APPENDICES FOR PROJECT PH0404

APPENDIX I

***Steering and Delivery Group*:**
A management structure for the project was agreed with the Defra project manager, consisting of a project delivery group with representatives of all three collaborating organisations that met as required to plan and monitor delivery of the work, and a project Steering group (convened by the Defra project manager) consisting of representatives of the major stakeholders (PHD, PHSI, CGA, Plant Health Consultants, Industry consultants) and the contractor's and Defra project managers.

The Project Delivery Group met on ten occasions (5th October 2005, 5th December 2005, 6th March 2006, 3rd July 2006, 15th August 2006, 28th February 2007, 30 th March 2007, 9 th November 2007, 9 th April 2007, 8th August 2008) to discuss work progress and future direction of the project.

Meetings of the Project Steering group were convened by the Defra project manager on two occasions (13th January 2006, 26 June 2008).

***Project delivery plan*:**
Research conducted under this project by the various partners was organised to generate the output required on a timescale that enabled the progressive and independent development of each module of the final control strategies. The order of delivery of each component (agreed by the project delivery Group) is summarised in Figure 1. This report has been structured to reflect this plan but each section refers to the specific milestones addressed by the work reported.

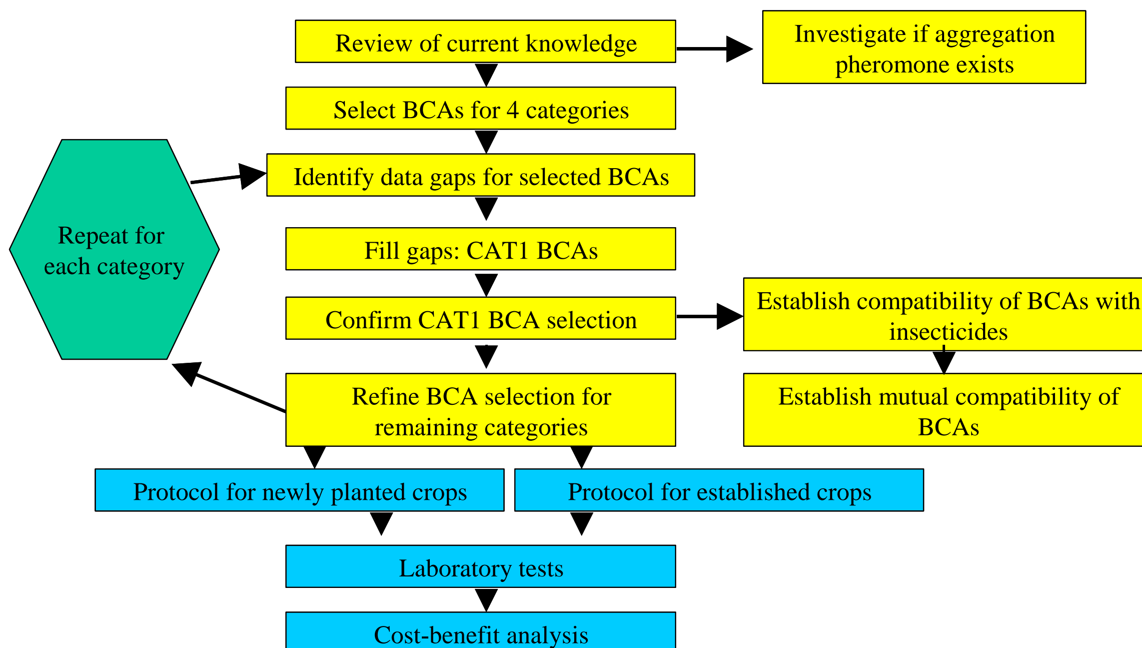


Figure 1. Delivery of the sub-components of Project

***Project delivery team*:**
The project delivery team was as follows:

Central Science Laboratory-
Keith Walters (Project Leader); Ray Cannon; Alistair Murray; Patrick Cox; Andrew Cuthbertson; Alan MacLeod; Lisa Blackburn; Helen Moran

Stockbridge Technology Centre-
Pat Croft; Nicola Nattriss

Rob Jacobson Consultancy Limited-
Rob Jacobson

Keele University (subcontractors to project)-
William Kirk; Gordon Hamilton

APPENDIX II

Existence of an aggregation pheromone in *T. palmi* (Milestone 7):

The component of the project investigating the possible existence of an aggregation pheromone produced by *T. palmi* was undertaken following a request from the Defra project officer.

It has been shown that adult male western flower thrips (*Frankliniella occidentalis*) produce a volatile aggregation pheromone that is attractive to both males and females (Kirk & Hamilton, 2004) and more recently two major male-specific volatiles produced by *F. occidentalis* have been identified as (*R*)-lavandulyl acetate and neryl (*S*)-2-methylbutanoate (Hamilton *et al*., 2005). Neryl (*S*)-2-methylbutanoate increased trap catches of males and females in field trials in commercial polytunnels (Gómez *et al*., 2006), and a commercial pheromone trap (Thripline$_{ams}$) for western flower thrips that uses this compound is now available. This species is rather similar in its biology to *T. palmi*. Both species have sternal glands (*areae porosae*) on the underside of the abdomen of adult males, which are assumed to be the site of release of aggregation pheromones (Hamilton *et al*., 2005), so it is possible that an aggregation pheromone also occurs in *T. palmi*. This component of the project aimed to investigate whether a male-produced pheromone occurs in *T. palmi* and, if so, to make a tentative chemical identification.

Experimental details:

Collection of Volatiles. Adult males, females or larvae were collected using a small aspirator, anaesthetised with carbon dioxide and transferred into a clean glass container (1.9 ml) which was then sealed with Teflon tape. The trips were illuminated from above with a bright light (60W tungsten filament lamp) to induce patrolling behaviour in males (Kirk & Hamilton, 2004). Patrolling occurs when the males swarm and this behaviour may be necessary for pheromone production. Head space volatiles were collected using the method of Kirk and Hamilton, (2004). Glass fibre disks were also used to collect volatiles, (Webster *et al*., 2000).

Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS analyses were carried out on HP 5890 II+ gas chromatograph coupled to HP 5972A mass spectrometer operated in electron impact (EI) (70 eV, 180°C), in full scan or in single ion monitoring (SIM) mode (scanning for ions at m/z 55, 69, 80, 234, 236 and 238).

SPME-collected, headspace volatile samples and synthetic standards were analysed with a DB5MS (30 m x 0.25 mm i.d., 0.25 µm film thickness) analytical column. Samples were introduced into the GC via a Merlin Microseal septum-less heated injector (170°C) fitted with a SPME glass injection sleeve (0.75 mm i.d.). SPME samples were allowed to desorb for 8 minutes before the glass fibre assembly was withdrawn. The GC was temperature programmed with an initial 2 min at 40°C, then an increase of 10°C min^{-1} to a final isothermal period at 250°C (10 min).

After completion of the entrainment experiments 70% EtOH was added to each of the vials of thrips and actual numbers of males, females and larvae present were checked later under a dissecting microscope. actual numbers of males, females and larvae present were checked under a dissecting microscope.

FIRST COLLECTIONS:

Entrainment on glass fibre disk: Male (n = 87♂, 3♀) and female (n = 49♀, 2LII) *T. palmi* were placed inside two 1.9 ml cleaned glass vials each containing a cleaned glass fibre filter disk. The glass vial was closed by placing a piece of Teflon tape over the open end of the vial. Thrips were allowed to walk over the cleaned glass fibre disk overnight (> 12 hours) after which time the vial and glass filter disk was extracted with pentane (1.9 ml), which was reduced in volume under air to 1 µl and analysed by GC/MS.

Controls consisting of pentane (1.9 ml) added to empty clean glass vials and then reduced in volume under air to 1 µl were also prepared for analysis by GC/MS.

Extract of whole *T. palmi* (10 ♀) in pentane: Two groups of ten ♀ *T. palmi* were placed into two 2 ml cleaned glass vials. Two ml of pentane were added to each vial. The extract from each vial was reduced to 1 µl under air and analysed by GC/MS.

Extract of whole *T. palmi* second instar larvae (LII) in pentane: Two groups of ten *T. palmi* LII were placed into two 2 ml cleaned glass vials. Two ml of pentane were added to each vial. The extract from each vial was reduced to 1 µl under air and analysed by GC/MS.

SECOND COLLECTIONS:

SPME and glass fibre filter entrainments: Combined Solid Phase Micro-Extraction (SPME) and glass fibre filter entrainments of adult (♂ & ♀) and larvae (LII) *T. palmi* were carried out. The glass filter fibre element of the experiment was carried out as described above and the SPME entrainment followed the method of Hamilton *et al.*, (2005). Adult males (n = 80♂, 3♀), females (n = 27♀, 1♂) and LII (n = 80LII, 2♂) were placed in separate groups in three 2 ml cleaned glass vials each containing a cleaned glass filter fibre. The open ends of the vials were sealed with a piece of Teflon tape. SPME fibres were pre-cleaned and kept in a small box that was open to the surrounding atmosphere. An SPME needle was pushed through each of the Teflon seals and then the fibre exposed to the headspace in the vial. A tungsten bulb was positioned over the vials and height set so that the air temperature surrounding the vials was 27°C overnight. The entrainment experiment was left to run throughout the night before analysis by GC/MS.

After completion of the experiment 70% EtOH was added to each of the vials and the actual numbers of males, females and larvae present were checked under a dissecting microscope.

THIRD COLLECTIONS:

SPME entrainment of male and female *T. palmi*: In order to obtain a better, library searchable mass spectrum with evidence of molecular weight for the male specific compound an attempt was made to collect and analyse the volatiles produced by larger numbers of male adults. To reduce the amount of contaminating volatiles that are adsorbed by the SPME fibres before use they were pre-cleaned and immediately sealed in heat cleaned borosilicate glass tubes. The glass tubes were opened and the SPME fibres exposed to *T. palmi* volatiles in vials that had been prepared as described above.

The samples were analysed by both normal Scan (SCAN) and single ion monitoring (SIM) mode on the mass spectrometer to attempt to obtain a molecular weight. The latter method is more sensitive when trying to measure the amounts of specific ions and allows a search for specific ions.

Comparison of *T. palmi* male specific compound with spectra of known monoterpene pentanoates: The spectrum obtained from the *T. palmi* male compound was compared with a mass spectral library of approximately 60 monoterpene pentanoates. Those standards in the library that had similar spectra and/or similar Kovat's indices to the compound from the adult male *T. palmi* were analysed again using the current mass spectrometer operating parameters. In addition a published mass spectrum of lavandulyl senecioate was obtained, a monoterpene pentenoate for comparison.

FOURTH COLLECTIONS:

Identification of molecular ion of *T. palmi* male compound by SIM: In order to identify the molecular ion which was predicted to be 236 based on analysis of the incomplete mass spectrum obtained so far a SIM experiment was carried out to determine the amount of m/z 236 ion relative to 238 and 234. The mass spectrometer was programmed to look for specific ions before the analysis of the thrips sample commenced and to further enhance the sensitivity it was programmed to search for a minimal number of ions. Therefore the instrument was programmed to look for some key ions at 55, 69, 80 and 83, all of which are present in the *T. palmi* male compound and if all were found to be present together in the same peak at the same R_t would confirm the presence of the *T. palmi* male compound. To determine the molecular weight the instrument was programmed to search for the ions 234, 236 and 238 (236 would be the molecular weight of a monoterpene pentenoate, 238 the molecular weight of a monoterpene pentanoate).

Results:

FIRST COLLECTIONS:

Entrainment on glass fibre disk: GC/MS analysis of both the extracts of glass filter disks are shown in Figure 1 (male top, female inverted below). The majority of the peaks are represented in both the male and female extracts. The arrow in the

chromatogram of the male extract indicates the position of an unusual terpene based compound found in the male extract but not in the female extract (R_t = 17.513 min). In the chromatogram of the female extract a compound with a similar Retention time (R_t = 17.487 min) appears but the mass spectrum is dissimilar and it is likely that this compound is represented by the slight front shoulder seen on the peak in the male extract. It was confirmed that 87 ♂ and 3 ♀ *T. palmi* were present in the "male" vial and that 49 ♀ and 2 larvae were present in the "female" vial.

GC/MS analyses of clean glass vials without *T. palmi* present showed that the compound of interest seen in the male *T. palmi* filter fibre extract was not present in the controls. Nor was it present in the extract of whole males.

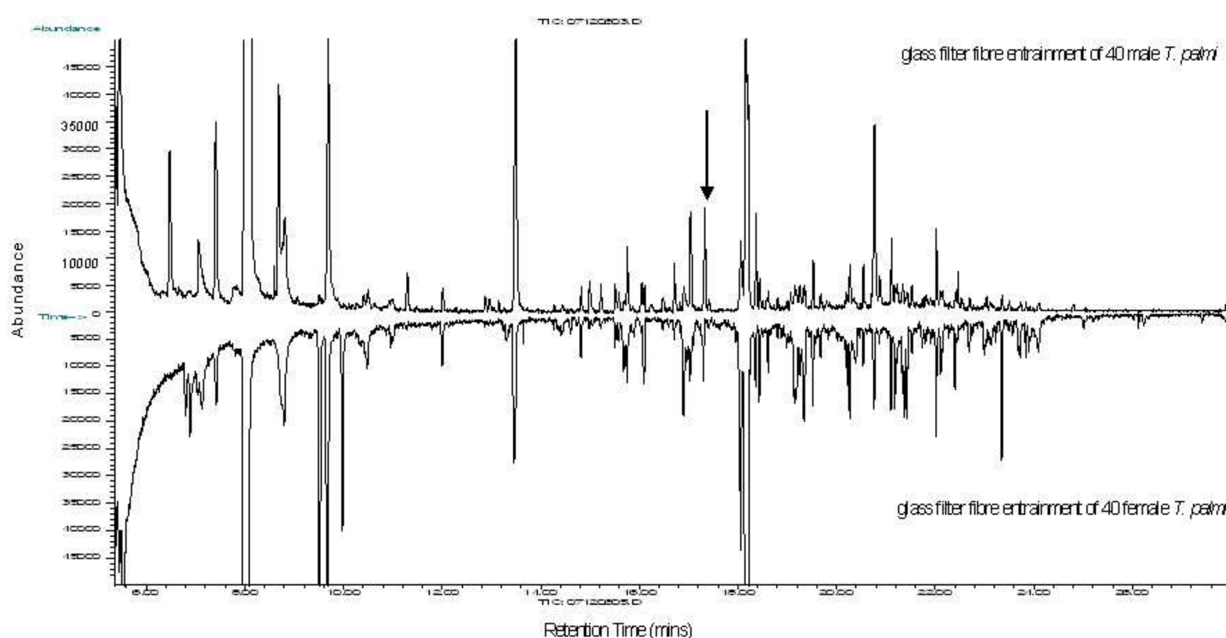


Figure 1. Comparison of total ion current (TIC) chromatograms of cleaned glass fibre filter disks exposed to 40 male (upper trace) and 40 female *T. palmi* (inverted lower trace). The arrow marks the peak of interest.

<u>Extract of whole *T. palmi* (10 ♀) in pentane</u>**:** The unusual peak found in the male extract from glass fibre disks was not found in either of these 2 female extracts, nor were any additional unusual peaks found in these extracts.

<u>Extract of whole *T. palmi* LII in pentane</u>**:** The unusual peak found in the male extract from glass fibre disks was not found in either of these 2 LII extracts. In addition no additional unusual peaks were found in these extracts, for example evidence for alarm pheromone in these extracts i.e. compounds similar to decyl acetate and dodecyl acetate might have been expected.

SECOND COLLECTIONS:

<u>SPME and glass fibre filter entrainments</u>**:** Thirty ♂ and 3 ♀ were present in the "male" vial, 27 ♀ and 1 ♂ were present in the "female" vial, and 30 larvae and 2 ♂ were present in the "larvae" vial.

Larvae SPME entrainments. (*R*)-Lavandulyl acetate was putatively identified at R_t = 14.601 min (library match 90) (peak area (pa) = 366,812 (units are arbitrary)) and an unidentified terpene eluted slightly earlier at R_t = 14.462 min (pa = 278,020).

Adult female SPME entrainments. (*R*)-Lavandulyl acetate was putatively identified at R_t = 14.607 min (library match 90) (peak area = 545,058) and an unidentified terpene eluted slightly earlier at R_t = 14.464 min (pa = 291,649).

Adult male SPME entrainments. (*R*)-Lavandulyl acetate was putatively identified at R_t = 14.644 min (library match 91) (peak area = 1,247,642) and an unidentified terpene eluted slightly earlier at R_t = 14.498 min (pa = 437,558).

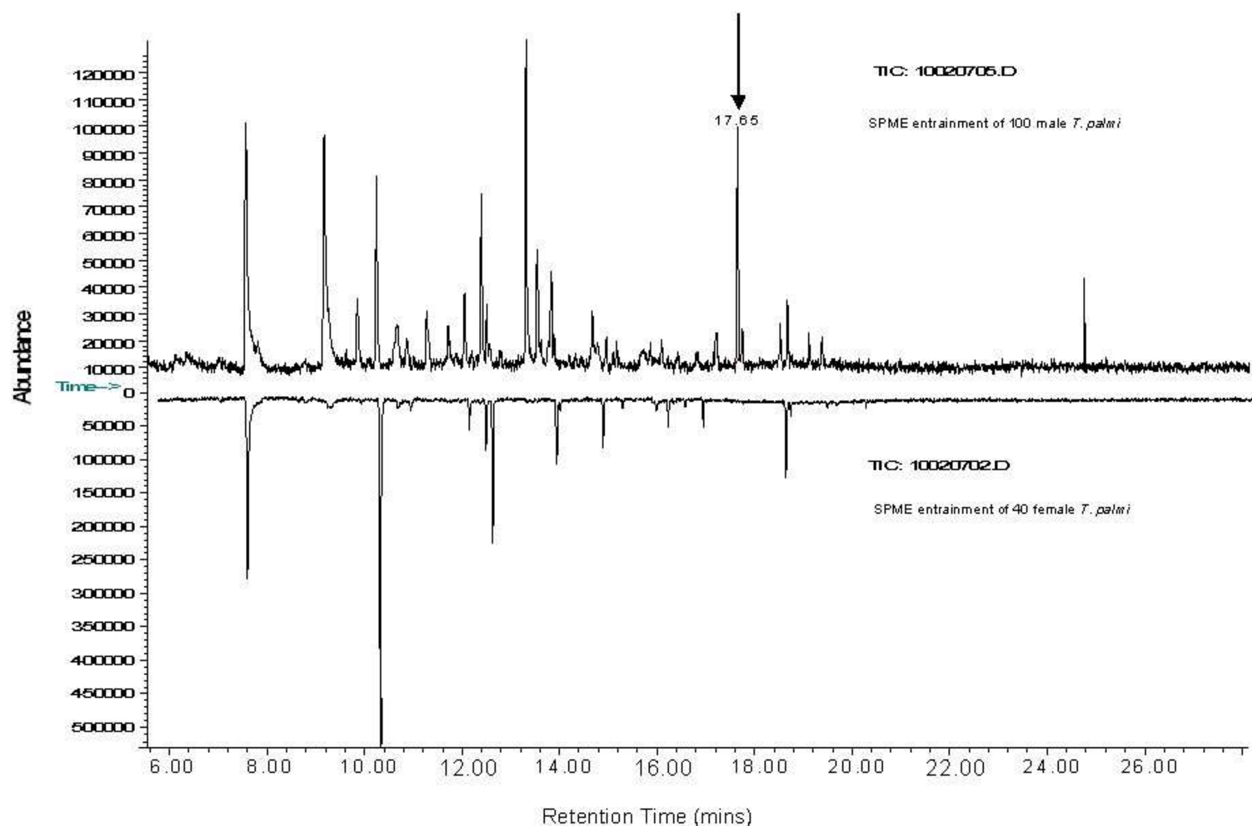


Figure 2. Comparison of total ion current (TIC) chromatograms of cleaned glass fibre filter disks exposed to 100 male (upper trace) and 40 female *T. palmi* (inverted lower trace). The arrow marks the peak of interest.

The male-specific peak which was identified previously in the glass fibre filter extract was also found in the SPME entrainment at R_t = 17.489 min. The abundance from only 26 males was very low (pa = 210,700) and a molecular weight was not obtained.

Adult female glass fibre filter extract: there was no (*R*)-lavandulyl acetate present in the extract.

Adult male glass fibre filter extract: no male specific compound present and no (*R*)-lavandulyl acetate present in the extract.

Larvae glass fibre filter extract: An analysis of this extract was not obtained because of a mass spectrometer fault, which occurred at the start of the analysis.

THIRD COLLECTIONS:

SPME entrainment of male and female *T. palmi*: Although the SIM mode analyses were unsuccessful the scan mode analyses were successful. Total ion current chromatograms (TIC) from the male and female entrainments are compared in Figure 2. The male chromatogram clearly shows the male-specific compound highlighted by an arrow at R_t = 17.645 min (peak area = 1,477,349). The mass spectrum of the male specific compound is given in Figure 3, and shows several significant features. The main ions at m/z 69, 93, 121, 136 and 154 are consistent with the identification of the compound as a monoterpene derivative and the ions at 83 and 55 suggest a C5 ester. No molecular ion is apparent, however the ions present at 83, and 55 are analogous to the ions in the WFT male sex pheromone at 85, and 57 and the difference of 2 mass units for each ion is indicative of the presence of a double bond in the acid moiety in the *T. palmi* compound compared to the WFT male aggregation pheromone. Taken together these data predict a monoterpene pentenoate with a molecular weight of 236.

Comparison of *T. palmi* male specific compound with spectra of known monoterpene pentanoates**:** The results of this investigation confirmed that there was no close match between the *T. palmi* male compound and any of the compounds in the library. The published mass spectrum of lavandulyl senecioate was dissimilar to the *T. palmi*

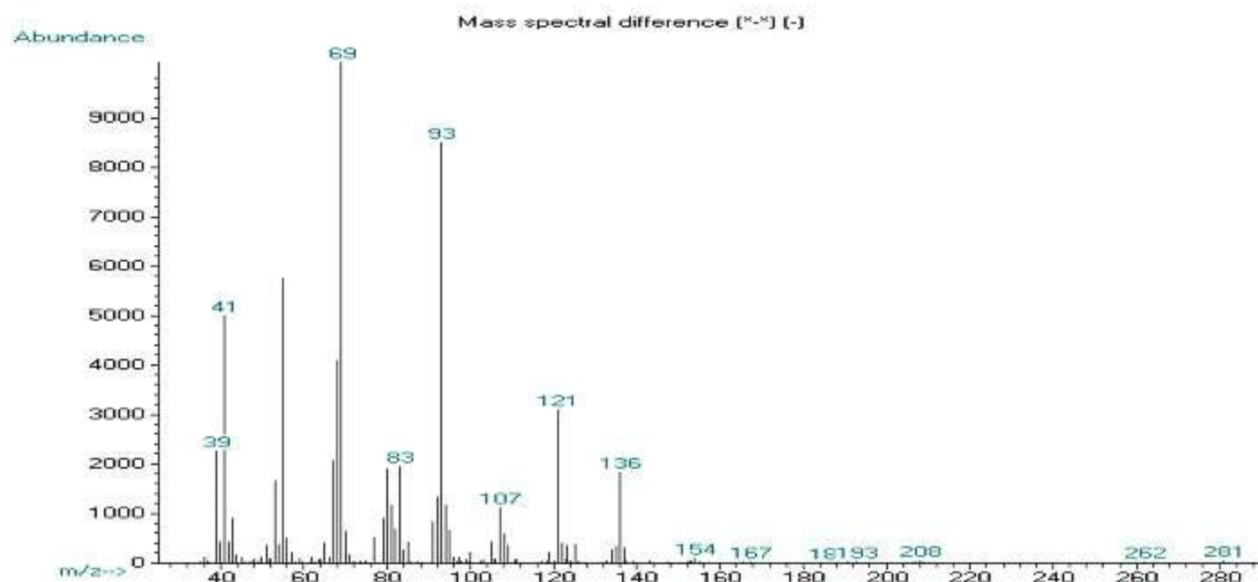
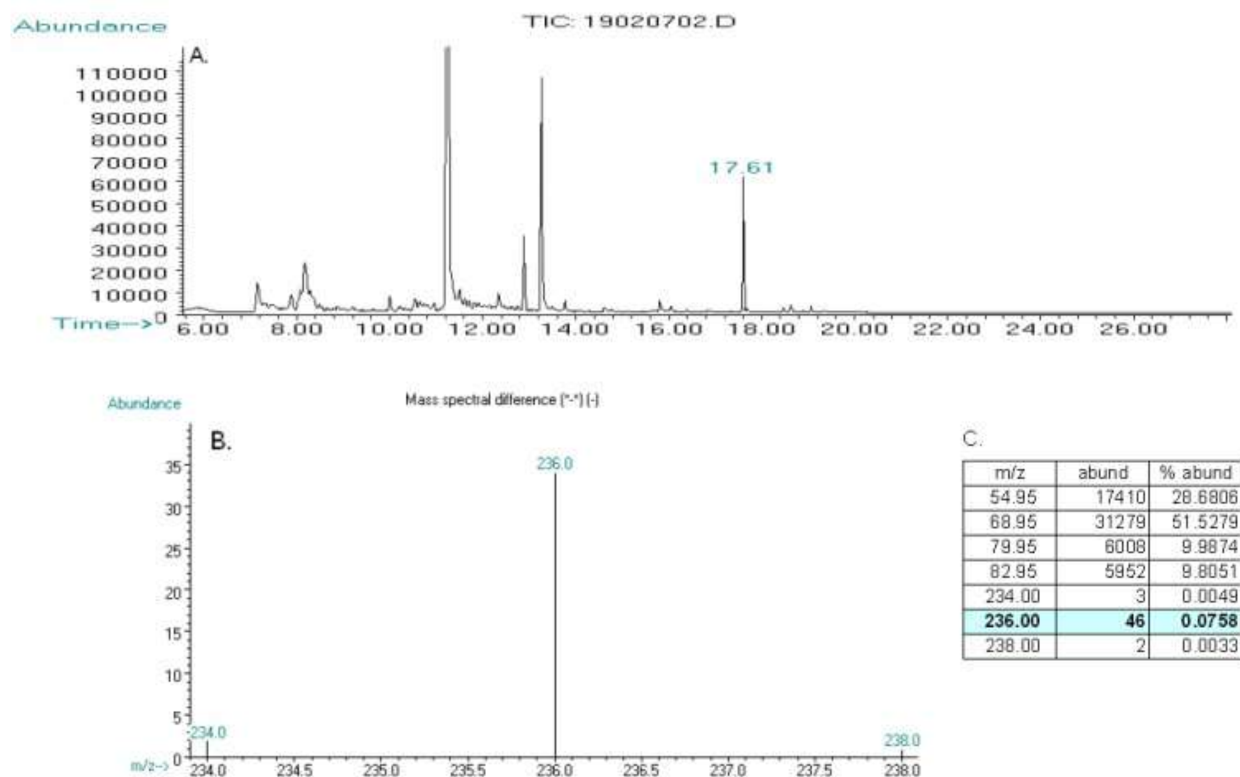


Figure 3. Mass spectrum of the male specific compound from *T. palmi* obtained by SPME entrainment of headspace volatiles from 100 males over 12 hours.

spectrum except that there were strong ions present at 83 and 55 and the molecular ion peak was present at m/z 236.

FOURTH COLLECTIONS:

Identification of molecular ion of *T. palmi* male compound by SIM: The results show that a peak was present at R_t = 17.605 min with significant ions at 55, 69, 80 and 83 indicating that this was the *T. palmi* male compound seen



m/z	abund	% abund
54.95	17410	28.6806
68.95	31279	51.5279
79.95	6008	9.9874
82.95	5952	9.8051
234.00	3	0.0049
236.00	**46**	**0.0758**
238.00	2	0.0033

Figure 4. A. Selected ion monitoring (SIM) chromatogram of SPME entrainment of 65 male *T. palmi.* B. Comparison of the relative amounts of ions 234 236 and 238 present in the peak at 17.61 min. C. Table of main ions, abundance and relative abundance.

in previous full SCAN analyses (Fig 11a). Detailed analysis of the ions at m/z 234, 236 and 238 showed that 236 was the predominant ion present (Fig 11b) and that the 236 ion was about 15 times more abundant than the 234 ion and 23 times more abundant than the 238 ion (Fig 11c). This provides strong evidence that the molecular weight of the male produced compound is 236.

Conclusions:

The results of the experiments carried out showed clearly that male *T. palmi* produce a volatile compound that is not produced by females or larvae II. All 3 entrainment

experiments including the glass fibre filter disk and SPME entrainments showed the presence of the compound in male extracts but not in female or larval extracts.

The compound is a monoterpene ester with a likely molecular weight of 236. The monoterpene part of this compound remains to be determined however the ester moiety is determined to be an unsaturated C5 (pentenoate) but again the precise structure remains to be elucidated.

These findings are consistent with our previous work on *Frankliniella occidentalis* where the male aggregation pheromone was shown to be neryl (*S*)-2-methylbutanoate, a monoterpene ester of nerol and 2-methyl butanoic acid (mw = 238).

Future work would aim to confirm the molecular weight of the *T. palmi* male compound by chemical ionisation mass spectrometry and to determine the precise structure of the compound. Given the very small amounts of material that are produced by the males we would do this by synthesising a library of candidate monoterpene pentenoates using commercially available monoterpene alcohols and pentenoic acids. We would then search the library for matches between the library compounds and the *T. palmi* compounds. This approach proved successful for identification of the aggregation pheromone of the western flower thrips *Frankliniella occidentalis*.

APPENDIX III

Examples of data obtained from the published literature and some unpublished sources to support IPM protocol development

A wide range of data and information from the published literature and unpublished sources have been utilised in support of the development of the IPM protocols. The extent of these data and the wide ranging elements of the protocols that are based on them preclude a comprehensive description being appropriate in the limited space available in this report. Instead the following illustrates the kind of the data/information obtained from these sources.

Modes of action of Calypso and Spinosad:
The following information was obtained from the Conserve Technical Brochure (Dow AgroSciences, 2002).

Spinosad acts in a unique way by modulating the acetylcholine receptors. In insects, the mode of action of spinosad is associated with excitation of the insect nervous system. Spinosad uniquely alters the function of nicotinic and GABA-gated ion channels in a manner consistent with the observed neuronal excitation. However, Spinosad does not interact with known binding sites for other nicotinic or GABAergic insecticides such as neonicotinoids, fiproles, avermectins and cyclodienes.

Thiacloprid is a neonicotinoid insecticide. It disrupts the nervous system by acting at nicotinic acetocholine receptors.

Thus Calypso has a different mechanism to Spinosad

The length of the residual effects of Spinosad:
The following information is summarised from Miles & Dutton (2001) and the Conserve Technical Brochure (Dow AgroSciences, 2002):

- BCAs for which there are no restrictions:
    - *Macrolophus caliginosus*, *Hyposaspis miles*
- BCAs which may be safely released on the day of spray application after the spray deposits are dry:
    - *Amblyseius californicus*, *A. cucumeris*, *Phytoseiulus persimilis*, *Aphidoletes aphidimyza*, *Chrysoperla carnea*, *Hippodamia convergens*, *Orius insidiosus*, *O. laevigatus*,
- BCAs which should not be released for one week after application of Conserve:
    - *Aphidius colemani*, *Diglyphus isaea*, *Encarsia formosa*, *Trichogramma brassicae*

Thus Spinosad should integrate well with the BCAs used against *T. palmi* but may have some secondary effects on agents used against other pests infesting cucumber crops (eg whitefly and aphid control with parasitoids). Hence prior to application within the *T. palmi* IPM system (described in the attached protocols), consideration

should be given to its potential effects on other non-chemical control systems that may be in current use for the control of other pests. If used, alternative approaches may need to be employed for these other pests.

Potential for using *Lecanicillium muscarium* as control agent for *T. palmi*:
Due to the capacity for *T. palmi* populations to increase in size rapidly, situations can arise under glasshouse conditions whereby biological agents such as predatory mites are unable to exert sufficient control. Under such circumstances measures resulting in a slowing of the rate of increase of the pest population (often by reducing the number of thrips by a single management action) can enable the predatory mites to become effective in controlling the population development without the need for increased release rates. A biological product that could be used in a similar fashion to a conventional insecticide and which had the potential to slow down pest population development was therefore sought.

The literature search conducted under this project identified the bio-insecticide Mycotal (*Lecanicillium muscarium*) as a suitable candidate (Cox *et al*., 2006). The published results of efficacy tests indicated that *Lecanicillium muscarium* provided a 37% reduction in numbers of *T. palmi* larvae (and up to 65% mortality of adults) under controlled conditions. This has yet to be confirmed in cropping situations but these levels of control are not dissimilar to those obtained for initial screening tests of fungal insecticides that have subsequently been developed into effective glasshouse control for other quarantine pests (Cuthbertson & Walters, 2005; Cuthbertson, *et al*., 2005).

Table 1: The effect of *Lecanicillium muscarium* on *T. palmi* survival in laboratory tests.

Host Plant	Temp	*T. palmi* larval mortality (control mortality)	*T. palmi* adult mortality (control mortality)	Reference
Chrysanthemum (Excised leaf)	20°C	45.0 ± 4.1 (2.0 ± 1.7)	-	Smith *et al.* (2005)
Cucumber (Excised leaf)	20°C	28.3 ± 5.1 (7.5 ± 2.5)	-	Cuthbertson *et al.* (2005)
Cucumber (Excised leaf)	25°C	35.0 ± 13.3 (11.6 ± 5.1)	-	Cuthbertson *et al.* (2005)
Chrysanthemum (Excised leaf)	20°C	35.9±15.7 (6.2±2.8)	-	Cuthbertson *et al.* (2005)
Chrysanthemum (Excised leaf)	25°C	43.3 ± 2.2 (15.5 ± 1.6)	65.6 ± 7.7 (11.1 ± 2.2)	North *et al.* (2006)

APPENDIX IV

Draft protocols for modular-based control strategies on cucumber (Milestone 13)

1. BACKGROUND

- *T. palmi* is a serious potential pest of a wide range of protected edible/ornamental crops in the UK, but containment/eradication measures based solely on intensive applications of agrochemicals are generally considered to be both undesirable and unsustainable.

- A previous outbreak in the UK has been successfully eradicated but the review of contingency planning for this non-native pest that followed highlighted the need for improved integrated management strategies. These protocols are an output from one of the consequential actions.

- The protocols describe IPM strategies for *Thrips palmi* infesting both newly planted crops and established crops. Within each of them are options for utilising a combination of biological and conventional chemical agents, or for organic crops biological agents alone. Their development has been based on information drawn from the scientific literature, supplemented with new research data and collated under Plant Health Division project number PH0404.

- The research resulting in this protocol was conducted in conjunction with Plant Health consultants and in discussion with representatives of PHD of Defra and the PHSI, and provides the basis for a flexible modular approach to the use of biological and chemical agents for the control of a major quarantine glasshouse pest, a first of its kind for use by the PHSI.

- Although brief explanatory text is provided with most actions, more detailed background information is available in the final report of the above project and in its emerging publications.

2. EQUIPMENT & PRODUCTS

- Conserve® SC (Active Ingredient**:** *Spinosad*)
- Natural pyrethrins (Pyrethrum 5EC)
- Thiacloprid (Calypso)

- *Amblyseius cucumeris* controlled release sachets
- *Amblyseius montdorensis*
- *Amblyseius swirskii*
- *Atheta coriaria*
- *Hypoaspis* spp
- *Lecanicillium muscarium*
- Blue sticky traps

3. METHODS

3.1. For newly planted crop:

- ACTION 1: Application of a short persistence chemical pesticide at standard commercial rates 2-3 days after planting out in the glasshouse
  - Objective - to reduce numbers of adult invaders and slow down egg laying
  - Products - Either spinosad (Conserve) or natural pyrethrins (Pyrethrum 5EC)
    - Important considerations:
      - Efficacy of any conventional pesticide product used should preferably be confirmed by bioassay prior to application.
      - Natural pyrethrins - it is intended that that these products will knock down adult *T. palmi*. Use as pesticide resistance may be present in the population
      - Both products depend on contact action and therefore good spray coverage is required. However, this should not be difficult on young cucumber plants.
      - Neither product should have persistent effects on the natural enemies used in the subsequent biocontrol programme (see Dow, Spinosad Technical Bulletin, 2001; Final report of research project PH0404).

- ACTION 2: Release *Amblyseius cucumeris* two days after the pesticide application. During the summer months a dual release with *A. swirskii* may increase impact and should be considered.
  - Objective – to provide protection against thrips nymphs hatching from eggs on leaves.
  - Products – *Amblyseius cucumeris* controlled release sachets are available from three biocontrol suppliers in the UK
    - Important considerations:
      - It is important that protection is provided to every plant. After planting, leaves may not touch for up to two weeks, so it is vital that a sachet is placed on each plant.
      - The cultures in the sachets should remain active for at least 6 weeks. To provide an overlap, further routine releases should be made at 4-5 week intervals.
      - In addition, samples of culture sachets should be taken at weekly intervals and examined to ensure that they are still active.

- ACTION 3: On a regular basis record numbers of *T. palmi* on blue sticky traps deployed throughout the period over which the integrated control strategy is being utilised and in accordance with the advice given in the PHSI handbook.
  - Objective – To monitor the population trends and provide guidance on further actions.
  - Products – Many suppliers
    - Important considerations:
      - Trap density should meet the advice given in the PHSI handbook
      - Traps should be checked weekly and sent to CSL York for identification/counting of *T. palmi*

            - By plotting the catch against time on a weekly basis it will be possible to determine whether the population is increasing, decreasing or static.

- ACTION 4: Release *Atheta coriaria* and / or *Hypoaspis miles*. to the floor beneath the crop 2-3 weeks after planting
    - Objective – To control *T. palmi* which drop to the floor to pupate
    - Products –
        - Important considerations:
            - It will be at least two weeks before the first generation of thrips produce pupae within the crop. Predators should not be released too soon or they will starve.
            - Even if the *T. palmi* population is small, after two weeks there should be alternative prey (e.g. shore fly larvae) to help these predators to survive.
            - Releases should be at commercially recommended rates.
            - An alternative approach would be the use of a sticky barrier on the floor (e.g. polybisobutylene). However, this is not compatible with biocontrol agents that are active on the floor or consistent with the results of current studies. A second alternative would involve the use of the entomopathogenic nematode *Steinernema carpocapsae*. However about 98% of the cucumber crops grown in the UK are currently produced using hydroponic systems, unsuitable for the use of nematodes. *S. carpocapsae* may, however, offer a useful alternative for the control of *T. palmi* on organic soil grown crops.

- ACTION 5: Between May and August, release c 10 *Amblyseius montdorensis* per plant on the uppermost leaves at the time when the plants reach the support wire (c 3 weeks after planting).
    - Objective – To provide additional protection in the tops of the plants during the summer when *T. palmi* are likely to be more prolific.
    - Products – Syngenta Bioline will have a "loose" *Amblyseius montdorensis* product and permit for release available in 2009 (Richard GreatRex pers. com., September 2008).
        - Important considerations:
            - *Amblyseius montdorensis* perform better at higher temperatures and are therefore suited to the tops of cucumber crops during the summer months.

Amblyseius montdorensis are scavengers and will survive on a wide range of invertebrate prey. Repeat releases should not be necessary (Steiner & Goodwin, 2002).

- ACTION 6: Remedial treatments during cropping: If population trends monitored using the blue sticky traps are not acceptable during the cropping period, the following actions may be taken.
    - Objective – To assist the biological control agents by slowing down pest population development.
    - Products - Mycotal (*Lecanicillium muscarium*) and / or thiacloprid (Calypso)
        - Important considerations:

        - *Lecanicillium muscarium* has been shown to provide a 33% reduction in numbers of *T. palmi* under controlled conditions (Cuthbertson *et al 2005*, although the lack of outbreaks of the pest has prevented the efficacy of the product being confirmed in cropping situations under UK conditions.
        - Thiachloprid is very harmful to some natural enemies when sprayed onto leaves. However, the side-effects can be much reduced when applied through the irrigation system and this would be the recommended course of action. As before, the lack of outbreaks of the pest has prevented the efficacy of the product against *T. palmi* being confirmed and application rates being defined. Both will require consideration in the event of the need to utilise this option.
        - Efficacy of any conventional pesticide product used should preferably be confirmed by bioassay prior to application.
        - Literature reports suggest that a third alternative for remedial treatments during the cropping period might involve the use of *Orius* spp. This action is not included in this protocol as cucumber crops do not produce pollen and *Orius* spp. requires pollen sources to thrive. In addition, the length of the life cycle indicates that the period required before strong population depression is achieved may be too long for the control of a quarantine pest in this situation.

- ACTION 7: Apply clean-up sprays at the standard commercial rate at the end (i.e. before crop destruction) of the crop.
    - Objectives – To reduce the risk of i) *T. palmi* being transmitted to other crops during crop disposal and ii) *T. palmi* carrying over to the following crop.
    - Products - spinosad (Conserve), natural pyrethrins (Pyrethrum 5EC) or thiacloprid (Calypso)
        - Important considerations:
            - Efficacy of any conventional pesticide product used should preferably be confirmed by bioassay prior to application.
            - Sprays to foliage should begin two weeks before crop removal taking note of harvest intervals
            - Repeat sprays at 4-5 day intervals depending on results of crop monitoring

3.2. For established crops:

- ACTION 1: Start to record numbers of *T. palmi* on blue sticky traps
    - Objective – To determine the initial size of the *T. palmi* population and thus provide a base line against which to measure the efficacy of subsequent actions.
    - Product details are provides in section 2 above.
    - Traps should be deployed throughout the period over which the integrated control strategy is being utilised and in accordance with the advice given in Standard Operating procedures for dealing with interceptions and outbreaks of *T. palmi* and in the PHSI handbook.

- ACTION 2: Pesticidal treatments
    - Objective - To reduce the *T. palmi* population to a level that may be controlled biologically
    - Products – Options are limited because there will already be biological control agents controlling other pests in the crop and they must not be harmed.
        - Efficacy of any conventional pesticide product used should preferably be confirmed by bioassay prior to application.
        - The first application should be Calypso (thiacloprid) applied through the irrigation system.
        - Determine the effect by monitoring numbers of *T. palmi* larvae on marked leaves and numbers of *T. palmi* adults on blue sticky traps.

- ACTION 3: Release *Amblyseius cucumeris* two days after the pesticide application
    - Objective – to provide continued protection against thrips nymphs on leaves.
    - Products – *Amblyseius cucumeris* controlled release sachets are available from three biocontrol suppliers in the UK
        - Important considerations:
            - Position the sachets in the middle of the crop canopy as exposure to direct sunlight at the top of the plant can dehydrate the cultures and reduce their effective life.
            - The cultures should remain active for at least 6 weeks. To provide an overlap, further routine releases should be made at 4-5 week intervals.
            - In addition, samples of culture sachets should be taken at weekly intervals and examined to ensure that they are still active (provide guidance in the form of a SOP).

- ACTION 4: If outbreak occurs between May and August, then release c 10 *Amblyseius montdorensis* per plant on the uppermost leaves
    - Objective – To provide additional protection in the tops of the plants.
    - Products – *Amblyseius montdorensis* (see sections 2 and 3.1, Action 5 for further details)
        - Important considerations:
            - *Amblyseius montdorensis* prefer higher temperatures and are therefore suited to the tops of cucumber crops during the summer months.

- ACTION 5: Continue to record numbers of *T. palmi* on blue sticky traps
    - Objective – To monitor the population trends and provide guidance on further actions.
    - Products – See section 2 above for suppliers, and section 3.1, Action 3 for further information.

- ACTION 6: Release *Atheta coriaria* and / or *Hypoaspis* spp. to the floor beneath the crop immediately
    - Objective – To control *T. palmi* which drop to the floor to pupate
    - Products – See section 2 above for suppliers and section 3.1, Action 4 for further details.

    - A sticky barrier on the floor (e.g. polybisobutylene) is an alternative, but may not be compatible with some biocontrol agents that spend part or all of their time on the floor.

- ACTION 7: Remedial treatments during cropping
    - Objective – To assist the biological control agents by slowing down pest population development.
    - Products - Mycotal (*Lecanicillium muscarium*)
        - Important considerations:
            - *Lecanicillium muscarium* has been shown to provide a 33% reduction in numbers of *T. palmi* larvae under controlled conditions. This has yet to be confirmed in cropping situations.
            - An alternative may involve the use of the insecticide thiachloprid. It has, however, already been recommended as part of this protocol (Section 3.2, Action 2) and multiple applications of the same product (or products with the same mode of action may not be desirable.

- ACTION 8: Clean-up sprays at the end of the crop.
    - Objectives: To reduce the risk of i) spreading *T. palmi* to other crops during crop disposal and ii) carrying *T. palmi* over to the following crop.
    - Products - Spinosad (Conserve), natural pyrethrins (Pyrethrum 5EC) or thiacloprid (Calypso)
        - Important considerations:
            - Efficacy of any conventional pesticide product used should preferably be confirmed by bioassay prior to application.
            - Sprays to foliage should begin two weeks before crop removal taking note of harvest intervals
            - Repeat sprays at 4-5 day intervals depending on results of crop monitoring

APPENDIX V

COST-BENEFIT ANALYSIS OF THE MODULAR-BASED CONTROL STRATEGIES ON CUCUMBER

The financial cost of actions for the control of *Thrips palmi* outbreaks in UK cucumber crops

Introduction

The contingency plan describes seven proposed actions for the control of *Thrips palmi* outbreaks in a newly planted UK cucumber crop and in an established cucumber crop.

Estimates of the costs of each action in each programme are made below and are then put into a commercial context by comparing to the gross margin for a typical cucumber crop grown in 2008.

Crop growing conditions

Cucumber plants are usually propagated by specialist plants raisers in November / December and transferred to production glasshouses in December / January. Fruit picking begins in late January / February and is then continuous for the life of the crop.

Most growers replace crops twice during the year. This enables them to supply good quality produce throughout an extended season. Each break in cropping can be restricted to less than three weeks and by staggering the timing of replants most growers have productive crops in their glasshouses from January to October.

Crops are grown in high quality Venlo-style glasshouses with computer controlled environments enriched with carbon dioxide to improve growth and yield. Day time temperature is maintained at a minimum of 21ºC via hot water pipe systems and the atmosphere is automatically ventilated at 23–24ºC. Nonetheless, temperatures may exceed 30ºC for short periods during hot weather. There may be a "pre-night" drop in temperature to 14-16 ºC for 4–5 hours to maintain plant vigour and the remainder of the night is usually set at 19ºC

Plants are grown in artificial, inert substrates, the most common being rockwool. These substrates are sterile and free from inherent disease problems. Nutrients are applied as liquid feeds through computer controlled irrigation systems.

Cucumber plants are usually grown by the cordon technique. The main stem is trained up a vertical string that is tied to a horizontal support wire positioned about 2m above the ground. Side shoots are removed from the main stem until it reaches the support wire. Two or three strong lateral shoots are then selected and the main growing point is removed. The side shoots are allowed to cascade downwards to a length of about 1m and their growing points are removed to encourage "sub-lateral" shoot development. Crop trimming is performed every 7 – 14 days when any additional unwanted side shoots are removed.

In estimating the financial costs of each action, the following assumptions have been made.
A series of three crops of cucumbers are grown each year.
The crops are of equal 14-15 week duration (nb: in practice this will vary to maintain continuity of production across the site).
The planting density is 1.5, 2.0 and 1.5 plants / m^2 in the first, second and third crops respectively.
Plants are grown in rockwool slabs and heating is provided by a conventional gas-fired boiler.

Costs for individual actions in the *T. palmi* contingency plan

When applying chemical sprays, it is essential to achieve good coverage of the crop foliage for optimum pest control. The volume of spray applied will therefore increase as the crop grows with approximately 3,000 litres per hectare being applied to mature crops. For the purpose of these calculations, it is assumed that labour to spray one hectare of mature crop costs £160 (including overheads). Another labour intensive activity is the placement of Amblyseius cucumeris sachets. For the purpose of these calculations, it is assumed that labour to attach one sachet to each plant costs £180 per hectare (including overheads).

Table 1 shows estimated costs for the Actions applied to a planted crop. Note that Action 7 are "clean-up" sprays applied at the end of the crop. Details showing how costs have been calculated are provided in a separate section (see 'Calculation of costs for chemical products').

Table 1: Cost estimates for contingency actions in a newly planted cucumber crop

Action	Product	Cost per 0.1ha application (£)	No. applications	Sum £
1	Conserve (low volume) (labour)	7 16	1	7 16
	Pyrethrum 5 EC (low volume) (labour)	16 16	1	16 16
2	Amblyseius cucumeris (labour)	132 - 176 18	3	396-528 54
3	Blue sticky traps	1.08	20	22
4	Atheta coriaria	375	1	375
	Hypoaspis miles (curative rate)	255	1	255
5	Amblyseius montdorensis	Not yet commercially available	-	-
6	Mycotal (labour)	5 16	1	5 16
	Calypso	6	1	6
7	Conserve (high volume) (labour)	35 16	1	35 16
	Pyrethrum 5 EC (high volume) (labour)	82 16	1	82 16
	Calypso (high volume) (labour)	12 16	1	12 16

The total cost of implementing one option from each of the Actions 1-6 and two options from Action 7 in Table 1 is estimated to be between £905 and £1,366 / 0.1ha per crop. There are three important considerations:

The most expensive Action (i.e. placement of A. cucumeris sachets) depends on the plant density in the crop.

The choice of chemical pesticides depends on previous use as well as cost. For example, Calypso is not chosen as a clean-up treatment if it has already been used mid-season.

Allowance has been made for the application of two different clean-up treatments in each crop.

The crops would be monitored closely after each Action and some repeat treatments may be necessary. Hence costs could be higher than estimated. The estimated cost for the full season is between £2,847 and £3,834.

Table 2 shows cost estimates for Actions applied to an established crop. Note that Action 7 are "clean-up" sprays applied at the end of the crop. Details showing how costs have been calculated are provided in a separate section (see 'Calculation of costs for chemical products')

Table 2: Cost estimates for contingency actions in an established cucumber crop

Action	Product	Cost per 0.1ha application (£)	No. applications	Sum £
1	Blue sticky traps	1.08	20	22
2	Calypso (via irrigation – rate to be confirmed)	6	1	6
3	Amblyseius cucumeris (labour)	132 - 176 18	Up to 3	396-528 54
4	Amblyseius montdorensis	Not yet commercially available	-	-
5	Atheta coriaria	375	1	375
	Hypoaspis miles (curative rate)	255	1	255
6	Mycotal (labour)	5 16	1	5 16
7	Conserve (high volume) (labour)	35 16	1	35 16
	Pyrethrum 5 EC (high volume) (labour)	82 16	1	82 16
	Calypso (high volume) (labour)	12 16	1	12 16

The cost of implementing one option from each of the Actions 1-6 and two options from Action 7 in Table 2 results in very similar costs (£903 to £1,410) to those from Table 1. This strategy would only apply to one crop as the subsequent crops would adopt the strategy designed for newly planted crops. Hence, the cost per season would be similar to the strategy for newly planted crops.

Pest control costs under standard circumstances

Estimated sales for typical / standard growing conditions and typical variable costs of glasshouse production i.e. without an outbreak of *T. palmi*, are shown in Table 3. Note that expenditure on pesticides (£550) and biological control (£600) make up approximately 4.2% of total variable costs. The estimated costs shown above for *T. palmi* control for the full growing season would represent approximately 9 – 12 % of the same variable costs.

Table 3: Typical gross margin budget for UK cucumber production, 2008 (per 0.1ha) (source: ADAS, 2008)

	£	£
Sales		30,160
Variable costs (marketing)		
Carriage / handling	4,500	
Variable costs (production)		
Gas	12,870	
Plants (bought in)	2,550	
Fertilizer	1,500	
Casual labour	1,500	
Rockwool slabs	938	
Water	805	
Biological control	600	
Pesticides	550	
Electricity	485	
Sundries	640	
Total variable costs		(26,938)
Gross margin		3,222

Status of the UK cucumber crop

From an analysis of historical data published in Basic Horticultural Statistics (Defra, 2008) it can be deduced that the UK production of cucumbers is in steady decline. Between 1997 and 2007, the planted area decreased from 186ha, worth £46.4 million to approximately 103ha, worth £33.7 million, a decline in area of almost 45% and in value of 27%. Although yields over this period increased from around 440t/ha to 480 t/ha it was not enough to maintain production at a steady level, and production over the period fell from almost 82,000 tonnes to just under 50,000 tonnes. Imports have more than made up for such a decline and have almost doubled from 60,000 tonnes in 1997 to 116,000 tonnes in 2007.

Calculation of costs for Chemical Products

Conserve SC
Cost of chemical = £145 /l
Applied at a rate of 800ml per 1,000 l water.
Volume of water applied depends on growth stage of crop, from 600 l /ha up to 3,000 l /ha, depending on crop height and structure.
Cost per application therefore ranges from £69.60 /ha to £348 /ha.
Limits: 3 applications per crop

Pyrethrum 5EC
Cost of chemical = £68 /l
Applied at a rate of 0.02 l per 5 water (0.004 l per l water)
NB: A SOLA (Number 3026/2006) was granted to allow this dilution of Pyrethrum 5EC to be applied to tomato crops at volumes up to 4,000 litres per hectare. It is assumed that an equivalent SOLA will be obtained for cucumber crops.
Volume of water applied depends on growth stage of crop, from 600 l / ha up to 3,000 l /ha.
Cost per application therefore ranges from £163.20 /ha to £816 /ha.
Limits: SOLA (Number 3026/2006) allows 8 applications per tomato crop and it is assumed that similar will be granted for cucumbers.

Calypso
Cost of chemical = £162 /l
Applied at a rate of 0.025 l per 100 l water. (0.00025 l / l water)
Volume of water applied depends on growth stage of crop from 600 l /ha up to 3,000 l /ha.
Cost per application therefore ranges from £24.30 /ha to £121.50 /ha.
Limits: Max 2 applications per crop

Calculation of costs for physical protection and monitoring products

Blue sticky traps
Cost of sticky traps = £270 for 1,500 traps (£0.18 per trap)
The density of 100mm x 200mm blue sticky traps used in the only previous T. palmi outbreak was 1 trap per 1,139m^2 in one of the glasshouses suffering the outbreak, and 1 trap per 1,538m^2 in the other house (Cannon et al., 2007).
The PHSI handbook notes that in an ideal situation, without constraints on the availability of resources, monitoring traps would be placed at optimal trapping densities, perhaps 1 trap every 100 to 300m^2, depending on the type of crop. However, in very large glasshouses, e.g. 2 to 3 ha in size, or in situations where there is more than one outbreak, a more pragmatic approach is required, and CSL advice should be sought to ensure trapping density is in line with available resources for processing samples.
Depending on circumstances, trap density could theoretically range from around 20 traps /ha (1 trap / 500m^2) to 100 traps /ha (1 trap / 100m^2).
No labour cost has been attributed to trapping because the traps will be inspected by PHSI.
Costs for traps range from £3.60/ha to £18.00 /ha (mean £10.80)

Calculation of costs for biological agents

Amblyseius cucumeris

£17.63 for 200 sachets = £0.08815 per sachet
One sachet per plant, applied every 4-5 weeks, will require three sachets per plant per crop.
Plant density is 15,000 plants per ha in the first and third crops and 20,000 plants in the second crop
Cost in first or third crop = £0.08815 x 45,000 = £3,966
Cost on second crop = £0.08815 x 60,000 = £5,289
Sachets per season = 3 x (15,000 + 20,000 + 15,000) = 150,000
Cost per season = £0.08815 x 150,000 = £13,222 / ha

Amblyseius swirskii

£47.85 for 50,000 individuals in a 500ml bottle containing 50,000 individuals
It can be applied at three rates:
Preventative (25 mites /m^2),
Curative for light infestation (50 mites /m^2)
Curative for heavy infestation (100 mites /m^2)
Cost per application per ha:
Preventative = 47.85 x (25 x 10,000 / 50,000) = £239.25
Curative light = 47.85 x (50 x 10,000 / 50,000) = £478.50
Curative heavy = 47.85 x (100 x 10,000 / 50,000) = £957.00

Amblyseius montdorensis

no prices available from Syngenta since they have no commercial license, it is only available for research.

Atheta coriaria

£37.50 for 500 individuals in a 1 l tube.
Apply 5 individuals per m^2 or up to 10 per m^2 at the foci of infestation or to speed up establishment.
50,000 individuals (100 tubes) would be needed per ha.
Cost per application = £3,750 / ha

Hypoaspis miles

£21.20 for 25,000 individuals in a 1 l tube.
Apply 100 mites/m^2 as a preventative measure or 300 mites/m^2 as a curative measure.
Cost per ha preventative application = 21.20 x (100 x 10,000 / 25,000) = £848 (40 tubes)
Cost per ha curative application = 21.20 x (300 x 10,000 / 25,000) = £2,544 (120 tubes)

Mycotal (Verticillium lecanii-m)

£32.94 for 500 gram
Apply at 0.1% with 3,000 l of spray per ha in high crops (such as cucumbers).
Kg per ha per application = 0.5 x 0.001 x 3,000 = 1.5
Cost per ha = 32.94 x 1.5 = £49.41

References

ADAS 2008. Gross margin budget for cucumbers, 2008. Produced by Dan Drakes cucumber and glasshouse consultant. Unpublished ADAS document.
Cannon, R.J.C., Matthews, L., Collins, D.W., Agallou, E., Bartlett, P.W., Walters, K.F.A., MacLeod, A., Sawson, D.D., Gaunt, A. 2007. Eradication of an invasive alien pest Thrips palmi, Crop Protection 26, 1303-1314
Defra 2008. Basic Horticultural Statistics
https://statistics.defra.gov.uk/esg/publications/bhs/2008/default.asp

Bibliography

Syngenta on-line factsheets
Amblyline cu CRS (Amblyseius cucumeris)
Amblyline M (Amblyseius montdorensis)
Hypoline M (Hypoaspis miles)
Staphyline C (Atheta)
Swirskiline AS (Amblyseius swirskii)

Koppert on-line factsheets
Mycotal (Verticillium lecanii-m)
Swirski-Mite (Amblyseius swirskii)
Thripex (Amblyseius cucumeris)

APPENDIX VI

Publications emerging from the project and other technology transfer

Papers published:

Cox, PD; Matthews, L; Jacobson, RJ; Cannon, R; MacLeod, A; Walters, KFA (2006). Potential for the use of biological agents for the control of *Thrips palmi* (Thysanoptera: Thripidae) outbreaks. *Biocontrol Science and Technology* **16**, 871-891.

Papers in preparation:

The potential of four species predatory mite species as components of an integrated pest management strategy for *Thrips palmi and Thrips nigropilosus.*

Mutual compatibility and predatory activity of *Atheta coriaria, Hypoaspis miles* and *Steinernema carpocapsae* against *Thrips palmi* and *Thrips nigropilosus*.

Reports/Presentations to Industry:

Presentation to the 8th Cucumber Growers Association Technical Conference and Annual General Meeting (East of England Showground, 20 November 2008). Preparing for the worst: A modular IPM Strategy for control of *Thrips palmi*. (Keith Walters)

Articles in Trade Press by Freelance Journalists:

Anonymous (2008). IPM solutions to *Thrips Palmi*. HDC News No. 149 (December 2008/January 2009), page 7.

Anonymous (2008). Research unveils IPM-based control methods for *Thrips palmi* on cucumbers. Horticulture Week, 5 December 2008, page 32.

Gillott, Ian (2009). Reasons to be cheerful. *Commercial Greenhouse Grower,* January 2009, 13-16.

Other activities:

1. The IPM Protocols are available for use by the Fera Plant Health and Seeds Inspectorate.

2. The IPM Protocols are currently being incorporated into National Contingency Plans for *Thrips palmi* outbreaks.

3. The final SID5 report for the project has been delivered to the Fera Plant Health Policy Programme.

4. The two SID4 reports required under this project were delivered to PHD.

5. Quarterly update reports have been delivered to PHD and Plant Health Consultants throughout the life of the project.

6. Presentations have been made to the PHSI representatives on the Project Steering Group in January 2006 and June 2008.

7. Responses have been provided to specific questions from Plant Health Consultants on a regular basis during the life of this project.

8. Presentations have been delivered at conferences on background data utilised in this project but generated under other studies (notably at the 2008 International Congress of Entomology).