC, Pickett JA, Wadhams LJ, Bennison JA (1998) Novel strategies for improving biological control of western flower thrips on protected ornamentals – attraction of western flower thrips to verbena plants. Proceedings Brighton Crop Protection Conference Pests and Diseases. Vol. 2. British Crop Protection Council, Farnham, UK. 417-422

Roditakis NE, Lykouressis DP (1996) Prospects for the use of volatile chemicals and a new pyrrole in integrated pest management of western flower thrips. Acta Hort 431, 513-520

SAS Institute (2015) The Statistical Procedure manual. North Carolina 27513: SAS Campus Drive, Cary.

Terry LI (1997) Host selection, communication and reproductive behaviour, pp 65-118 in T Lewis (ed) Thrips as Crop Pests. CAB International, Oxon, UK

Terry LI, DeGrandi-Hoffman G (1998) Monitoring western flower thrips (Thysanoptera: Thripidae) in "Granny Smith" apple blossom clusters. Can Ent 120, 1003-1016

Teulon, DAJ, Davidson MM, Nielsen M-C, Perry NB, van Tol RWHM, De Kogel W-J (2008) The potential use of lures for thrips biological control in greenhouses: Practice and theory. In Proceedings Third International Symposium on Biological Control of Arthropods, Christchurch, New Zealand. PG Mason, DR Gillespie and R Vincent (Eds).

United States Department of Agriculture, Forest Service, Morgantown, WV, USA. pp 301-308

Van Tol RWHM, James DE, de Kogel W-J, Teulon DAJ (2007) Plant odours with potential for a push-pull strategy to control the onion thrips, *Thrips tabaci*. Entomol Exp Appl 122, 69-76

CHAPTER 5. GENERAL DISCUSSION AND CONCLUSIONS

5.1 GENERAL DISCUSSION

The deciduous fruit industry requires an effective, environmentally sustainable integrated pest management strategy for the western flower trips (WFT), *Frankliniella occidentalis* Pergande. The fact that standard chemical control has not been effective in preventing oviposition damage, as demonstrated in this study, and increasing consumer demands for residue-free, sustainably produced fruit (Kumar 2012) are driving the search for alternative pest management measures that are environmentally sustainable and can reduce producers' reliance on toxic chemicals.

Correct identification of pest insects and knowledge of the seasonal dynamics of the pest and its synchrony with the vulnerable stages of the crop are prerequisites for the implementation of effective pest management strategies (Hill 2008). This study confirmed that WFT oviposition causes the pitting damage observed on plums and that the apparent failure of insecticide applications to prevent pansy spot and pitting damage, caused by WFT oviposition, was due to the fact that WFT entered plum blossoms even before the petals opened. By the time that spray applications were made at 20% bloom, much of the damage had already been done. If insecticidal control is to be effective in reducing WFT oviposition damage, monitoring must start as soon as the flower buds begin to swell and the first spray application should be made as soon as the blossoms reach balloon stage, if WFT are present.

Establishing economic damage thresholds for treatment is an integral element of integrated pest management programs. Such thresholds, based on the number of WFT adults or larvae per flower or fruit, have been established for WFT on vegetable crops such as field grown peppers (*Capsicum annuus,* Solanales: Solanaceae), eggplant (*Solanum melongena,* Solanales: Solanaceae) and tomato (*Lycopersicon esculentum,* Solanales: Solanaceae) in Florida, USA (Demirozer et al. 2012) and peppers in greenhouses (Bán et al. 2012 as quoted in Elimem et al. 2014). No treatment threshold has yet been established for WFT on deciduous fruit, since no consistent significant relationship has been found between sticky trap counts and WFT damage on table grapes in South Africa (De Villiers and Pringle 2007; Allsopp 2010), nectarines in Canada (Pearsall 2000) or plums, as reported in this study (Chapter 2). Even if a consistent relationship was found between WFT counts in blossoms and fruit damage, it would be too late to achieve effective control to prevent oviposition damage. In the absence of a threshold level, sticky trap counts serve mainly to indicate the presence or absence of WFT in an orchard.

The presence of WFT on weeds, ground covers and wild vegetation in and around orchards in South Africa when plum blossoms and fruit are vulnerable to WFT oviposition and feeding, the presence of honey bees brought into orchards for pollination and increasing market demands for residue free fruit complicate chemical control of WFT. Modifying WFT behaviour by means of plant essential oils that are non-toxic to humans to reduce or prevent crop damage presents an environmentally sustainable alternative (Agelopoulos et al. 1999; Cook et al. 2007).

The concept of a push-pull strategy for insect pest management was conceived by Pyke et al. (1987). Push-pull typically uses non-toxic behavioural stimuli to make the crop less attractive to the pest (push), while luring it towards an attractive alternative (pull) from where it can be removed (Cook et al. 2007). The individual components are usually not as effective as insecticides at reducing pests, but by deploying the push and pull components in tandem, their efficacy is increased (Cook et al. 2007 and references therein). While many attractants for thrips, and WFT in particular, have been identified (Table 1.1 in Chapter 1), new volatiles are still being added. Teulon et al. (2014) showed that several lactones, particularly γ-decalactone and δ-decalactone, attract WFT, while Abdullah et al. (2015) identified a pine pollen volatile (*S*)-(−)-verbenone as an attractant for WFT. Many of these volatiles are attractive to more than one species of thrips, therefore the choice of volatile for a push-pull strategy will depend on which thrips species occurs on the target crop.

The use of trap crops or lure plants for WFT control on various vegetable and ornamental crops cultivated under protection has been proposed. The use of two *Verbena* cultivars ('Sissinghurst Pink' and 'Tapien Pink') to lure WFT away from Ivy-leaf Geraniums (*Pelargonium peltatum*) and pot chrysanthemums (*Dendranthema* spp.) was proposed by Bennison et al. (1999). Matsuura et al. (2006) planted the verbena cultivars 'Pink Parfait' and 'Fancy Parfait' alongside chrysanthemum cv. 'Jimba' in a greenhouse in Japan and found that not only was WFT colonisation of chrysanthemums reduced until flower bud initiation, but also the incidence of tomato spotted wilt virus. The chrysanthemum cultivar 'Swingtime' was shown to effectively attract WFT away from the crop chrysanthemum cultivar ('Charm') until it came into flower when used together with additional lures of *E-β*-Farnesene (Bennison et al. 2003). No trap crop for WFT has yet been proposed for use in or around deciduous fruit orchards. Pearsall (2000) found that WFT still oviposited in less attractive flowers when more attractive flowers were present, and concluded that an attractive ground cover would only be successful in reducing WFT in nectarine blossoms if used in conjunction with a powerful deterrent spray on the nectarine blossoms. Demirozer et al. (2012) also concluded that companion plants like *Bidens* and *Helianthus,* which are more attractive to WFT than the fruiting vegetable crops,

should be used in a push-pull strategy together with ultraviolet-reflective mulches or kaolin as the push to manage WFT and tospoviruses in fruiting vegetable in Florida (USA). The olfactometer bioassays described in Chapter 4 showed that the volatiles of clover flowers and plum blossoms are both very attractive to WFT females; 69% of WFT were attracted to clover flower volatiles and 65% and 71% to balloon stage and open plum blossoms, respectively. Direct comparison of clover flower volatiles with plum blossoms volatiles in olfactometer studies were precluded by the limited volumes of volatiles obtained through air entrainment, but the bioassays with single volatile bouquets showed that white clover has potential as a trap crop for WFT in local orchards. In view of the findings by Pearsall (2000) and Demirozer et al. (2012), clover could be a successful trap crop for WFT in plum orchards if used in conjunction with a deterrent in a push-pull strategy.

Planting white clover adjacent to orchards presents various management challenges to ensure that it flowers before the plums do and to avoid competition with plum blossoms for pollinators. It may be necessary to control the WFT on the clover with an insecticide and cut the clover flowers before bees are brought in for pollination. In view of these constraints, further investigation to identify more potential trap crops is advised.

The concept of using semiochemical lures for mass trapping to control thrips has been explored for various vegetable and ornamental crops cultivated under protection. Sampson and Kirk (2013) reported that mass trapping with blue sticky traps and Thripline™ ams pheromone lures kept the WFT population below the economic threshold in semi-protected strawberries. Elimem et al. (2014) showed that ten blue sticky traps equipped with WFT male sex pheromone capsules (Lures Atlas-Agro®) reduced the WFT population in greenhouse grown peppers in Tunisia to the economic threshold of one thrips per pepper flower and concluded that it could be used to manage WFT in these crops. Broughtom et al. (2015) investigated the potential of semiochemicals for mass trapping of WFT in greenhouse roses. They concluded that although the addition of Thripline™ ams or Lurem-TR increased WFT catches on yellow sticky traps significantly, their use for mass trapping to reduce cosmetic thrips damage to roses has yet to be demonstrated. Teulon et al. (2008) suggested that the use of semiochemicals for mass trapping of thrips would require a very strong lure and would probably only be really effective in conjunction with other mortality factors such as biocontrol agents. Although the addition of lures has also been shown to increase trapping efficiency of WFT in nectarine and apple orchards in Western Australia (Broughton and Harrison 2012) and in field grown French beans in Kenya (Muvea et al. 2014), the continuous influx of thrips from surrounding vegetation and the lack of significant reduction in damage suggest that mass trapping in open cultivation may not be a feasible management practice.

All of the strategies discussed above rely only on the use of a lure or 'pull' element, without using any deterrents on the crop itself. Various plant essential oils have been demonstrated to deter WFT feeding and oviposition on leaf discs in laboratory assays (Sedy and Koschier 2003; Koschier et al. 2007; Bruhin 2009; Egger et al. 2014). This study was the first to show that suspensions of thymol (10%), methyl salicylate (1% and 10%) and carvacrol (1% and 5%) significantly reduced the oviposition rate of WFT when applied to individual plum blossoms. However, obtaining similar levels of deterrence in semi-field and field situations appear to present many difficulties. The fact that the wetting agents, Triton X-100 and Citrex® mineral oil, were not able to maintain the essential oils in stable suspensions, resulting in uneven deposits and phytotoxic damage at higher essential oil concentrations, means that the true potential of thymol, methyl salicylate and carvacrol as oviposition deterrents for WFT on plum blossoms could not be determined in semi-field and field conditions. Despite this limitation, the results of the bioassays with individual plum blossoms indicate that the use of these oils as the "push" element in a push-pull strategy is worth pursuing, provided that a suitable formulation can be found.

The effect of a deterrent comes into play after an insect has landed on a prospective host plant (Dethier et al 1960), therefore any oviposition deterrent, be it a plant essential oil or other compound, used to provide the "push" in a push-pull system for WFT management in plums will have to be applied to the blossoms. Isman (2000) cautioned that the essential oils most effective against pests are often phytotoxic and would require serious attention when products are formulated for use in agriculture. Reitz et al. (2008) tested the efficacy of three plant essential oils, geraniol, lemongrass oil (*Cymbopogon flexuosus*) and tea tree (*Melaleuca alternifolia*) oil in combination with kaolin as repellents of WFT in field grown tomatoes in North Florida. Although laboratory tests showed strong repellent effects for all three oils against WFT adults, there was no clear effect on WFT populations in the field. However, when applied in combination with kaolin, the incidence of tomato spotted wilt virus was reduced, although thrips abundance was not reduced. It was thought that the high volatility of the oils, resulting in limited persistence in the field, and the repeated influx of thrips from surrounding areas masked the short term deterrent and/or antifeedant effects of the essential oils, and that kaolin interfered with thrips feeding behaviour, only resulting in reduced tomato spotted wilt incidence. Micro-encapsulation of active compounds, such as insecticides and insect pheromones, enables sustained release of active ingredients in an environmentally sustainable way (Masuda 2011), and could potentially solve the problem of delivering effective doses of essential oils in a sprayable form without phytotoxic effects.

Van Tol et al. (2006) demonstrated the principle of a classical push-pull system using sweet marjoram essential oil as a deterrent and ethyl i*so*-nicotinate as the attractant for *T. tabaci* in a pasture field. At present, the closest to a push-pull system for WFT is included in an integrated pest management program for WFT on fruiting vegetables in Florida (Demirozer et al. (2012). Ultraviolet-reflective mulches that have been shown to effectively repel WFT adults from colonizing fruiting vegetable crops, were recommended as a 'push' component, rather than essential oils, and companion planting of species of *Bidens* and *Helianthus* were recommended as the 'pull'. Similarly, Tyler-Julian et al. (2014) used ultraviolet-reflective mulches and kaolin as the 'push' components and sunflower companion plants as the 'pull' against *F. bispinosa* in bell peppers.

Since a number of lures for WFT are commercially available, it would appear that the fact that push-pull strategies for WFT management have not become established in practice is due, in part, to a lack of suitable deterrents. A major contributor to the slow rate of commercialisation of plant essential oils as crop protectants is the lack of critical knowledge regarding their efficacy and optimal application methods, including appropriate formulations (Reitz et al. 2008). Isman (2006) also identified sustainability of the botanical resource to provide a reliable and constant supply, standardisation of chemically complex extracts and regulatory approval as barriers to commercialisation. Formulation development requires highly specialised skills, however, and falls in the domain of companies specialising in this field, therefore it was not included in this study.

5.2 CONCLUSIONS

This study provides crucial information to improve the monitoring of WFT, timing and efficacy of early-season insecticidal control of WFT in South African plum orchards. The positioning of blue sticky traps close to the ground and the use of commercially available thrips lures to improve monitoring could provide more accurate indications of WFT movement into and their presence in orchards early in the season. Potential oviposition deterrents based on essential oils and white clover as a trap crop that could be developed for use in a push-pull strategy to manage WFT more sustainably were identified, provided that suitable formulations of the essential oils can be developed. Micro-encapsulation of the essential oils may provide a solution to finding a suitable formulation and warrants investigation. Identification of a suitable trap crop and development of a push-pull system is not dependent on identification of the volatiles in plum and clover flower bouquets that are attractive to WFT, but it would provide insight into the chemical basis responsible for the attraction. . The use of white clover as a trap crop for WFT adjacent to orchards and the possibility of adding commercial thrips lures

to enhance attraction of WFT require further investigation. Implementation of a push-pull system for WFT management in deciduous fruit can only become a reality when these objectives have been achieved. The use of ultra-violet reflective mulches and kaolin as deterrents for WFT should also be considered in further research towards achieving a fully integrated management strategy for WFT on deciduous fruit.

5.3 REFERENCES

Abdullah ZS, Greenfield BPJ, Ficken KJ, Taylor JWD, Wood M (2015) A new attractant for monitoring western flower thrips, *Frankliniella occidentalis* in protected crops. SpringerPlus 4:89 DOI 10.1186/s40064-015-0864-3

Agelopoulos N, Birkett MA, Hick AJ, Hooper AM, Pickett JA, Pow EM, Smart LE, Smiley DWM, Wadhams LJ Woodcock CM (1999) Exploiting semiochemicals in insect control. Pestic Sci 55, 225-235

Allsopp E (2010) Seasonal occurrence of western flower thrips, *Frankliniella occidentalis* (Pergande), on table grapes in the Hex River Valley, South Africa. S Afr J Enol Vitic 31, 49-57

Bán G, Fetykó K, Tóth F (2012) Application of mass-collected, non-selected arthropod assemblages to control pests of greenhouse sweet pepper in Hungary. N. West. J. Zool 8, 139-153

Bennison JA, Maulden K, Dewhirst S, Pow E, Slatter P, Wadhams LJ (2003) Towards the development of a push–pull strategy for improving biological control of western flower thrips on chrysanthemum. In: Marullo R, Mound L (Eds). 7th International Symposium on Thysanoptera: ''Thrips, Plants, Tospoviruses: the Millenial Review'', 2–7 July 2001, Reggio Calabria, Italy. CSIRO Entomology, Canberra, Australia, 199–206

Bennison JA, Pow EM, Wadhams LJ, Maulden KA, Wardlow LR, Buxton JH (1999) Improving biological control of western flower thrips, *Frankliniella occidentalis*, on greenhouse ornamentals. Proceedings sixth International Symposium on Thysanoptera, Antalya, Turkey, April 27 to May 1. Akdeniz University, Antalya, Turkey. 19-24

Broughton S, Cousins DA, Rahman T (2015) Evaluation of semiochemicals for their potential application in mass trapping of *Frankliniella occidentalis* (Pergande) in roses. Crop Prot 67, 130-135

Broughton S, Harrison J (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 Prot 42:156-163

Bruhin D (2009) Direct and Indirect Effects of Methyl Salicylate and Methyl Jasmonate on *Frankliniella occidentalis* Pergande on Pot Chrysanthemum. MSc Thesis, Universität für Bodenkultur, Vienna, Austria

Cook SM, Khan ZR, Pickett JA (2007) The Use of Push-Pull Strategies in Integrated Pest Management. Annu Rev Entomol 52, 375-400

Demirozer O, Tyler-Julian K, Funderburk J, Leppla N, Reitz S (2012) *Frankliniella occidentalis* (Pergande) integrated pest management programs for fruiting vegetables in Florida. Pest Manag Sci 68, 1537-1545

Dethier VG, Browne LB, Smith CN (1960) The designation of chemicals in terms of the responses they elicit from insects. J Econ Entomol 53, 134-136

De Villiers M, Pringle KL (2007) Seasonal occurrence of vine pests in commercially treated vineyards in the Hex River Valley in the Western Cape Province, South Africa. Afr Ent 15, 241-260

Egger B, Spangl B, Koschier EH (2014) Habituation in *Frankliniella occidentalis* to deterrent plant compounds and their blends. Entomol Exp Appl 151, 231-238

Elimem M, Teixeira da Silva JA, Chermiti B (2014) Double-attraction method to control *Frankliniella occidentalis* (Pergande) in pepper crops in Tunisia. Plant Protect Sci 50, 90-96

Hill DS (2008) Principles of pest control, pp 21-31 in Pests of Crops in Warmer Climates and Their Control. Springer Verlag, New York, USA

Isman MB (2000) Plant essential oils for pest and disease management. Crop Prot 19, 603-608

Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 51, 45-66

Koschier EH, Hoffmann D, Riefler J (2007) Influence of salicylaldehyde and methyl salicylate on post-landing behaviour of *Frankliniella occidentalis* (Pergande). J Appl Entomol 131(5), 362-367

Kumar S (2012) Biopesticides: A Need for Food and Environmental Safety. J Biofertil Biopestici 3: e107. doi:10.4172/2155-6202.1000e107

Masuda M (2011) Microencapsulation of Pesticides for Controlling Release from Coatings. Unpublished PhD thesis, Chalmers University of Technology, SE-412 96 Göteborg, Sweden

Matsuura S, Hoshino S, Koga H (2006) Verbena as a trap crop to suppress thrips-transmitted *Tomato spotted wilt virus* in chrysanthemums. J Gen Plant Pathol 72, 180-185

Muvea AM, Waigani MM, Kutima HL, Osiemo Z, Nyasani JO, Subramanian S (2014) Attraction of pest thrips (Thysanoptera: Thripidae) infesting French beans to coloured sticky traps with Lurem-TR and its utility for monitoring thrips populations. Int J Trop Insect Sci 34, 197-206

Pearsall IA (2000) Damage to nectarines by the western flower thrips (Thysanoptera: Thripidae) in the interior of British Columbia, Canada. J Econ Entomol 93, 1207-1215

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

Reitz SR, Maiorino G, Olson S, Sprenkel R, Crescenzi A, Momol MT (2008) Integrating plant essential oils and kaolin for the sustainable management of thrips and tomato spotted wilt on tomato. Plant Dis 92, 878-886

Sampson C, Kirk WDJ (2013) Can mass trapping reduce thrips damage and is it economically viable? Management of the western flower thrips in Strawberry. PloS ONE 8, e80787. http://dx.doi.org/10.1371/journal.pone.0080787

Sedy KA, Koschier EH (2003) Bioactivity of carvacrol and thymol against *Frankliniella occidentalis* and *Thrips tabaci*. J Appl Entomol 127, 313-316

Teulon DAJ, Castaňé C, Nielsen M-C, El-Sayed AM, Davidson MM, Gardner-Gee R, Poulton J, Kean AM, Hall C, Butler RC, Sansom CE, Suckling DM, Perry NB (2014) Evaluation of new volatile compounds as lures for western flower thrips and onion thrips in New Zealand and Spain. N. Z. Plant Prot 67, 175-183

Teulon, DAJ, Davidson MM, Nielsen M-C, Perry NB, van Tol RWHM, De Kogel W-J (2008) The potential use of lures for thrips biological control in greenhouses: Practice and theory. In Proceedings Third International Symposium on Biological Control of Arthropods, Christchurch, New Zealand. PG Mason, DR Gillespie and R Vincent (Eds). United States Department of Agriculture, Forest Service, Morgantown, WV, USA. pp 301-308

Tyler-Julian K, Funderburk J, Frantz G, Mellinger C (2014) Evaluation of a push-pull strategy for the management of *Frankliniella bispinosa* (Thysanoptera: Thripidae) in bell peppers. Environ Entomol 43, 1364-1378

Van Tol RHWM, James DE, de Kogel WJ, Teulon DAJ (2006) Plant odours with potential for a push-pull strategy to control the onion thrips, *Thrips tabaci*. Entomol Exp Appl 122, 69-76