## AHDB Horticulture

## STANDARD RESEARCH REPORT

**Project title:** Development of the solid-injection technique to identify thrips pheromones for monitoring and mass trapping in UK horticulture

**Project number:** CP 194

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**Location of project:** Keele University

**Industry Representative:** none

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*The results and conclusions in this report are based on an investigation conducted over a four-month period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.*

# AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.


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# GROWER SUMMARY

## Headline

An improved method has been developed for the identification of thrips pheromones for use in monitoring, push-pull or mass monitoring.

## Background

Thrips can cause considerable amounts of damage to protected and semi-protected crops in the UK. Since the western flower thrips (*Frankliniella occidentalis*) arrived in the UK in 1986 (Kirk & Terry, 2003), this species has been the main pest thrips in these crops. Before 1986, the onion thrips (*Thrips tabaci*) was the main pest and several other species were known as minor pests. In recent years, UK strawberry growers have reported that species other than the western flower thrips have been invading crops and causing damage. The reported species include: rose thrips (*Thrips fuscipennis*), rubus thrips (*Thrips major*), flower thrips (*Frankliniella intonsa*) and onion thrips (*Thrips tabaci*). Thrips are often invasive and so new species (e.g. *Thrips setosus*) may arrive and spread in the UK in future.

Aggregation pheromones attract both males and females and so can be used to increase trap catches and thus monitor a pest or reduce population size (Kirk, 2017). Some commercial products using thrips aggregation pheromones are already available from several companies. Our group at Keele identified the first aggregation pheromone in thrips in 2002 (Hamilton, Hall & Kirk, 2005). This was for the western flower thrips (*Frankliniella occidentalis*). Since then we have identified the aggregation pheromone of the melon thrips (*Thrips palmi*), which is an invasive species from SE Asia, and the bean flower thrips from Africa (*Megalurothrips sjostedti*). A group in China has identified the aggregation pheromone of the flower thrips (*Frankliniella intonsa*). However, the standard method of identification that we used (entrained volatiles eluted in solvent) has many limitations because it takes a long time and it is only possible when hundreds of live males are available at one time and can be separated from other species when alive. In addition, the technique needs large amounts of pheromone and it is hard to collect enough pheromone because thrips are so small. It is therefore not practical for most thrips species.

The identification of new thrips pheromones could be speeded up and extended to more species if a technique could be developed that is more sensitive and needs very few adult males at a time. Such a technique, the "solid-injection technique", was invented almost 50 years ago at Keele (Morgan & Wadhams, 1972; Bagnères & Morgan, 1990), but it is not widely used because the injection device is not commercially available and sample injection

cannot be automated. It can only be used for insects that are less than about 2 mm across. This project will develop this technique further in order that the sensitivity of the technique will outweigh the limitations.

Our aim is to develop this method to be able to detect and identify the male-produced aggregation pheromone of high priority thrips species in UK horticulture. It will also allow rapid identification of invasive thrips that arrive and become a problem in future.

## Summary

We have developed a method that can be used to identify the aggregation pheromone of new species of thrips. These pheromones can be used to trap thrips. The new method has the advantage that it is very sensitive to the very small amounts of pheromone that thrips produce and can be used with as few as 10 individual thrips for each sample. The method is also cheap and portable. We are in the process of using the method to identify the aggregation pheromone of the onion thrips (*Thrips tabaci*).

## Financial Benefits

None as yet

## Action Points

None as yet

# SCIENCE SECTION

# Objective 1: Confirm detection of the known pheromone of adult male western flower thrips using the solid-injection technique.

## Introduction

Adult male western flower thrips (*Frankliniella occidentalis*) produce an aggregation pheromone consisting of a major component (neryl (*S*)-2-methylbutanoate) (N2MB) and a minor component ((*R*)-lavandulyl acetate) (LA). This thrips species is reared continuously at Keele University, thus allowing us to collect samples of males for pheromone samples at any time. Since the pheromone components are known, we can easily measure how much pheromone is obtained, even close to the limits of detection. Working with a known pheromone is the best way to develop the method before applying it to species for which the pheromone is not known.

We first tested whether we could detect pheromone from the bodies of adult males. This would be the simplest approach, but it is only possible if thrips store pheromone. The approach can be developed by improving sensitivity of detection and/or increasing the amount of pheromone. The second approach, if the males do not store enough pheromone, is to collect pheromone as it is produced. This process is more involved. The approach can also be improved by improving sensitivity of detection and/or increasing the amount of pheromone released and collected.

## Materials and Methods

### Rearing

The western flower thrips (*Frankliniella occidentalis*) was reared on pot chrysanthemum in rearing cages at 25±2°C. Entrainment of pheromone was carried out in the same room at 24°C.

### Gas chromatograph - mass spectrometer (GC-MS) analysis

Two different setups were used for GC-MS analysis of the pheromone. The main setup, which was used for most analyses, had a quadrupole MS. The second setup had a Time of Flight (TOF) MS, which would be expected to be more sensitive.

A solid-sample injector (Morgan & Wadhams, 1972; Bagnères & Morgan, 1990) was used to introduce samples to the inlet port of the GC. This device was custom-made. It has the advantage that the analysis does not involve any solvents, so volatiles are not lost and any

trace contaminants in the solvent are avoided. It is therefore likely to be more sensitive for small biological samples. We also took other measures to improve sensitivity, such as using a new column in the GC and changing the inlet liner of the GC.

The first setup (referred to here as the 'quadrupole MS') was an HP6890 GC instrument, modified to equip the solid-sample injector, linked to an HP5973 Network Mass Selective Detector (Agilent). The capillary column was coated with DB-5MS (30 m x 0.25 mm internal diameter, 0.25 μm film thickness). Helium (1 ml min$^{-1}$) was used as the carrier gas. The temperature of the inlet port, in which the solid-sample injector was inserted was set at 250°C and the injection was splitless. After placing the sealed capillary in the solid-sample injector, it was left in the inlet for 3 min, before crushing the glass capillary, and hence releasing any compounds. Compounds were then transferred by the carrier gas to the column. The temperature of the oven was held at 50°C for 2 min, before increasing to 200°C at 25°C min$^{-1}$, then to 220°C at 3°C min$^{-1}$ and then to 320°C at 30°C min$^{-1}$ and held for 5 min. The mass spectrometer was operated in the EI mode at 70 eV, scanning from 40 to 160 atomic mass units (i.e. ion mass) for the reduced scan range. In SIM mode, we selected ions *m/z* 69, 93, 121 and 136.

The second setup (referred to here as the 'TOF MS') was a Pegasus BT1 instrument (Leco Ltd, Hazel Grove, UK) with a GC connected to a TOF MS. The inlet temperature was 250°C. The same oven temperature program and carrier gas was used. The capillary column type was also DB-5MS (30 m x 0.25 mm internal diameter, 0.25 μm film thickness). The Pegasus BT1 was on loan from Leco, and has not been modified to fit the solid-sample injector, so we could not use it for solid injection.

**Limit of detection and calibration of the GC-MS**

The analysis of a range of concentrations of synthetic neryl (S)-2-methylbutanoate, which is the major compound in the aggregation pheromone of the western flower thrips (*F. occidentalis*), on the quadrupole MS revealed that the lowest detected concentration was 2,220 pg (Fig. 1), whereas on the TOF MS we could detect as low as 9 pg (Fig. 2). However, we estimate that on the TOF MS the limit of detection could be lower (approximately 1 pg). We could use the solid-sample injector on our quadrupole machine, but we could not use it on the TOF MS because it was on loan from the manufacturer and so we could not make the necessary modifications to allow use of the solid-sample injector.
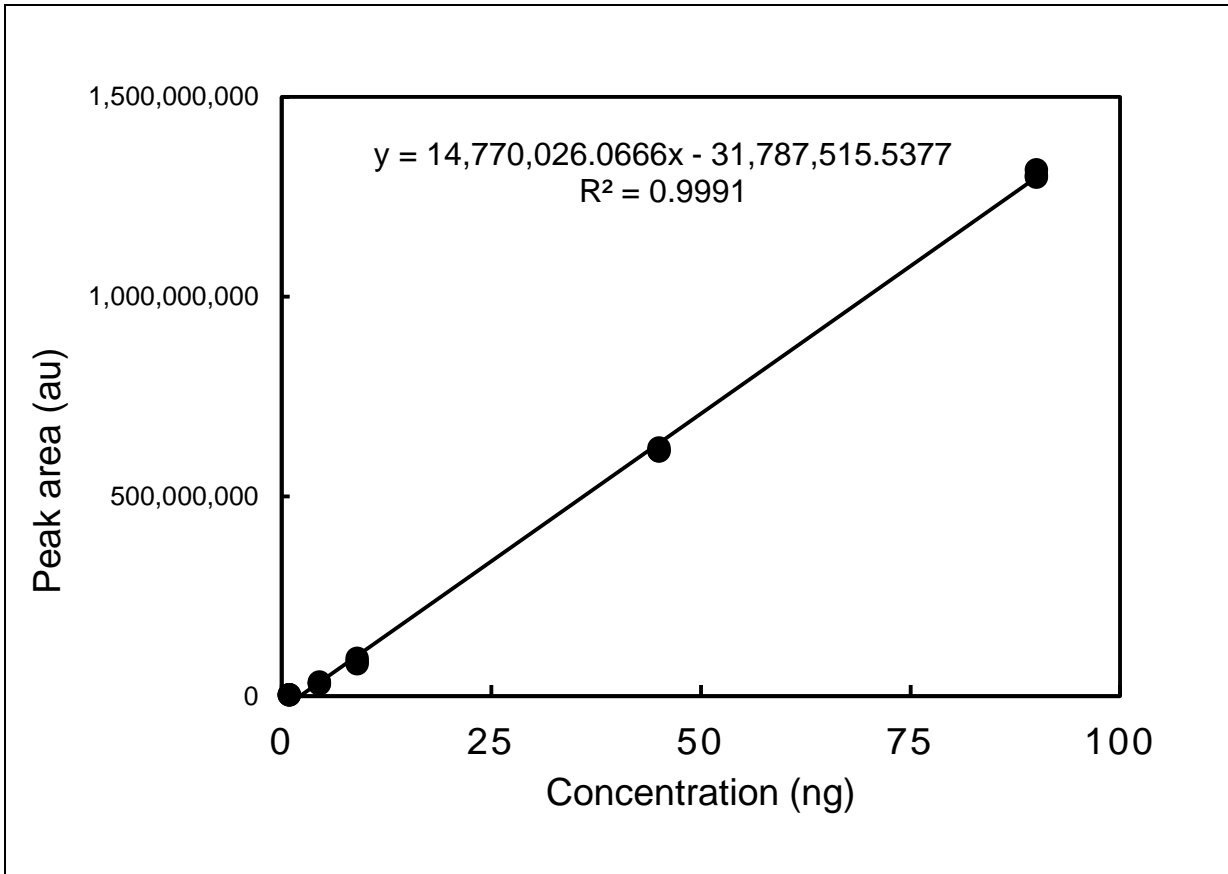
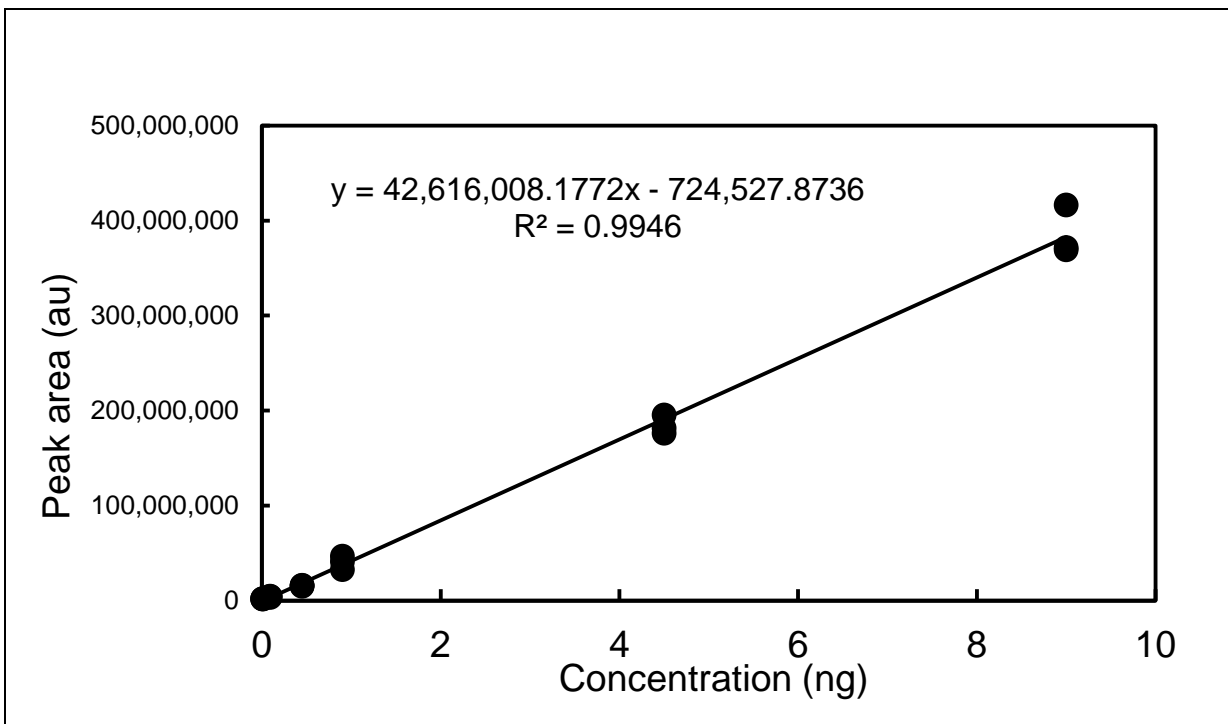**Figure 1**. Synthetic major compound - calibration curve on the quadrupole MS



**Figure 2**. Synthetic major compound - calibration curve on the TOF MS

Synthetic N2MB and LA external standard solutions were separately dissolved in hexane as the solvent and analysed with the Auto Liquid Sampler (ALS) on both types of mass spectrometer (quadrupole and TOF) in order to determine their retention time and mass spectrum (i.e. characteristic pattern of ion fragments). Additionally, the major compound was also used to produce the calibration curve that we used to determine the amount in our samples. For this purpose, eight different concentrations were prepared (90 ng $\mu$l$^{-1}$, 45 ng $\mu$l$^{-1}$, 9.0 ng $\mu$l$^{-1}$, 4.5 ng $\mu$l$^{-1}$, 0.9 ng $\mu$l$^{-1}$ for the quadrupole and 9.0 ng $\mu$l$^{-1}$, 4.5 ng $\mu$l$^{-1}$, 0.9 ng $\mu$l$^{-1}$, 0.45 ng $\mu$l$^{-1}$, 0.09 ng $\mu$l$^{-1}$ and 0.009 ng $\mu$l$^{-1}$ for the TOF). This allowed us to test the lowest concentration of major compound that the quadrupole MS and TOF MS can detect. All standard samples were run in a sequence with three injections for each concentration. A control sample containing hexane only was added to the sequence. On both mass spectrometers the data were acquired using a reduced scan range.

The standard analysis of both the minor and major compound provided us with their retention time and the ion fragmentation pattern. We used this to determine the presence or absence of these molecules in our samples. We could also calculate the amount in the samples and thus estimate the amount on whole bodies (in pg male$^{-1}$) or the release rate (in pg male$^{-1}$ h$^{-1}$).

**Solid-sample injector and sample preparation**

The solid-sample injector consists of a hollow tube (3 mm OD, 2 mm ID) with small holes at the bottom (Fig. 3). The device further has a plunger that can be pushed down this hollow tube. The whole unit is sealed with the help of an O-ring. The hollow tube section of the solid sampler is inserted in the GC inlet. When the plunger is lifted up, there is access to the hollow tube, and a glass capillary containing the sample (see below) can be dropped into the tube. The plunger is then lowered to stop just above the glass capillary, and the system is sealed by tightening a nut housing the O-ring. After a certain time, the plunger is pushed down further to crush the glass capillary and this releases all the compounds from the sample in the capillary, which are then transferred to the GC column.

**Figure 3**. The solid-sample injector in the GC inlet: (A) with plunger inserted; (B) with glass capillary being dropped into the tube.
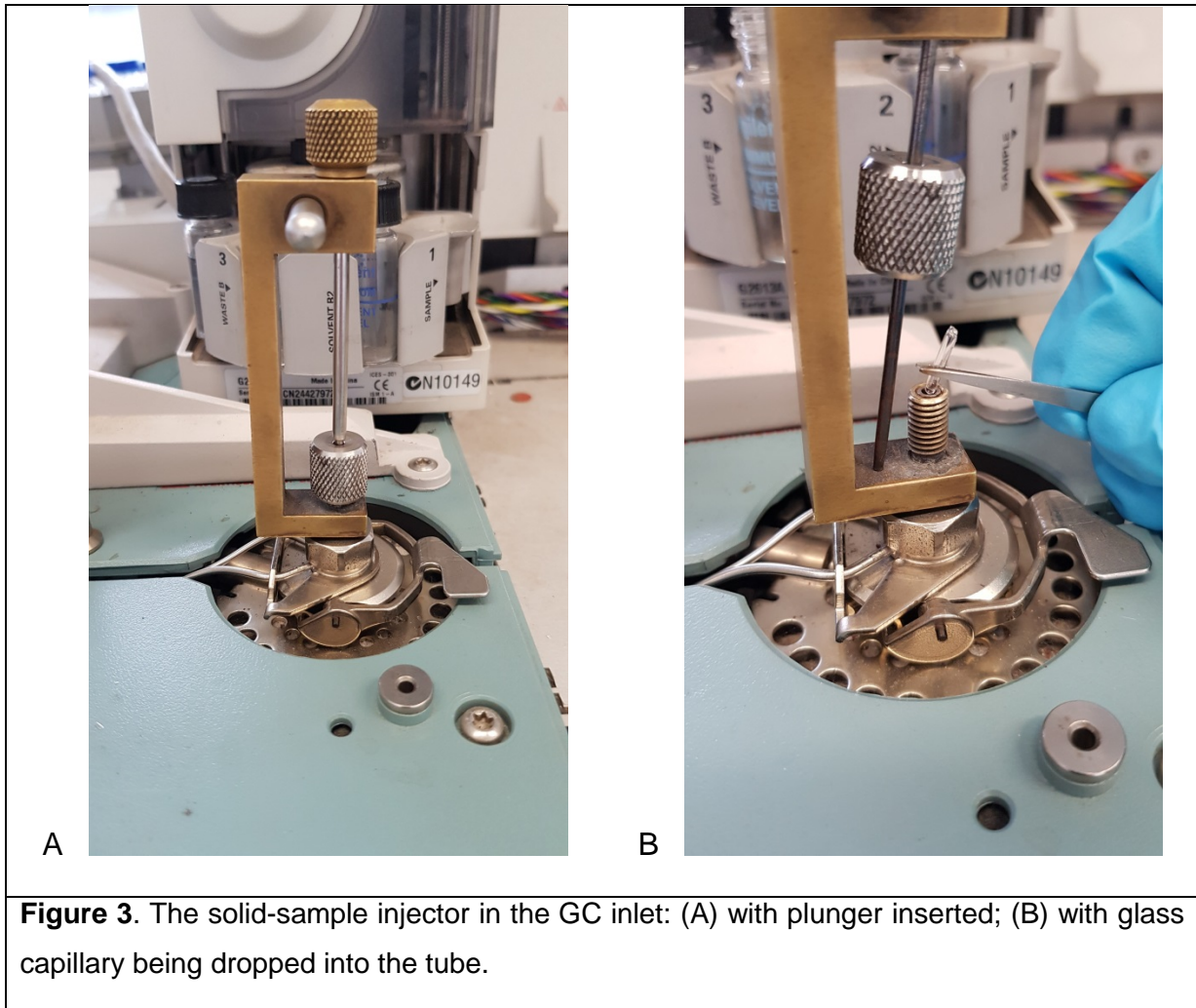
For solid injection, glass capillary tubes (OD 1-2 mm) were cut to length (10-20 mm), cleaned in hexane for 20-30 min, drained and baked overnight at 80°C. They were sealed at one end using a small blowtorch.

**Analysis of extracts of whole bodies**

Adult males were collected from the culture, killed by freezing and placed in the glass capillary tubes using a fine brush. There were 10 replicates each with 10 adult males. The glass capillary tubes were sealed at the top end using a small blowtorch and then analysed using the solid injection technique on the quadrupole MS.

To test whether more pheromone could be obtained by puncturing the cuticle of the insects, thus allowing more pheromone to be extracted, a further 10 replicates were prepared in the same way, but after freezing each thrips was cut in two.

According to Hamilton, Hall & Kirk (2005), patrolling behaviour may be necessary for pheromone production. To test whether more pheromone could be extracted from whole bodies by allowing the adult males to patrol, live adult males were anaesthetised with

carbon dioxide for 15 s and placed in the glass capillary tubes. The tubes were then sealed with a small blowtorch. Any pheromone produced while the males were patrolling would be captured inside the glass tube. The males were left in the glass capillary tubes for 4-6 h. Illumination with a desk lamp was used to encourage patrolling. Ten samples of 10 males were analysed by the solid-injection technique on the quadrupole MS.

A series of adjustments to the collection method were made to try to increase pheromone production. We also adjusted the settings of the mass spectrometer to increase the sensitivity. The MS was changed to SIM mode, which scans only for selected ions of interest. This is more sensitive for the selected ions, but at the cost of losing information about other ions. We scanned for the ions (69, 93, 121 and 136) that we know are in the fragmentation pattern of the major pheromone compound, neryl (*S*)-2-methylbutanoate (Hamilton, Hall & Kirk, 2005). However, although this can suggest the presence of the pheromone, there is not enough information for a positive identification. We also repeated the experiment using the reduced scan mode (40-160 amu) instead of the full scan mode (40-800 amu). The reduced scan gains sensitivity by omitting masses in ranges unlikely to be of interest. This is not as sensitive as SIM mode, but there is enough information for positive identification of the major compound from the fragmentation pattern.

### Analysis of headspace volatiles by SPME

To check that the male thrips were actually producing aggregation pheromone, we used entrainment with SPME (solid-phase microextraction) fibres (divinylbenzene (DVB)/ carboxen/polydimethylsiloxane (PDMS), 57328-U, Supelco). This is the method by which the aggregation pheromone of the western flower thrips (*F. occidentalis*) was originally identified at Keele (Hamilton, Hall & Kirk, 2005). Ten males were transferred with a fine brush into clean 2 ml glass vials with a screw-cap lid containing a septum. The septum was pierced and the SPME fibre was pushed through and exposed to the insects. The headspace volatiles were collected for 2 h. The vials were placed horizontally under the bright light of a desk lamp to induce patrolling behaviour. One set of entrainments was analysed on the quadrupole MS (3 replicates) and another set on the TOF MS (6 replicates) using the reduced scan mode.

For comparison, the same 10 males that had been exposed to the SPME fibre (5 replicates) were immediately transferred into glass capillaries and analysed with the solid-injection technique to test whether the any of the released pheromone remained in the body. These were run with the reduced scan mode.

**Analysis of headspace volatiles with Tenax beads**

Tenax® TA beads (Supelco, 20832-U, Poole, UK) can be used to collect volatile compounds. They are cheaper and more versatile than SPME fibres and can be transferred into glass capillaries and used for the solid-injection technique. A small amount of clean Tenax beads was weighed out into a clean 2 ml glass vial and the lid was immediately screwed into place to prevent adsorption of extraneous volatiles. Males were collected from the culture and transferred into the Tenax vials following the same procedure as with SPME entrainment and then placed under a bright desk light to induce patrolling behaviour. The Tenax was exposed to the males for 4 h. At the end of the entrainment the vials were placed in the freezer for at least 30 min to kill the insects. The contents of the vials was then transferred carefully to a glass capillary tube, sealed at the top end and analysed by the solid-injection technique. We entrained the males using different amounts of Tenax (2 mg, 5 mg or 10 mg) in order to optimize the sample preparation process and to test whether the Tenax uptake properties are quantity-related. The samples were analysed using the reduced scan method. Blank samples without thrips were used as a control and analysed in the same way. Each set of samples was entrained on different days at a different time of day to test whether there is variation in the amount of pheromone released.

For all experiments, blank vials with Tenax and no thrips were used as controls to check that no volatiles were present.

## Results

**Analysis of extracts of whole bodies**

In order to identify the presence of the minor and major compounds in our samples, we needed to know their retention times and the ion fragmentation patterns to be able to locate them in the GC-MS output from our samples. Synthetic standards of the two known pheromone compounds were injected on the quadrupole MS. The minor compound eluted at 7.282 min and the major compound eluted at 8.935 min (Fig. 4).
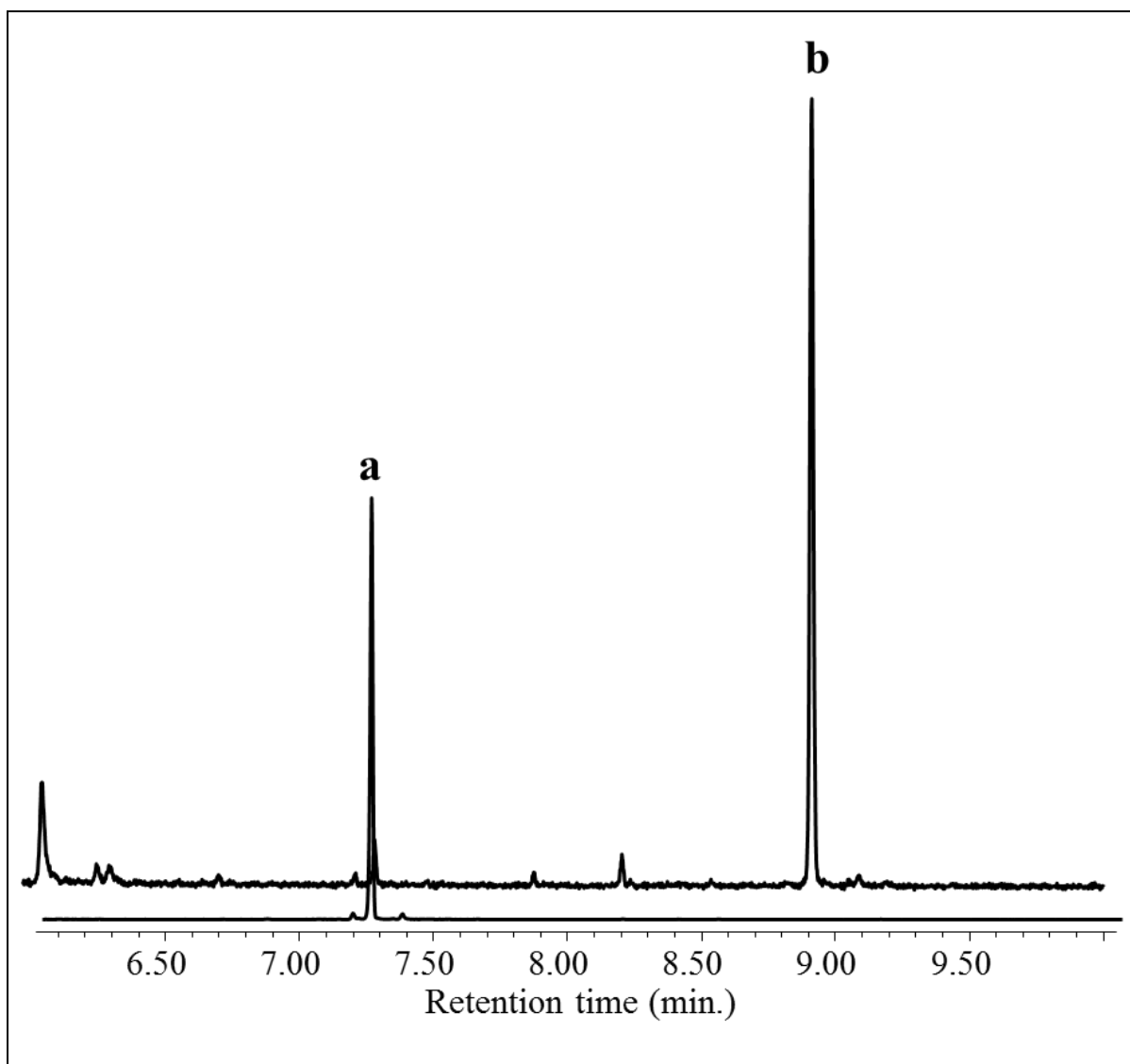
**Figure 4**. Overlaid GC chromatograms of synthetic standards of the minor compound ((*R*)-lavandulyl acetate) and the major compound (neryl (*S*)-2-methylbutanoate) to show their retention times on the quadrupole MS. Peak a: minor compound ($t_R$ = 7.282 min). Peak b: major compound ($t_R$ = 8.935 min).

Analysis of intact whole-body samples of adult male western flower thrips (*F. occidentalis*) failed to detect peaks for the two pheromone compounds (Table 1). We focused on the major compound (N2MB) because this compound is always produced in larger amounts than the minor compound and thus would be easier to detect. Since the limit of detection on the quadrupole MS is 2,200 pg, we know that our extracts from the 10 males produced <220 pg of N2MB from each male on average.

**Table 1**. The amounts of neryl (*S*)-2-methylbutanoate (N2MB) detected in samples of 10 whole bodies of adult male western flower thrips (*F. occidentalis*)

| Approach | No. of replicates | Positive detection of N2MB | Amount of N2MB (pg male$^{-1}$) |
|---|---|---|---|
| Intact body | 10 | 0 of 10 | <220 |
| Cut body | 10 | 0 of 10 | <220 |
| Induced patrolling | 42 | 0 of 42 | <220 |

The approach was modified by cutting the dead thrips in two before extraction in case this could release more pheromone from inside the body, but again no peaks were detected for N2MB (Table 1).

The approach was modified again by allowing live males to patrol within the glass capillary for 4-6 h before extraction, but again no peaks of N2MB were detected (Table 1). This approach was adjusted by varying the length of the glass capillary and settings on the quadrupole MS, but still no peaks were detected. Use of SIM mode on the MS showed that some of the right ions were present at the right retention time for N2MB, suggesting that some of the compound may have been present, but this was not enough to positively identify the presence of the compound. Since the limit of detection on the quadrupole MS is 2200 pg, each male would need to have produced more than 55 pg h$^{-1}$ on average to be detected (based on 10 males for 4 hours in the vial per sample). Males have previously been found to produce 100-300 pg male$^{-1}$ h$^{-1}$ (Kirk & Hamilton, 2004; Dublon, Hamilton & Kirk, 2008), but the restricted space in the tube probably prevented normal behaviour. Also, the heat while sealing the glass capillary killed or otherwise affected some of the thrips and would have affected their behaviour.

**Analysis of headspace volatiles by SPME**

To check that the male thrips were actually producing pheromone and to check the release rates, we used the same SPME method that we had used previously to identify the aggregation pheromone of adult male western flower thrips (*F. occidentalis*) (Hamilton, Hall & Kirk, 2005). The method had only been used to identify the pheromone and no attempt had been made in the past to study how the pheromone release rate varied with time or conditions. Entrainment of only 10 males for 2 h showed the clear presence of both pheromone compounds (N2MB and LA) (Fig. 5).
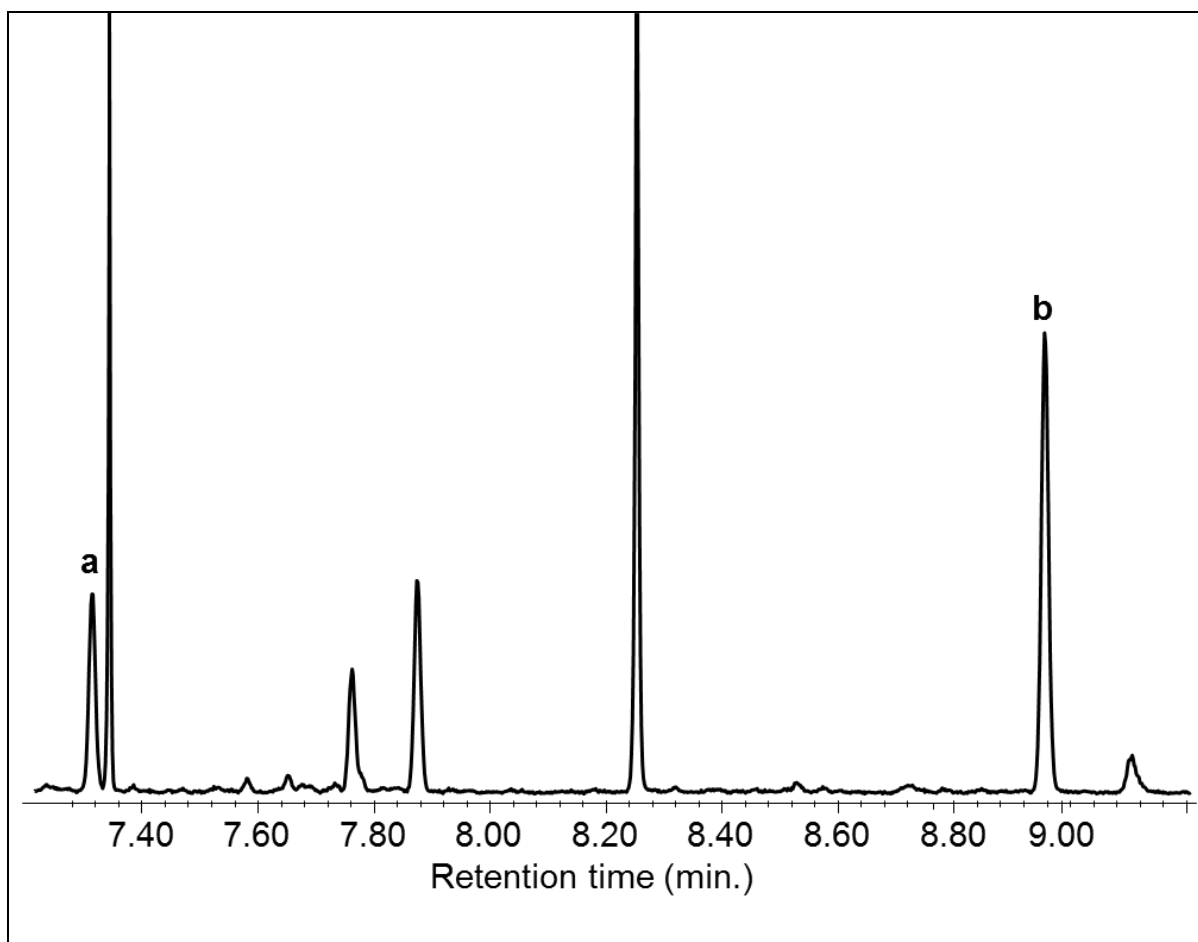
**Figure 5**. GC chromatogram of the WFT aggregation pheromone compounds detected with an SPME fibre on the quadrupole MS. Peak a: the minor compound ((*R*)-lavandulyl acetate). Peak b: the major compound (neryl (*S*)-2-methylbutanoate).

As expected, more of the major compound (N2MB) was released than of the minor compound (LA) (Fig. 5, Table 2). The three entrainments that were run on the quadrupole MS had different release rates of the two compounds compared with the entrainments that were run on the TOF MS. The samples were run on different days and the difference may be attributable to variation in the thrips rather than an effect of different machines. Since we do not know whether the SPME fibres were adsorbing all the pheromone that was produced, the calculated release rates should be considered as minimum release rates. Thus, the three entrainments on SPME that were run on the quadrupole MS gave average minimum release rates of 200 pg male$^{-1}$ h$^{-1}$ and those that were run on the TOF MS gave average minimum release rates of 624 pg male$^{-1}$ h$^{-1}$.

**Table 2.** The amounts of neryl (*S*)-2-methylbutanoate (N2MB) and (*R*)-lavandulyl acetate (LA) detected on an SPME fibre with the quadrupole MS and the TOF MS, using 10 males for 2 h.

| | SPME + quadrupole MS | SPME + TOF MS |
|---|---|---|
| No. of replicates | 3 | 6 |
| N2MB positive detections | 3 of 3 | 6 of 6 |
| N2MB detected range (ng) | 3.4-4.4 | 1.2-23.1 |
| N2MB detected mean (ng) | 4.0 | 12.5 |
| N2MB release rate mean (pg male$^{-1}$ h$^{-1}$) | 200.4 | 623.7 |
| LA positive detections | 3 of 3 | 4 of 6 |
| LA detected range (ng) | 2.2-2.6 | 0.2-1.5 |
| LA detected mean (ng) | 2.4 | 0.6 |
| LA release rate mean (pg male$^{-1}$ h$^{-1}$) | 120.9 | 29.06 |

The bodies of males that had been exposed to SPME and were shown later to have been producing pheromone were also analysed by solid injection, but no pheromone peaks were detected from the whole bodies.

**Analysis of headspace volatiles with Tenax beads**

Since the SPME entrainments confirmed that the thrips were producing pheromone, even though it was not detectable on whole-body samples, we developed a cheap, convenient, flexible and portable method of entrainment that uses Tenax beads and small numbers of males. The pheromone compounds from live males were entrained with Tenax beads in a small glass vial and analysed with the solid-injection technique on the quadrupole MS. Both the major and minor pheromone compounds were detected and, as expected, more of the major compound was detected than of the minor compound (Fig. 6).
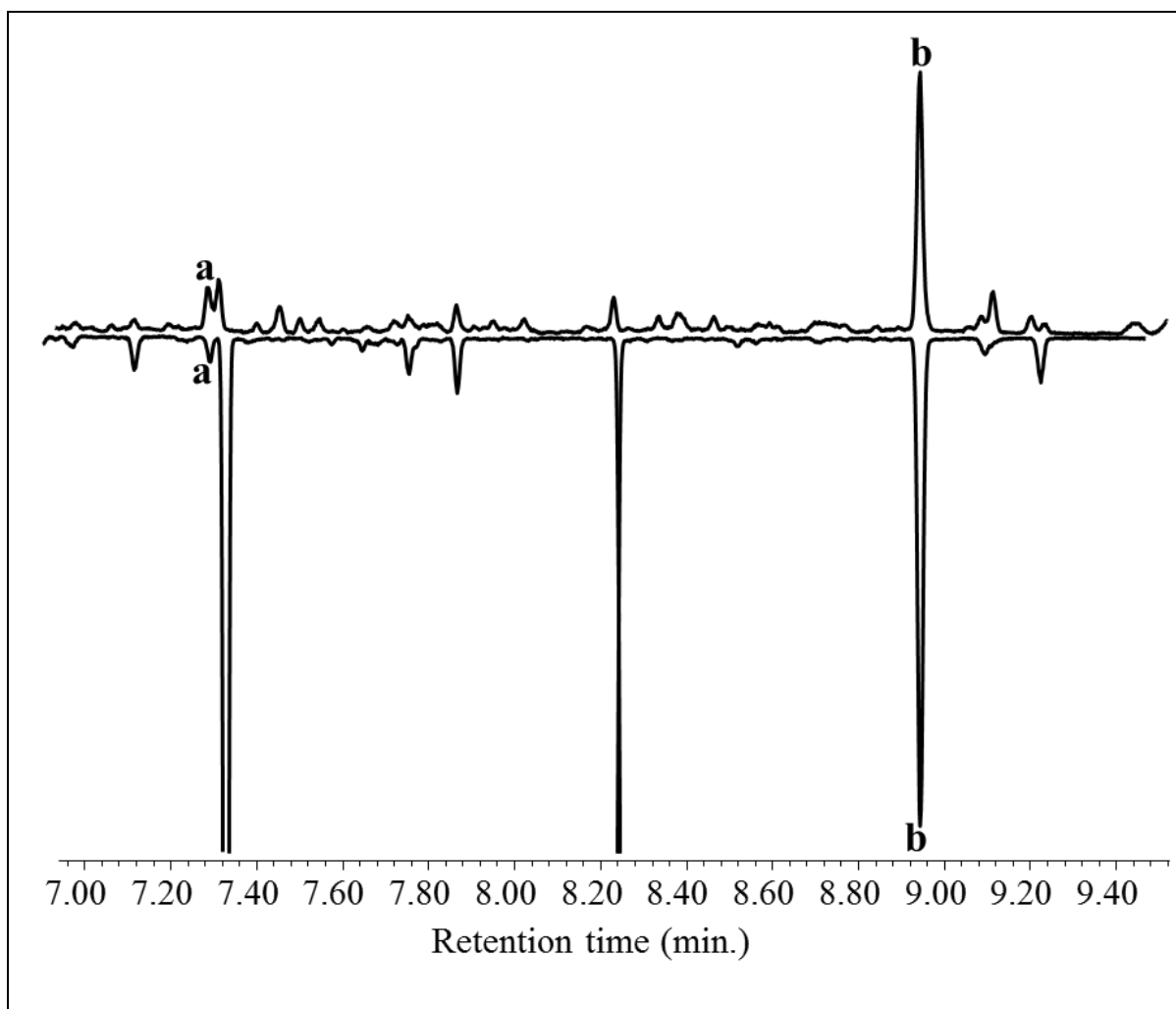
**Figure 6**. GC traces of headspace volatiles collected on Tenax beads (upper chromatogram) or an SPME fibre (inverted lower chromatogram) exposed to 10 adult males and run on the quadrupole MS. Peak a: the minor compound ((*R*)-lavandulyl acetate). Peak b: the major compound (neryl (*S*)-2-methylbutanoate).

The amount of Tenax (2 mg, 5 mg) made no difference to the amount of pheromone collected (Tables 3-4). This is as expected because the very small amounts of pheromone that are entrained are not likely to saturate 2 mg of Tenax. The use of 10 mg was abandoned because it could not all fit in a glass capillary. Increasing the exposure time for the sample in the inlet port made no obvious difference to the amount detected.

A striking feature is the considerable variability in the amounts detected within and between samples (Tables 3-4). Although samples were run on many different days and at different times of day, there was no obvious pattern to the variation.

**Table 3**. The amounts of the major compound (N2MB) entrained on different amounts of Tenax at two male densities. The means and ranges are for the positives.

| Tenax (mg) | No. of males | No. of replicates | N2MB positive detection | N2MB detected range (ng) | N2MB mean amount (ng) | Mean release rate (pg male$^{-1}$ h$^{-1}$) |
|---|---|---|---|---|---|---|
| 10 | 10 | 5 | 3 of 5 | 2.1-2.2 | 2.1 | 54.6 |
| 5 (set 1) | | 4 | 3 of 4 | 2.1-2.6 | 2.4 | 60.2 |
| 5 (set 2) | | 5 | 5 of 5 | 2.2-2.6 | 2.4 | 61.3 |
| 2 (set 1) | | 5 | 2 of 5 | 2.15-2.17 | 2.16 | 54.1 |
| 2 (set 2) | | 5 | 5 of 5 | 2.2-3.5 | 2.6 | 64.9 |
| 2 (set 3) | | 5 | 2 of 5 | 2.2-2.8 | 2.4 | 60.6 |
| 2 (set 4) | | 4 | 3 of 4 | 2.16-2.19 | 2.18 | 54.5 |
| 2 * | | 5 | 3 of 5 | ** | ** | ** |
| 2 | 5 | 5 | 3 of 5 | ** | ** | ** |

*This set of samples was exposed for 5 min in the inlet port to test whether the prolonged exposure would desorb more compound from the Tenax beads. All other replicates were exposed for 3 min.

**The peak areas were too small to be quantified.

**Table 4**. The amounts of the minor compound (LA) entrained on different amounts of Tenax at two male densities. The means and ranges are for the positives. The calibration curve for N2MB was used as a rough guide to convert peak areas to amounts.

| Tenax (mg) | No. of males | No. of replicates | LA positive detection | Quantifiable peaks | LA detected range (ng) | LA mean amount (ng) | Mean release rate (pg male$^{-1}$ h$^{-1}$) |
|---|---|---|---|---|---|---|---|
| 10 | 10 | 5 | 0 of 5 | - | - | - | - |
| 5 (set 1) | | 4 | 3 of 4 | 2 of 3 | 2.2-2.3 | 2.3 | 57.5 |
| 5 (set 2) | | 5 | 2 of 5 | 1 of 2 | 2.2 | 2.2 | 55.3 |
| 2 (set 1) | | 5 | 0 of 5 | - | - | - | - |
| 2 (set 2) | | 5 | 4 of 5 | 1 of 4 | 2.4 | 2.4 | 59.8 |
| 2 (set 3) | | 5 | 1 of 5 | 0 of 1 | - | - | - |
| 2 (set 4) | | 4 | 0 of 4 | - | - | - | - |
| 2 * | | 5 | 0 of 5 | - | - | - | - |
| 2 | 5 | 5 | 0 of 5 | - | - | - | - |

*This set of samples was exposed for 5 min in the inlet port to test whether the prolonged exposure would desorb more compound from the Tenax beads. All other replicates were exposed for 3 min.

The solid-injection analysis of the headspace volatiles collected from 10 live females entrained on 2 mg of Tenax confirmed that the two male-specific aggregation pheromone

compounds are absent in the trace of female volatiles (Fig. 7), as found earlier by Kirk & Hamilton (2004) using SPME.
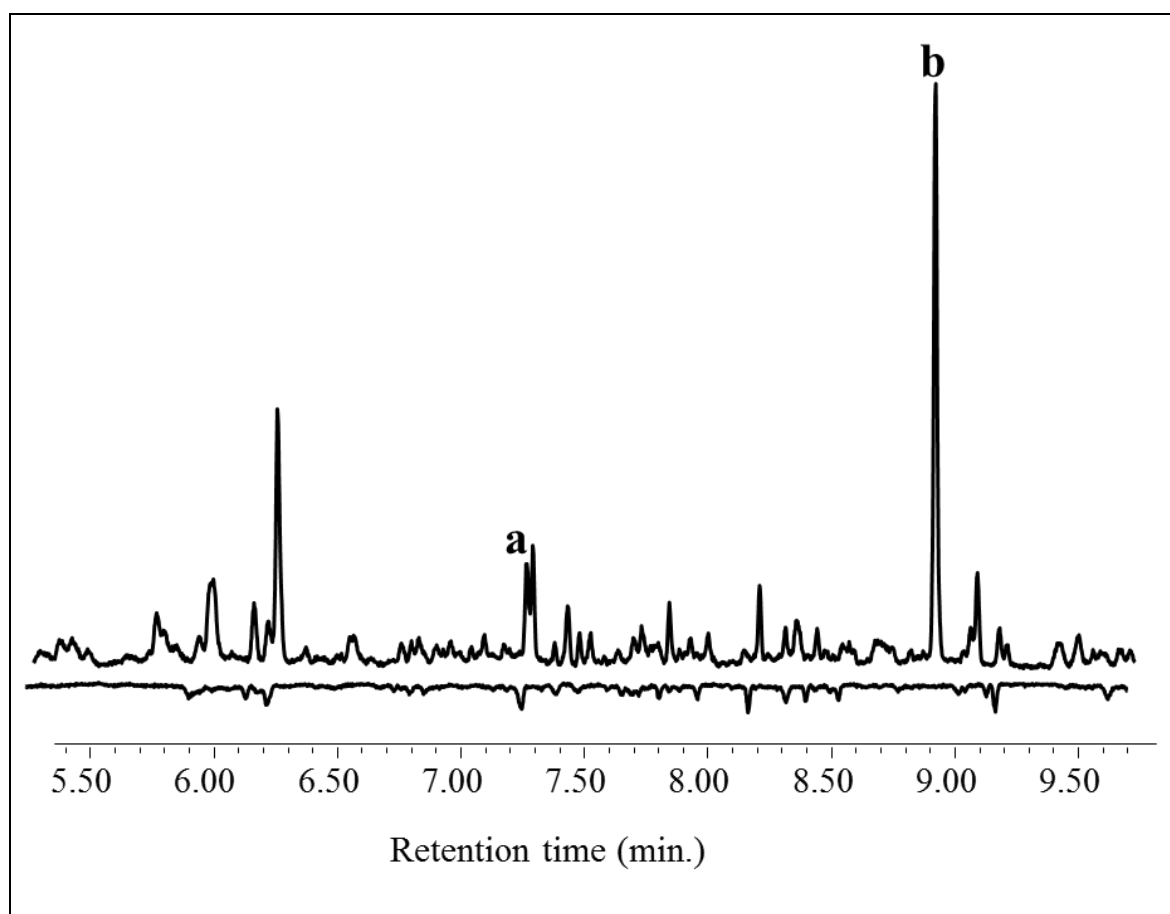


**Figure 7**. GC traces of headspace volatiles collected on Tenax beads exposed to adult males (upper chromatogram) and adult females (inverted lower chromatogram). Peak a: minor compound (($R$)-lavandulyl acetate). Peak b: major compound (neryl ($S$)-2-methylbutanoate).

## Discussion

It has previously been suggested that male western flower thrips (*F. occidentalis*) may not store pheromone and might only produce it on demand (Kirk & Hamilton, 2004). This was confirmed in a series of experiments using different methods that tried without success to extract pheromone from whole bodies of males.

When 10 live adult males were left in a sealed glass vial with 2 mg of Tenax in a bright light for 4 h, enough pheromone was entrained for identification by solid injection on a quadrupole MS. However, the use of solid injection on a TOF MS would greatly increase the

sensitivity. By this new method, the quadrupole MS should be able to detect pheromones produced at rates exceeding 55 pg male$^{-1}$ h$^{-1}$, whereas the TOF MS should be able to detect pheromones at rates exceeding only 9 pg male$^{-1}$ h$^{-1}$. The thrips aggregation pheromones identified so far have involved either one or two compounds and the major compounds have been produced at rather similar rates (at least 100 pg male$^{-1}$ h$^{-1}$). However, there is no guarantee that other species will produce pheromone at the same rate, so the more sensitive the method, the better.

An important difference between this research and previous research is that pheromone was obtained from samples of only 10 males. Previous research used 30-70 males (Kirk & Hamilton, 2004) or 15 males (Dublon, Hamilton & Kirk, 2008). Our results suggest that the amount of pheromone produced by each thrips might even increase at lower male densities, so there may be little gained from larger samples of thrips. The use of fewer males will make it much easier to obtain samples of just one species because these usually have to be reared in small numbers to exclude other species. Thrips collected from the field are often a mixture of species and they cannot be identified reliably when alive.

The thrips could perhaps be entrained for longer than 4 h in some species, but this is not always an option because the males usually die after a few hours in a glass vial.

There was considerable variation in the amount of pheromone produced by the samples of 10 males, but there was no obvious pattern to explain it. Thrips show considerable day to day variability in behaviour, such as readiness to fly (O'Leary, 2005), even if reared under constant conditions, so variability in pheromone production is to be expected. This shows that even if no pheromone is initially detected from thrips entrainments it is worth persevering because variability is to be expected. The new method not only makes it easier to identify new pheromones of high priority thrips species in UK horticulture, but also offers a way to study how thrips use aggregation pheromones because it can now be done with small samples. A better understanding of thrips behaviour could also enhance the use of pheromones in pest management.

## Objective 2: detect the presence of pheromone compounds by solid injection of adult males for at least one pest thrips for which the pheromone is unknown

### Introduction

In Objective 1, we developed and tested a method that can detect aggregation pheromone compounds in the western flower thrips (*F. occidentalis*). The method used samples of only

10 males, which are fairly easy to collect. It is also portable, such that collection vials can be sent by courier and once they are exposed to thrips the vials can be returned by courier for analysis at Keele. This project ran over winter from November 2019 to February 2020 when it would be impossible to locate enough adult males of any further species in the field, so in order to try to identify a new pheromone we located a culture of the onion thrips (*Thrips tabaci*) that is maintained year-round at a university in Budapest, Hungary. The species has several strains (Fail, 2016), which differ in behaviour, so we intend to test the two strains that have males.

Since it turned out that the aggregation pheromone was not detectable on whole bodies of adult male thrips, because they do not store enough pheromone, the development of the method took longer than had been expected. We were therefore only able to courier the prepared collection vials to Budapest in the last week of the project. The spread of the coronavirus has since led to the closure of universities in Budapest, with the consequence that the thrips cultures have been stopped, which has now prevented the collection of further pheromone samples in Budapest. Samples from one strain of onion thrips (*T. tabaci*) were collected just in time and have now been received in the UK. We plan to analyse the samples at another UK university with a more sensitive TOF MS that can be used with solid injection. This analyser can detect as little as about 9 pg of pheromone in a sample. In comparison, the Quadrupole MS at Keele can only detect about 2200 pg. However, we cannot do this analysis until the coronavirus crisis is over.

## Materials and Methods

### Preparation of sample vials

A small amount (2 mg) of clean Tenax was weighed out into a clean 2 ml glass vial and the lid was immediately screwed into place to prevent adsorption of extraneous volatiles. These prepared vials were then sent by next-day courier to Budapest where they were to be exposed to thrips as follows:

- 10 vials each with 10 adult female onion thrips (*T. tabaci*) strain L1
- 10 vials each with 10 adult male onion thrips (*T. tabaci*) strain L1
- 10 vials each with 10 adult female onion thrips (*T. tabaci*) strain T
- 10 vials each with 10 adult male onion thrips (*T. tabaci*) strain T
- 10 vials not exposed to thrips (controls)

### Collection of pheromone

The thrips were collected from the culture in Budapest and transferred into the vials and then left under a bright desk light to induce male patrolling behaviour. The Tenax was

exposed to the thrips for 4 h. At the end of the entrainment, the vials were placed in the freezer for at least 30 min to kill the insects. The vials were then returned to Keele by courier.

At Keele, the samples will be prepared for analysis by solid injection in an OPTIC-4 Multi Mode Inlet (GL Sciences, Eindhoven, the Netherlands) on a Pegasus BT1 instrument (Leco Ltd, Hazel Grove, UK) with a GC connected to a TOF MS. The injection will be carried out at another university in the UK.

## Results

The samples for male and female onion thrips (*T. tabaci*) strain T and controls have now been sent from Budapest and received in the UK. The collection of samples for male and female onion thrips (*T. tabaci*) strain L1 was prevented by the ending of the Budapest culture as a result of university closure to slow the spread of coronavirus.

## Discussion

If the males produce enough pheromone, we should be able to detect it. Further sample collections may be needed to verify the identification. For example, if the compound or compounds are chiral (have left-handed and right-handed forms), it will be necessary to separate these by running further samples on a chiral column. Once a compound or compounds have been identified, it can be synthesised and added to traps in the field to test whether it increases trap catch.

## Conclusions

- The aggregation pheromone of the western flower thrips (*Frankliniella occidentalis*) is produced on demand by adult males. The amount present on the body at one time is <220 pg male$^{-1}$, whereas adult males typically released about 50-600 pg male$^{-1}$ h$^{-1}$.

- We developed the solid-injection technique to try to detect and identify aggregation pheromone from whole bodies of male thrips, but the amount on the body was too small to be identified from small numbers of thrips.

- We successfully modified the technique to collect pheromone as it is produced, using an adsorbent polymer (Tenax). Enough pheromone is produced by 10 males for analysis and identification.

- The technique can be applied to thrips species that have not had their aggregation pheromone identified. It should allow pheromone identification with a quadrupole MS

if the males produce at least 55 pg male$^{-1}$ h$^{-1}$ or with a TOF MS if males produce at least 0.2 pg male$^{-1}$ h$^{-1}$. Synthetic pheromones can then be used to trap thrips.

## Acronyms

amu    Atomic mass unit (ion size)

au    Arbitrary units used to measure peak areas on a gas chromatograph.

ALS    Auto Liquid Sampler

GC    Gas Chromatograph

LA    (*R*)-Lavandulyl acetate, the minor component of the aggregation pheromone of the western flower thrips (*Frankliniella occidentalis*)

MS    Mass spectrometer

N2MB    Neryl (*S*)-2-methylbutanoate, the major component of the aggregation pheromone of the western flower thrips (*Frankliniella occidentalis*).

ng    Nanogram. A thousand millionth of a gram ($10^{-9}$ g).

pg    Picogram. A thousandth of a nanogram or a million millionth of a gram ($10^{-12}$ g).

t$_R$    Retention time

SIM    Selected Ion Monitoring.

SPME    Solid-Phase Microextraction. See SPME below.

TOF    Time of Flight analyser

WFT    Western flower thrips (*Frankliniella ocidentalis*)

## Glossary

**Carrier gas**. An inert gas that pushes the analysed sampled through the column towards the detector.

**Column**. A coiled silica-based capillary tube in the GC that separates compounds.

**Detector**. The device that measures how many ions of each molecule have reached it and at what rate.

**Fragmentation pattern**. The way a molecule breaks up into smaller charged fragments (ions). This gives a characteristic 'fingerprint' for a compound, referred to as a mass spectrum.

**Full scan mode**. Mode whereby all the ions formed in the analysis are detected. This includes ions that could potentially be noise or irrelevant compounds.

**Gas Chromatograph (GC)**. A device that separates the components of a mixture using a stationary and mobile phase. The stationary phase is contained in the column and is a liquid or solid chemical that can selectively attract components in the sample mixture. The mobile phase is a carrier gas.

**Headspace volatiles**. Compounds that are present in the gaseous phase in a certain volume, such as pheromones.

**Mass Spectrometer (MS)**. An instrument (often used as a detector in chromatography) that detects ions and their masses.

**Mass spectrum**. See fragmentation pattern.

**Quadrupole mass spectrometer**. A type of mass analyser in mass spectrometry. The molecules are electrically charged and subsequently fragmented into smaller charged fragments. The quadrupole separates the ions based on their mass to charge (m/z) ratio and records the abundance of each type of ion. The manner of separation in the quadrupole is to (repeatedly) filter ions of a certain mass (each cycle is called a scan). In this process, ions that are not being filtered in one cycle (scan) are lost, reducing the sensitivity of a quadrupole compared with a TOF mass spectrometer (see below).

**Reduced scan mode**. Mode where a smaller range of masses are selected to be detected by the mass analyser. A typical full scan mass range is from 40-600 amu, whereby in our reduced scan mode the range was from 40-160 amu.

**Retention time (t$_R$)**. The time required for a compound to travel through the column to the detector. These are used to help recognise compounds.

**SIM mode**. Selective ion monitoring mode. A mode of use of the MS that increases sensitivity by only detecting certain ions.

**Solid sample injector**. Mode that enables solid samples to be analysed

**SPME**. SPME uses a fibre coated with a liquid (polymer), a solid (sorbent), or a combination of both. The fibre coating extracts compounds from a sample onto the fibre coating. The SPME fibre is then inserted directly into the gas chromatograph for desorption and analysis.

**Tenax**. Porous polymers widely used as an adsorbent in air collection applications.

**TOF mass spectrometer**. A type of mass analyser in mass spectrometry. The Time of Flight also separates the ions based on their mass to charge (m/z) ratio and records the abundance of each type of ion. The manner of separation is measuring the time each ion

travels a certain distance (flight tube). During each cycle **all** ions are detected, hence increasing the sensitivity.

## Knowledge and Technology Transfer

10-11 December 2019. William Kirk. BBSRC Horticulture and Potatoes Initiative (HAPI) Final Dissemination Event, Leeds, UK. *Development of the solid-injection technique to identify thrips pheromones for monitoring and mass trapping in UK horticulture.*

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