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SID 5 Research Project Final Report

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Project identification

1. Defra Project code: PH0404
2. Project title: A modular approach to integrated control of Thrips palmi Karny
3. Contractor organisation(s): Central Science LaboratorySand HuttonYork, YO41 1LZ
4. Total Defra project costs (agreed fixed price): £ 226007
5. Project: start date .... 01 October 2005

   end date .... 28 February 2009

6. It is Defra's intention to publish this form.
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Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

Thrips palmi Karny is a widespread pest of vegetable and ornamental crops, which is often spread on the trade in plants and plant products. If outbreaks occur in the UK containment and eradication is required but the species is difficult to control because of its small size, thigmotaxic behaviour, rate of reproduction and insecticide resistance. Containment/eradication measures based on intensive use of agrochemicals are undesirable and unsustainable and contingency planning has highlighted the need for improved integrated management strategies. The overall aim of this project was to develop IPM programmes for *T. palmi* that substantially increase reliance on biological control agents (BCAs), within the constraints of a finite budget. More specifically the work aimed to evaluate the potential of existing and novel biological control agents within four categories: a) prophylactic use of foliage and flower inhabiting BCAs to suppress establishment potential/population development; b) prophylactic use of generalist BCAs to suppress establishment potential/population development of *T. palmi* life stages that inhabit soil; c) use of BCAs to eradicate/contain thrips in foliage and flowers; d) use of BCAs to eradicate/contain thrips in soil. In addition the project aimed to establish the compatibility of selected BCAs with chemical insecticides used in the host crop (cucumber) and mutual compatibility of the selected BCAs; to investigate if male *T. palmi* produce an aggregation pheromone that could be used in an IPM programme; to evaluate the potential of using the selected control agents in combination rather than individually; to design preliminary IPM strategies for the control of *T. palmi*. The work focused mainly on the development of an IPM strategy for use in cucumber crops and quarantine restrictions resulted in work being conducted using *T. palmi* and in some case a "model" species *T. nigropilosus*.

A literature search was conducted to establish a basis for selection of BCAs for further investigation, and the results were published (Cox *et al*., 2006). Based on the information obtained, eight candidate agents were identified together with important data gaps that limited the potential for developing an IPM strategy that utilised them. In addition, five conventional pesticides were selected including products for the control of both *T. palmi* and other (indigenous) pests and diseases that would continue to need to be addressed if a *T. palmi* outbreak occurred.

The feeding rates (on *T. palmi*) of four species of predatory mites for control of the pest on the aerial portion of the crop (*Amblyseius cucumeris, A. degenerans, A. montdorensis, A.swirskii*) were investigated in the laboratory. The results confirmed that all four species had potential to suppress populations of the target thrips species and that none had consistently higher potential than the others. One of the predatory mites (*A. degenerans*) is known to be most successful when deployed in pollen rich crops thus was of limited use in cucumber but was retained as an option to support the investigation of extrapolation of the techniques to other crops.

Experiments were conducted to establish survival (compatibility) of the four predatory mites when exposed to direct applications of pesticides or to dry residues of those pesticides on leaf surfaces. The results indicated that good survival of *A. cucumeris, A. montdorensis* and *A.swirskii* was recorded following exposure to both direct applications and dry residues of Thiacloprid (Calypso) and Pymetrozine (Chess). Good survival of *A. cucumeris* and *A. montdorensis* was also recorded following exposure to dry residues of Imazalil (Fungaflor), and of *A. montdorensis* when exposed to dry residues of Abamectin (Dynamec). These results, coupled with analogous data for Spinosad (Conserve) and Abamectin which indicated relatively high mortality of most of the predatory mites species following exposure, provided the basis for the use of either sequential or parallel applications/releases of conventional chemical pesticides and BCAs in the IPM protocols developed under this project. Thus, this component of the work established that careful design of the IPM protocols would enable the combined use of conventional pesticides and BCAs for the control of both *T. palmi* and indigenous pests (e.g. aphids) and diseases (e.g. fungal diseases) of cucumber crops.

A series of laboratory experiments investigating the potential use of more than one species of predatory mite in parallel rather than individually indicated that all species appeared to be mutually compatible with no evidence of mutual interference being recorded. Highest mortality rates of thrips were recorded when they were exposed to combinations of predators including *A. cucumeris* & *A. degenerans*; *A. cucumeris* & *A. montdorensis*; *A. degenerans* & *A. montdorensis*; and all four mite species. This information, coupled with other aspects of the biology and behaviour of each species (e.g. *A. swirskii* was more active at high temperatures and therefore may greater impact during UK summers, and is a generalist predator that could maintain itself on alternative prey if *T. palmi* is not present thus supporting its use in prophylactic control components of the protocols) informed the strategic deployment of predatory mites at key points in the population development cycle, in the IPM protocols developed.

The impact on *T. palmi* and *T. nigropilosus* of three BCAs that offered potential for control of the pest life stages that inhabit the surface layer of soil (a predatory beetle, *Atheta coriaria*; a predatory mite, *Hypoaspis miles*; an entomopathogenic nematode, *Steinernema carpocapsae*) were investigated in a series of laboratory experiments. No significant difference in the *T. palmi* mortality was recorded following exposure to any of the three BCAs and percentage mortality of the pest was high and significantly different from controls.

Following the results of the impact assays a laboratory investigation of the mutual compatibility of *A. coriaria*, *H. miles* and *S. carpocapsae* was conducted. No detrimental interactions were recorded between adults of *A. coriaria* and *H. miles*. However, exposure to adult *H. miles* reduced survival of immature *A. coriaria* (although exposure of immature *H. miles* to adult *A. coriaria* did not result in increased mortality of the former when compared to controls). Thus the parallel use of these two predators in an IPM strategy could result in significant impact on the population establishment of *A. coriaria* used for prophylactic control of *T. palmi* and should be approached with caution. Exposure to soil to which *S. carpocapsae* had been applied had no adverse effect on adult *A. coriaria* or adult *H. miles* with survival rates being similar to those in the controls in both cases.

The use of multiple species of soil dwelling BCAs in parallel was also investigated using the model thrips species *T. nigropilosus*. When the thrips were exposed to a combination of adult *A. coriaria* and adult *H. miles*, mortality of the thrips prey was significantly lower than when exposed to either species on their own. No mortality of the predators was apparent (supporting earlier results), suggesting another mechanism such as behavioural interference was acting and providing further evidence of the need for caution if parallel use of these two predators is considered.

The possible existence of an aggregation pheromone was also investigated. Male *T. palmi* produces a chemical that is not produced by females or 2nd instar larvae. Chemical analysis indicated the compound is an ester of a monoterpene alcohol and an unsaturated 5 carbon fatty acid, i.e. a monoterpene pentenoate with a molecular weight of 236. This is analogous to the situation in *Frankliniella occidentalis* where males produce the aggregation pheromone neryl (*S*)-2-methylbutanoate. This compound is also an ester of a monoterpene alcohol (nerol) and a saturated 5 carbon fatty acid (methylbutanoic acid), i.e. it is a monoterpene pentanoate with a molecular weight of 238.

Project findings were used to refine and confirm the selection of BCAs and conventional pesticides for the IPM protocols, and data was collected on growth rate and leaf area size of cucumber crops to clarify release rates of the BCAs. Draft protocols for IPM strategies on both newly planted cucumber crops and established crops were drafted. Whole plant experiments were conducted to test key components of the protocols and control results showed that during the period over which the experiment was conducted (1 month), total (adults + juveniles) thrips populations increased to a mean of >200/plant. In contrast, application of a combined treatment using an insecticide (Conserve) followed by one release of a single predatory mite species reduced this population to <10 thrips/plant (irrespective which predatory mite species was used: *A. cucumeris*, *A. degenerans*, *A. montdorensis, A. swirskii*). Analysing the data for adult and juvenile thrips separately indicated that all treatment combinations controlled both immature and adult thrips effectively. A cost benefit analysis was conducted

Whole plant studies were conducted to investigate the potential for extrapolating the cucumber IPM protocols to aubergine crops. Significant control of *Thrips* spp. was achieved using similar methods in aubergine to those employed in cucumber, showing potential for transference of the IPM method to other crops. However, significant differences in individual components on the two plants (e.g. predatory mites were less effective on aubergines) suggest that direct extrapolation between crops may carry risks and targeted work to tailor the protocols will be required. Thus the IPM strategy established under this project are transferable to a range of crop types, but the specific components will currently require further testing in each case.

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
   - the scientific objectives as set out in the contract;
   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Transfer).

BACKGROUND

Thrips palmi Karny is a widespread pest of vegetable crops in tropical and subtropical regions of the world (Kirk & Terry, 2003). Since the 1970s, it has become an invasive pest, spreading from south-east Asia to Japan, the Pacific Islands, the Caribbean Islands, South America, southern USA, northern Australia and northern Africa (Murai, 2002). It has also increased in abundance within south-east Asia. The species is regularly intercepted at quarantine on plant material being imported into Europe and outbreaks in Europe have been eradicated with difficulty (MacLeod *et al.*, 2004). With further spread in Europe and Africa the species would have a worldwide distribution across all continents. The species survives in the field in warmer climates, but in cooler climates, such as northern Europe, it will not survive the winter outdoors and is restricted to glasshouses (McDonald *et al.*, 1999, 2000). However, the rapid national and international movement of horticultural material means that it can spread very effectively between glasshouses and presents a major threat in such regions.

The life cycle of *T. palmi* is typical of a terebrantian thrips (Lewis, 1973). The parthenogenetically or sexually produced egg is laid into plant tissues with subsequent development through two active larval instars that feed on foliage and in flowers, followed by two relatively inactive, non-feeding stages: the propupa and pupa. Pupation may occur both on and off plants, often in the soil or growing medium. Thus for successful control of thrips an IPM strategy will require agents that can suppress larvae and adults on the aerial parts of the plant and others that can suppress pupal survival in growing media.

T. palmi breeds on a very wide range of plant species. It causes considerable damage to cucumber, aubergine, melon, pumpkin and sweet pepper. Other major crops that are affected include major world crops from the tropics and subtropics, such as rice, soyabean and cotton, and other crops as diverse as tobacco, cowpea, chrysanthemum, citrus, mango and potato. The species is particularly difficult to control because of its small size and thigmotaxic behaviour that results in it spending a substantial proportion of its time in recesses on plants. The species breeds very quickly and is resistant to many insecticides. Damage is mainly caused by feeding, which destroys plant tissue and so scars and stunts vegetables, making them unsaleable. Large outbreaks can defoliate and stunt plants and prevent crop production. The species is also known to transmit several serious plant diseases that affect crops. These are all in the tospovirus group and include: Tomato spotted wilt virus, Groundnut bud necrosis virus, Melon yellow spot virus, Watermelon bud necrosis virus and Watermelon silver mottle virus (Fujisawa *et al.*, 1988; Whitfield *et al.*, 2005). The host range of these diseases is not as narrow as the names suggest. For example, Tomato spotted wilt virus (TSWV) affects a very wide range of crop plants, not just tomatoes.

In the UK *T. palmi* is a serious potential pest of a wide range of protected edible/ornamental crops in the UK, but containment/eradication measures based on intensive applications of agrochemicals are generally considered to be both undesirable and unsustainable. Contingency planning for this non-native pest has highlighted the urgent need for improved integrated management strategies. The overall aim of this project is to develop IPM programmes for *T. palmi*, substantially increasing reliance on biological agents, within the constraints of a finite budget.

From the published literature and results of Defra project PH0168 it is clear that reliable non-chemical control of *T. palmi* will not be achieved simply by releasing greater numbers of the BCAs currently in commercial use. In nature, herbivorous invertebrates are usually constrained by a range of natural enemies that attack different life cycle stages and/or operate at different prey population densities. More effective and sustainable control of *T.* palmi will similarly depend on a suite of natural enemies with complementary life histories and behaviour, built on a solid foundation of knowledge of the tritrophic interactions between plants, pests and natural enemies. In addition, as the different life cycle stages of *T. palmi* may be found in different locations within the crop habitat (adults and larval instars on foliage or flowers, pupae within soil) biological control of *T. palmi* is likely to be enhanced by maximising the proportion of the pest’s life cycle that is exposed to natural enemies by introducing different natural enemies for each habitat. Recent consultancy observations and work on other thrips species in cucumber crops has also suggested that a combination of prophylactic and remedial control measures provide a basis for robust control programmes that would also be appropriate for a wide range of other crops.

The efficacy of an IPM strategy can be enhanced by the use of pest pheromones. No suitable pheromone is currently known for *T. palmi*, although there has been speculation about the existence of an aggregation pheromone (Hamilton *et al*., 2005). If ultimately characterised and synthesised, such a pheromone could be used for monitoring, mass trapping or mating confusion. 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 project also aimed to investigate whether a male-produced pheromone occurs in *T. palmi* and, if so, to make a tentative chemical identification.

The aim of the current work is to take advantage of previous research findings to develop IPM-based eradication programmes against *T. palmi* in key glasshouse crops by:

1. Evaluating the potential for control of *T. palmi* of suites of biological agents, within four categories:
    a. prophylactic use of foliage and flower inhabiting natural enemies of suppress population development and reduce establishment potential
    b. prophylactic use of generalist natural enemies in the surface layer of soil to suppress population development and reduce establishment potential
    c. use of invertebrate natural enemies to help eradicate/contain *T. palmi* populations
    d. use of entomopathogens to help eradicate/contain *T. palmi* populations
2. Establishing compatibility of selected BCAs with chemical insecticides used in the host crop (cucumber) and mutual compatibility of the selected BCAs
3. Investigating whether a male produced aggregation pheromone that could be used in an IPM programme occurs in *T. palmi*.
4. Evaluating the potential advantages of using the selected control agents in combination rather than individually in the above categories.
5. Designing, evaluating and refining preliminary modular-based control strategies for the control of T. palmi under quarantine cage conditions
6. Assessing the potential for using the modular-based approach as an IPM strategy in another crop

Work underpinning development of a suite of control modules for cucumber crops involving control agents for each category listed above will be described. In future research projects, those modules best suited to other crops might ultimately be selected and integrated into an optimum control strategy for that crop which maximises the control exerted at all levels. The principles underpinning the approach will be researched/established using cucumbers, but the potential for extrapolation to make the system generic for all protected crops will be reviewed.

Difficulties relating to the withdrawal of active ingredients and products from the market, scarcity of registration applications for new products, and public concern over environmental issues, result in the need for improved biocontrol/IPM measures for quarantine pests. The research described in this proposal represents the first investigation of a flexible modular approach to the use of biological and chemical agents for the control of a major quarantine glasshouse pest. The inclusion of a Plant Health consultant in the delivery team ensured that the work is directed at quarantine issues of immediate concern and takes account of unique considerations and constraints relating to requirements for containment and eradication of quarantine pests. Additionally PHD, PHSI, CSL, consultants and growers will obtain further benefits from the technology transfer framework utilised by the project to optimise delivery of results to end users.

More widely, the results of this project will reduce the UK protected crop industry's dependence on chemical insecticides, addressing important policy drivers such as the European Union Thematic Strategy "Sustainable Use of Pesticides" that promotes the need to maximise the efficiency of pesticide use while at the same time developing more sustainable alternatives. It thus contributes to Defra's aims and objectives by providing sustainable pest management programmes based on a combination of compatible and complementary biologically based control measures and improving the competitiveness of the UK industry by satisfying retailer and consumer requirements for a reduction in the amount of chemical toxins used within the produce supply chain.

PROJECT MANAGEMENT

The objectives of the project were: To take advantage of previous research findings to further develop IPM-based control/eradication programmes against *T. palmi* infesting cucumber crops and to consider constraints on extrapolating findings to other crops (by preparing draft contingency plans for *T. palmi* eradication in cucumber, aubergine and sweet pepper) within the constraints of a finite budget by:

1. Evaluating the potential for control of *T.* palmi of existing and novel biological control agents, within the following categories:

    a. prophylactic use of foliage and flower inhabiting natural enemies to suppress population development and reduce establishment potential
    b. prophylactic use of generalist natural enemies in the surface layer of soil to suppress population development and reduce establishment potential
    c. use of invertebrate natural enemies to help eradicate/contain *T. palmi* populations
    d. use of entomopathogens to help eradicate/contain *T. palmi* populations
2. Establishing compatibility of selected BCAs with chemical insecticides used in the host crop (cucumber), and mutual compatibility of the selected BCAs.
3. Investigating whether a male produced aggregation pheromone that could be used in an IPM programme occurs in *T. palmi*.
4.Evaluating the potential advantages of using the selected control agents in combination rather than individually in the above categories.
4. Designing, evaluating and refining preliminary modular-based control strategies for the control of *T. palmi* under quarantine cage conditions
5, Assessing the potential for using the modular-based approach as an IPM strategy in other crops (selected by agreement with steering group but possibly including aubergine or sweet pepper) and report on this via draft contingency plans.

Full details of the management structure for this project inclusing the operation of steering and delivery groups, details of the project delivery team and the project delivery plan are provided in Appendix I.

Milestone delivery:
All milestones listed in the SID3 were achieved in full, as detailed in annual SID 4 project reports and the SID5a report.

METHODOLOGY AND RESULTS

A) SELECTION OF AGENTS FOR USE IN THE MODULAR IPM APPROACH

Candidate agents for control of T. palmi (Milestone 1):
To ensure that work under this project was based on the most up to date scientific information from relevant research areas, the project commenced with a literature search investigating the potential for the use of biological agents for the control of *T. palmi* outbreaks. This was supplemented with information from unpublished sources, including reports of other relevant research projects and contacts with national and international experts made by the Science Delivery Group members. A detailed report (26 pages) was prepared and submitted to the Project Steering Group when it met in January 2006. In addition, the findings have been published as a review paper (Cox *et al.* 2006). Accordingly, and with the objective of reducing the length of this report, full details of the findings will not be presented here. Instead a copy of the published paper has been provided as a supplementary document to this report.

The abstract of this paper summarises the findings of the literature review as follows: *Thrips palmi* is a major pest of many crops in the tropics and sub-tropics, and is a serious threat in other parts of the world including the UK protected horticulture industry. Widespread use of insecticides against *T. palmi* throughout the world coupled with the restricted range of products available makes it essential to find alternative systems for control. The scattered information on its natural enemies, particularly predators and parasitoids, is reviewed and their potential for use in the eradication of *T. palmi* as part of IPM strategies in the UK is considered. Natural enemies selected for detailed consideration in the review include: *Orius* spp., *Amblyseius* spp., *Hypoaspis* spp., *Phytoseiulus* spp., *Franklinothrips* spp., *Bilia* spp., *Ceranisus* spp., *Campylomma* spp., *Deraeocoris* spp., *Anthocoris nemoralis* and *Atheta coriaria*. Recommendations for further investigations are made, including screening and efficacy testing of candidate predators and parasitoids, using semiochemicals to enhance their effectiveness, and assessing the compatibility of chosen species with other components of an IPM system.

Following the publication of this review, the Defra Project Officer responded to the discussion of the potential use of semiochemicals in systems for the control of *T. palmi* by extending the project to incorporate an investigation of the possible existence of an aggregation pheromone specific to the species to provide a basis for decision making on future work. Details of the state of knowledge in this sector are provided in the relevant section of this report.

Selection of agents for investigation (Milestone 2):
Based on this literature review, biocontrol agents for investigation as potential components of the IPM system were selected for each of the four modules detailed above. All agents selected are available commercially to ensure that they can be purchased at short notice in the event of outbreaks occurring.

Prophylactic control on flowers/foliage:
Amblyseius cucumeris (an established and effective agent for the control of other thrips species; the agent had the advantage of there being an existing delivery system that was in commercial use)
Amblyseius degenerans (likely to be most successful in pollen rich crops and was therefore selected for investigation in relation to extrapolation of the IPM approach beyond cucumbers).
Amblyseius montdorensis (See below under Remedial control on flowers/foliage)

Amblyseius swirskii (a generalist predator that could maintain itself on alternative prey if *T. palmi* is not present; therefore ideal for use in the prophylactic control module. This species was more active than others at high temperatures and therefore may have a higher potential for use during summer in the UK under conditions that will favour *T. palmi*.)

Prophylactic control on the ground:
Atheta coriaria (This soil dwelling staphylinid beetle has very high predation rates on the pupae of *F. occidentalis.* It was also known to attack many other soil-dwelling pests and therefore had the potential to survive in the absence of the primary thrips target species. At least one biocontrol producer was developing a rearing unit with a view to supplying a commercial product into the UK market at the time agent selection was undertaken)
Hypoaspis miles (A generalist soil-dwelling mite, which has been shown to predate upon thrips pupae in the soil and other growing substrates. Up to 30% reduction in numbers of F. occidentalis had been attributed to this predator. It was already available as a commercial product.)

Remedial control on flowers/foliage:
Amblyseius montdorensis (Schicha (1979) reported that this Australian species was a superior predator of thrips and therefore capable of providing remedial control. It was reported to consume greater numbers of WFT nymphs than *A. cucumeris* and was capable of attacking larger individuals. It was a leaf dwelling, rather than flower dwelling mite, thus giving added potential for the control of *T. palmi*. At the start of this project, the project team was informed that a licence was being sought to release *A. montdorensis* in the UK and suppliers predicted that it could become the leading product for thrips control. Thermal biology characteristics also indicated that *A. montdorensis* would be a safe candidate for introduction as a glasshouse biocontrol agent in the UK, adding confidence that it might become commercially available (Hatherly *et al*., 2004). The licence was confirmed during the life of the project.
Orius spp. (Several *Orius* spp. are known to be effective biological control agents and are employed widely. Two species are native to the UK and therefore can be used without a licence issued by ACRE. Most reports of efficacy against *T. palmi* involve species not widely used (or available) in the UK. The tendency of some species to abandon partly consumed prey if another prey item is located results in more thrips being killed than are required to reach maturity, increasing their efficacy as biological control agents. However *Orius* also feed on other BCAs used for thrips control such as *A. cucumeris* (Cox *et al*., 2006), reducing the potential cumulative impact on the pest population if both are used together. In addition, considering the length of the life cycle it can take up to two months to build up a satisfactory population of *Orius* on a UK crop (Jacobson 1994) presenting a risk to rapid control of quarantine pest outbreaks. Secondly *Orius* spp. Need pollen or plentiful prey in order to establish. Therefore they are not recommended for use on crops that do not have pollen (e.g. chrysanthemums). Some species or strains have a reproductive diapause at low temperatures and short daylengths and do not thrive before April of after September in the UK. Diapause can be averted using artificial light, but this adds further complication to their use as a component of a complex IPM system. Finally few insecticides can be used in combination with *Orius* spp. It was concluded that, despite these constraints, *Orius* spp remained an option for use in the IPM system under development. However, a range of factors resulted in predatory mites being a primary option. *Orius* spp. remain additional candidates but funding constraints result in the necessary work to further assess potential not being conducted under this project.
Beauveria bassiana and *Lecanicillium muscarium* (The use of entomopathogenic fungi was reported to show promise at the start of the project and *Beauveria bassiana* had shown good compatibility with *A. cucumeris.* At that time it was not approved for use in the UK however, and no clear approval date was available. Published evidence of the efficacy of *Lecanicillium muscarium* as a control agent for *T.* palmi in the laboratory was available in the literature (see section D below) and it was readily available from commercial sources. Thus this species was adopted as the candidate entomopathogenic fungi for the IPM system under development)

Remedial control on the ground:
Atheta coriaria (The reported high predation rates indicated that this predator had potential to provide remedial control of *T. palmi*. As the agent was being actively developed by commercial companies, it was adopted for use in this IPM niche.)
Steinernema carpocapsae (entomopathogenic nematode with known efficacy as a control agent of other soil dwelling pests; has the advantage of being applied using existing equipment available on nurseries and acting as a “bio-insecticide”; good potential activity)

Fuller details of the basis for these selections are given in Cox *et al.* (2006).

Candidate (conventional) pesticides for control of T. palmi (Milestone 2):
Five pesticides used in cucumber production at the start of this project were selected for investigation as potential components of the IPM system for *T. palmi*.

Abamectin as Dynamec (Syngenta Crop Protection UK Ltd, Cambridge, UK): This was one of a very small number of established products approved for the control of *Frankliniella occidentalis* on cucumbers in the UK. It was said to be effective against *F. occidentalis* nymphs and to provide useful reduction in numbers of adults.

Pymetrozine as Chess WG (Syngenta Crop Protection UK Ltd.): Although not effective against thrips, this was an important product for the control of pirimicarb-resistant aphids in IPM programmes. It was therefore important that it be compatible with any biocontrol agents selected for use against *T. palmi*. Products tested in this project covered other essential aspects of cucumber production rather concentrating solely on thrips control products.

Spinosad as Conserve (Fargro Ltd., UK): This was a new insecticide to the UK with known activity against thrips. It was derived from a naturally occurring soil dwelling bacteria. The novel chemistry made it a particularly useful product to build into resistance management strategies.

Thiacloprid as Calypso (Bayer CropScience Ltd., Cambridge, UK): This product was new to the UK market and known to be effective against thrips. It had been utilised by PHSI in *T. palmi* eradication programmes (see section H below) in ornamental crops.

Imazalil as Fungaflor 100EC (Certis, Amesbury, UK): Testing of the compatibility of the selected predatory mites with a fungicide as well as a range of insecticides was considered essential if the IPM strategy developed was to be a viable option for growers against a background of the level of disease risk encountered in cucumber production. This product was selected as being one of the most widely utilised fungicides in UK cucumber production.

B) Essential data for development of the modular IPM Programme

Data gaps identified for selected control agents/pesticides for each defined category (Milestone 3 and 4):

The results of the literature review conducted for this project (Cox *et al.* 2006), confirmed the findings of initial reviews of the subject relating to essential data gaps that would need to be filled before IPM protocols could be drafted. Although much relevant information was available both in the literature and from unpublished sources, key information was required in the following areas.

To establish if any of the four mites selected for further investigation, (*Amblyseius cucumeris, A. degenerans, A. montdorensis, A.swirskii)* had the potential to exert greater impact on *T. palmi* populations than the others, data was required on feeding rates on this prey species. Although much data was available on consumption rates of these mites, data allowing statistical comparison of their impact on *T. palmi* was lacking.

To provide a basis for integrating the use of conventional pesticides with the predatory mites in an IPM system, data on the compatibility of selected pesticides (following both direct and indirect exposure) was required.

Information on the mutual compatibility of predatory mites if used in parallel within the IPM system was identified as requiring further investigation.

Although good data was available in the literature on other prey species, further data on the impact (feeding rates) of *Atheta coriaria, Hypoapsis miles* and *Steinernema carpocapsae* on *T. palmi* pupae was required. Similarly data on the mutual compatibility of *Atheta coriaria, Hypoapsis miles* and *Steinernema carpocapsae* was identified as a needing further investigation.

Steinernema carpocapsae has known efficacy as a control agent of other soil dwelling pests; good potential activity but needs further screening against *T. palmi*. This was not identified as an essential requirement under this project, but was noted for future reference.

Information on the mutual compatibility of the selected soil dwelling natural enemies if used in parallel within the IPM system was also identified as requiring further investigation.

B(i) Categories addressing foliar/flower dwelling stages of the pest: Feeding rates on* T. palmi *of selected predatory mites for each IPM category (Milestone 5, 9 and 11) :

Following the review of potential biological control agents for use in a modular integrated pest management (IPM) system for *T. palmi* (Cox *et al.* 2006), four mites were selected for further investigation, (*Amblyseius cucumeris*, *A. degenerans*, *A. montdorensis*, *A.swirskii*). To generate comparative data on their consumption of different pest life stages as part of the basis for establishing if an individual species had the potential to exert greater impact on *T. palmi* populations, experiments were conducted to establish their feeding rates.

In addition, as quarantine restrictions render whole plant experiments to establish the efficacy of components of the IPM protocols under development difficult to conduct because of licence constraints, a similar range of experiments were conducted on a second (non-quarantine) "model" thrips species. These used the same methodology to establish if comparative feeding rates of *T. nigropilosus* were analogous to those recorded when the mites were offered *Thrips palmi*.

Experimental details:

A single predatory mite was placed on cucumber leaf discs, together with 10 *T. palmi* as prey (all thrips were at a single same life stage of the given species). The leaf discs, thrips and mites were contained in an experimental arena (Tashiro (1967), and incubated in a controlled environment cabinet at 21 ± 2°C, 65% relative humidity (r.h.), and 16:8 Light:Dark (L:D). The number of thrips that were dead or missing, and the number of thrips with clear symptoms of feeding damage were recorded after five days to determine consumption rates. Thrips that have been attacked and fed upon were defined as those that appear ‘sucked out’ and hence flattened in structure. The experiment was replicated 15 times. Each feeding trial for each species of mite will be replicated 15 times.

The controls (15 replicates for each species of mite) consisted of an identical experimental procedure to those used for each treatment, with the exception that a predatory mite was not placed in the experimental arenas.

The above procedure was repeated for each of three *T. palmi* life stages (1^{st} and 2^{nd} instar larvae and adults) and for each mite species separately. The whole experiment was repeated using *T. nigropilosus*

The number of thrips either dead or missing out of a total of those recorded as alive, dead or missing, was analysed as a binomial Generalised Linear Model (GLM), with a logit link, using Genstat 10. The model included the two species of thrips, the three life stages, the four predatory mite species and control, and interactions between them.

The number of thrips eaten (with symptoms of feeding damage) out of a total of those recorded as alive, dead or missing, was analysed as a binomial Generalised Linear Model (GLM), with a logit link, using Genstat 10. The model included the two species of thrips, the three life stages, the four predatory mite species, and interactions between them. Control responses were not included in the analysis since the numbers eaten were all zero (there were no predators).

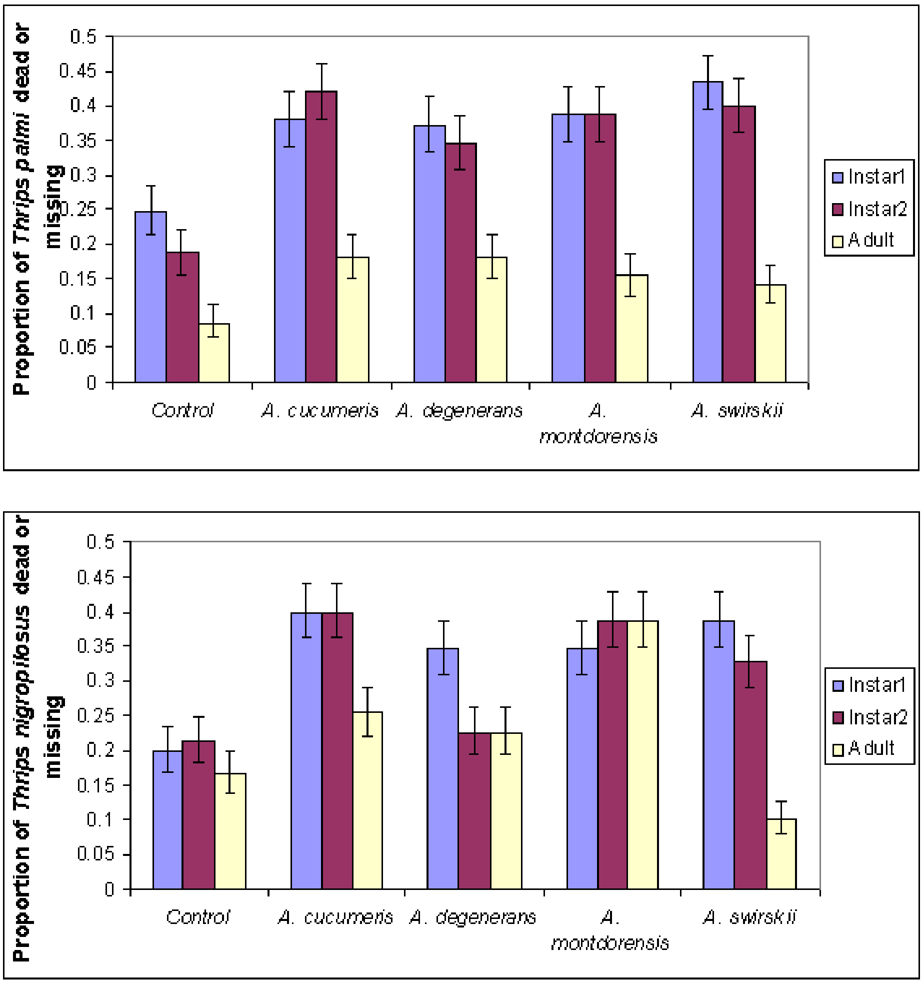


Fig 1. The proportion of *Thrips palmi* or *Thrips nigropilosus dead* or missing following exposure to four species of predatory mites.

Results:

Dead or Missing: The results of this analysis are summarised in Fig. 1. The regression analysis for the number of thrips either dead or missing demonstrated a statistically significant effect (deviance ratio of F=9.11, df=29, 420, p<0.001). The accumulated analysis of deviance showed a statistically significant interaction between Stage and Predatory Mite (F=3.11, df=8,420, p=0.002) and the probabilities associated with these two as main effects were both <0.001 respectively. There is also a significant interaction between Stage and Thrips (F=9.57, df=2,420, p<0.001) and between Thrips and Predatory Mite (F=2.42, df=4,420, p=0.046). There was no statistically significant effect of thrips (p=0.742).

Post-hoc pair-wise comparisons are not available with GLM but a comparison of two means can be made using the tabulated standard errors of the differences for t-tests. These pairwise comparisons for Mite species within life stage and Thrips species (i.e. *A. cucumeris* eating 1st instar *T. palmi* vs *A. swirskii* eating 1st instar *T. palmi* etc) showed that, for *T. palmi*, all four mite species were significantly different from the control for the 1st and second instars and that *A. cucumeris* and *A. degenerans* were significantly different from the control for adults. All except *A. degenerans* were significantly different from the control for the 2nd instar. Only *A. montdorensis* was significantly different from the control for the Adults. *A. degenerens* had significantly fewer 1st instar *T. nigropilosus* dead or missing than *A. montdorensis* and *A. cucumeris*.

Pairwise comparisons between life stages within mites and thrips species (i.e. *A. cucumeris* against 1st instar *T. palmi* vs *A. cucumeris* against adult *T. palmi* etc) showed no differences between 1st and 2nd instars for *T. palmi* for any of the mites or the control. All had a significant difference between both 1st and 2nd instars and the adults.

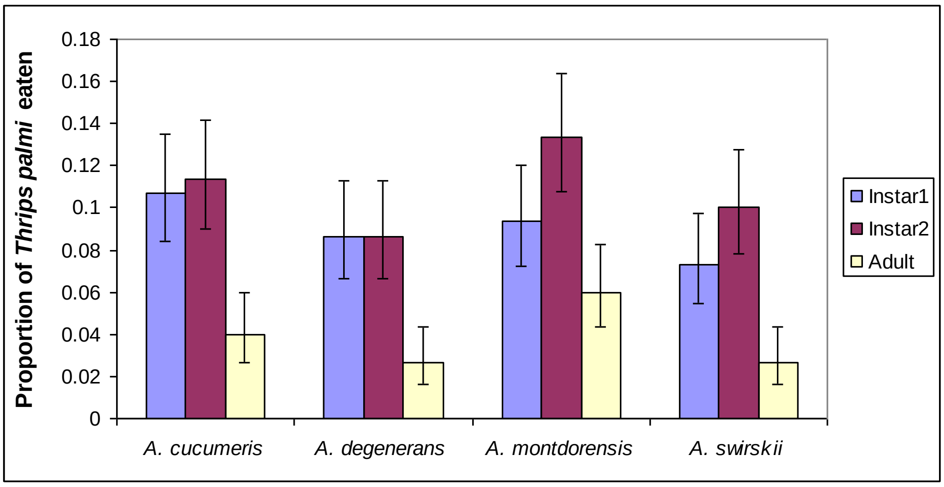
Thrips Eaten: The results of this analysis are summarized in Fig. 2. The regression analysis of the number of thrips eaten demonstrated a statistically significant effect (deviance ratio or F=3.58, df=23, 336, p<0.001. The accumulated analysis of deviance showed a statistically significant interaction between Stage and Predatory Mite (F=2.43, df=6,336, p=0.024) and the probabilities associated with these two as main effects were <0.001 and 0.004 respectively. There was no statistically significant effect of Thrip (p=0.616), nor were any of the other interactions significant.

Post-hoc pairwise comparisons are not available with GLM but a comparison of 2 means can be made using the tabulated standard errors of the differences for t-tests. These pairwise comparisons for Mite species within life stage and Thrips species (i.e. *A. cucumeris* eating 1st instar *T. palmi* vs *A. swirskii* eating 1st instar *T. palmi* etc) showed that the only significant difference was that *A. montdorensis* consumed a significantly higher number of adult *T. nigropilosus* (P<0.05) than the other three mite species.

The results of pairwise comparisons between life stages within mites and *Thrips* species (i.e. *A. cucumeris* eating 1st instar *T. palmi* vs *A. cucumeris* eating adult *T. palmi* etc) for *A. cucumeris* and *A. degenerans* there were significant differences between both 1st and 2nd instars and adult *T. palmi* (p<0.05) and for *A. montdorensis* and *A. swirskii* there were significant differences between and 2nd instars and adult *T. palmi* (p<0.05).

Conclusions:

Dead and Missing thrips: Analysis of the data collected on dead and missing thrips showed no significant differences



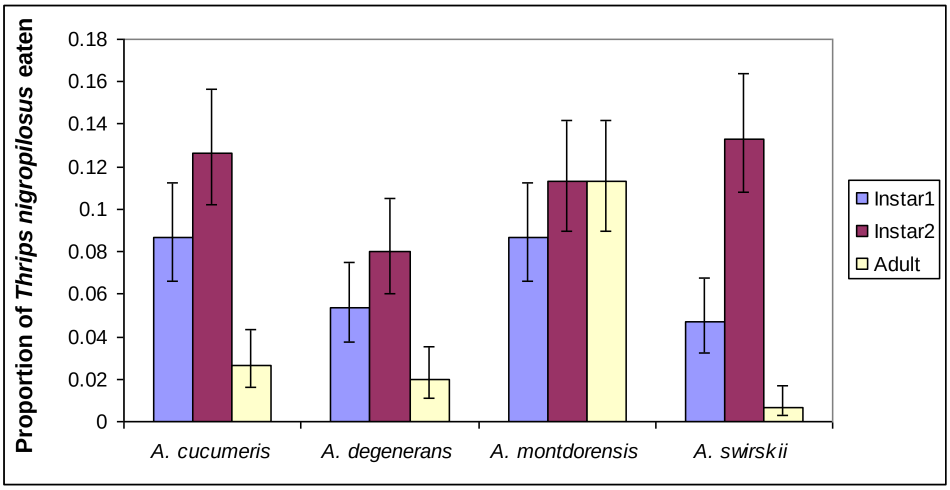


Fig 2. The proportion of *Thrips palmi* or *Thrips nigropilosus* eaten following exposure to four species of predatory mites.

between consumption rates of mites when fed on the two thrips species (p=0.742). Significant differences were recorded between Mite species (p<0.001) and between life stages (p<0.001) and the interaction term between life stage and mite was also significant (p=0.002). There was also a significant interaction between Stage and Thrips (p<0.001) and between Thrip and Pred atory Mite (p=0.046).

T. palmi *as prey*: When *T.* palmi was offered as prey, consumption rates of *T. palmi* indicated that all four mite species were significantly different from the control for the 1st and 2nd instars resulting in lower prey survival. Adult thrips survival was significantly lower following exposure to *A. cucumeris* and *A. degenerans* than in the control. For all 4 mite species there was no difference between consumption rate of 1st and 2nd instar thrips. For all four mite species significantly more 1st and 2nd instars were consumed than adults.

T. nigropilosus *as prey*: When 1st instar *T. nigropilosus* was offered as prey, the number of dead or missing thrips were significantly higher than in controls following exposure to all four mite species. Significantly lower survival of 2nd instar prey was recorded following exposure to all species of predatory mites except *A. degenerans*. Survival of adults was only significantly different from controls following exposure to *A. montdorensis*.

There were significantly fewer 1st instar thrips dead or missing following exposure to *A. degenerens* than after exposure to *A. montdorensis* and *A. cucumeris.* Significantly more adult thrips were dead or missing following exposure to *A. montdorensis* than was recorded for the other three mite species, and exposure to *A. swirskii* was followed by significantly lower numbers of dead or missing adult thrips than was recorded for the other three mite species.

A. cucumeris and *A, swirskii* consumed significantly more 1st and 2nd instar thrips than adults *and A. degenerens* consumed significantly higher 1st instar than 2nd instars or adult thrips. There were no significant differences between life stages for either *A. montdorensis* or the controls.

Thrips eaten:
There were no significant differences between consumption rates between thrips species (p=0.616). There were significant differences between mites (p=0.004) and between life stages (p<0.001) and the interaction term between life stage and mite was also significant (p=0.024)

The only significant differences recorded between mite species indicated that *A. montdorensis* consumed a significantly higher number of adult *T. nigropilosus* (P<0.05). *A. cucumeris* and *A. degenerans* consumed significantly more 1st and 2nd instars than adult *T. palmi A. montdorensis* and *A. swirskii* consumed significantly more 2nd instars than adult *T. palmi*. *A. swirskii* consumed significantly more 2nd instars than 1st instars, and more 1st and 2nd instars than adult *T. nigropilosus* (P<0.05). *A. cucumeris* consumed significantly more 1st and 2nd instars than adult *T. nigropilosus* (P<0.05). *A. degenerans* consumed significantly more 2nd instars than adult *T. nigropilosus* (P<0.05). There were no significant differences between the number of the different life stages of *T. nigropilosus* that *A. montdorensis* consumed.

Thus none of the four mite species was found to have consistently higher potential as a component of the IPM strategy under development. It was also concluded that use of *T. nigropilosus* in whole plant experiments would provide useful information on the performance of components of the IPM strategies under development but that care must be taken in interpreting results, particularly in situations where differences are close to conventional 5% significance levels.

Compatibility of predatory mites selected for each IPM category with conventional pesticides (Milestones 6 and 12):
The investigation of the compatibility of the four selected predatory mite species with commonly used conventional pesticides for insect and disease control on cucumbers in UK glasshouses included establishment of responses to both direct and indirect (i.e. exposure to dry residues) exposure of predators to the products. The techniques utilised were modified from those established by Head et al. (2000) and subsequently used widely in published studies of compatibility of conventional pesticides and biocontrol agents.

Experimental details:
The predatory mites used in this study (*(Amblyseius degenerans, A. cucumeris, A. swirskii, A. montdorensis)Amblyseius cucumeris, A. degenerans, A. montdorensis*, *A. swirskii*) were obtained from a commercial supplier (Syngenta Bioline). Pwere obtained from a commercial supplier (Syngenta XXXXX). Pesticides were also obtained from commercial sources and were diluted and applied at the recommended dose rate for cucumber plants in the UK. Five pesticides were investigated: Abamectin as Dynamec (concentration 0.5ml/L; active ingredient (a.i.) content: 1.8% w/w; source: Syngenta Crop Protection UK Ltd, Cambridge, UK); Imazalil as Fungaflor 100EC (1.0ml/L, a.i. 10% w/w, Certis, Amesbury, UK); Thiacloprid as Calypso (0.45 ml/L, a.i. 48% w/w, Bayer CropScience Ltd., Cambridge, UK); Pymetrozine as Chess WG (0.2g/L, a.i. 50% w/w, Syngenta Crop Protection UK Ltd.); Spinosad as Conserve (0.8ml/L, a.i. 12% w/w, Fargro Ltd., UK).
Testing of the compatibility of the selected predatory mites with a fungicide as well as a range of insecticides was considered essential if the IPM strategy developed is to be a viable option for growers against a background of the level of disease risk encountered in cucumber production.

Experiment 1 – Direct application: Topical application of pesticides was conducted using an Auto-Load Potter Precision Laboratory Spray Tower (Burkhard Scientific, Hertfordshire) fitted with a medium atomiser. Experiment 1 - Direct application:
Topical application of pesticides was conducted using an Auto-Load Potter Precision Laboratory Spray Tower (Burkard Scientific, Hertfordshire) fitted with a medium atomiser.
Five mites were transferred to each of five glass slide cover slips housed within a 9cm diameter Petri dish. The selected pesticide was then topically applied to the mites before they were transferred to 4cm diameter leaf disks that had been floated on water in another 9cm Petri dish, and with bee pollen added as a food source. Leaf discs were cut from a cucumber plant Five mites were transferred to each

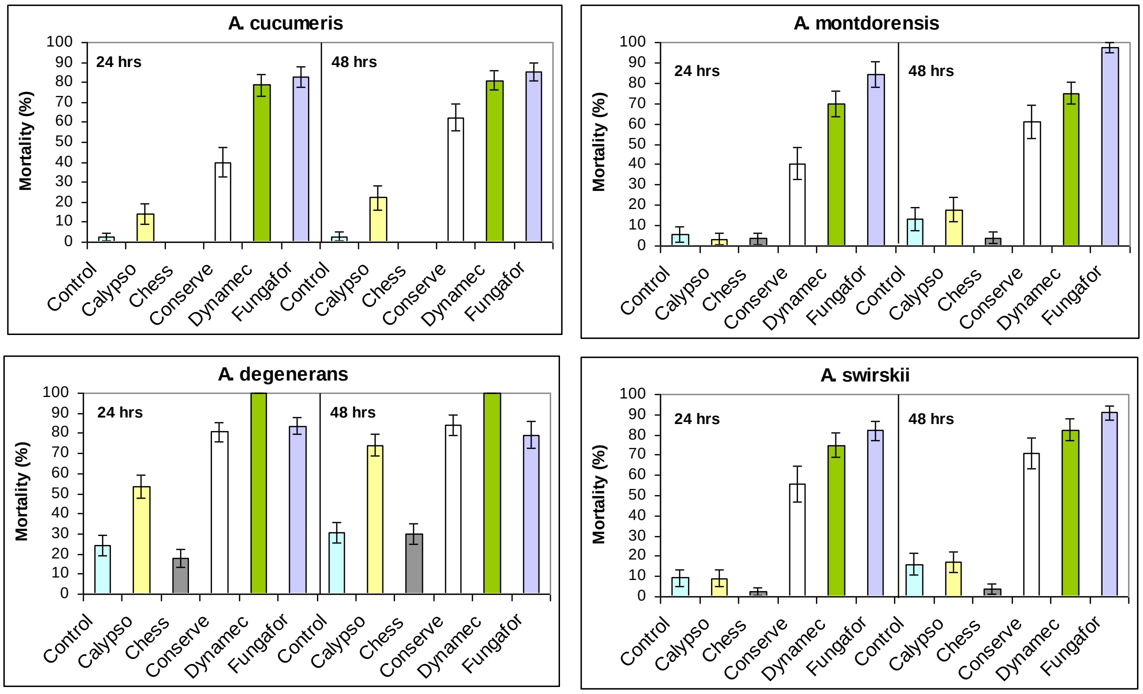


Fig. 3. The mortality of *Amblyseius cucumeris*, *A. montdorensis*, *A. degenerans*, and *A. swirskii* at 24 and 48 hours after direct application of Thiacloprid (Calypso), Pymetrozine (Chess), Spinosad (Conserve), Abamectin (Dynamec) and Imazalil (Fungaflor)

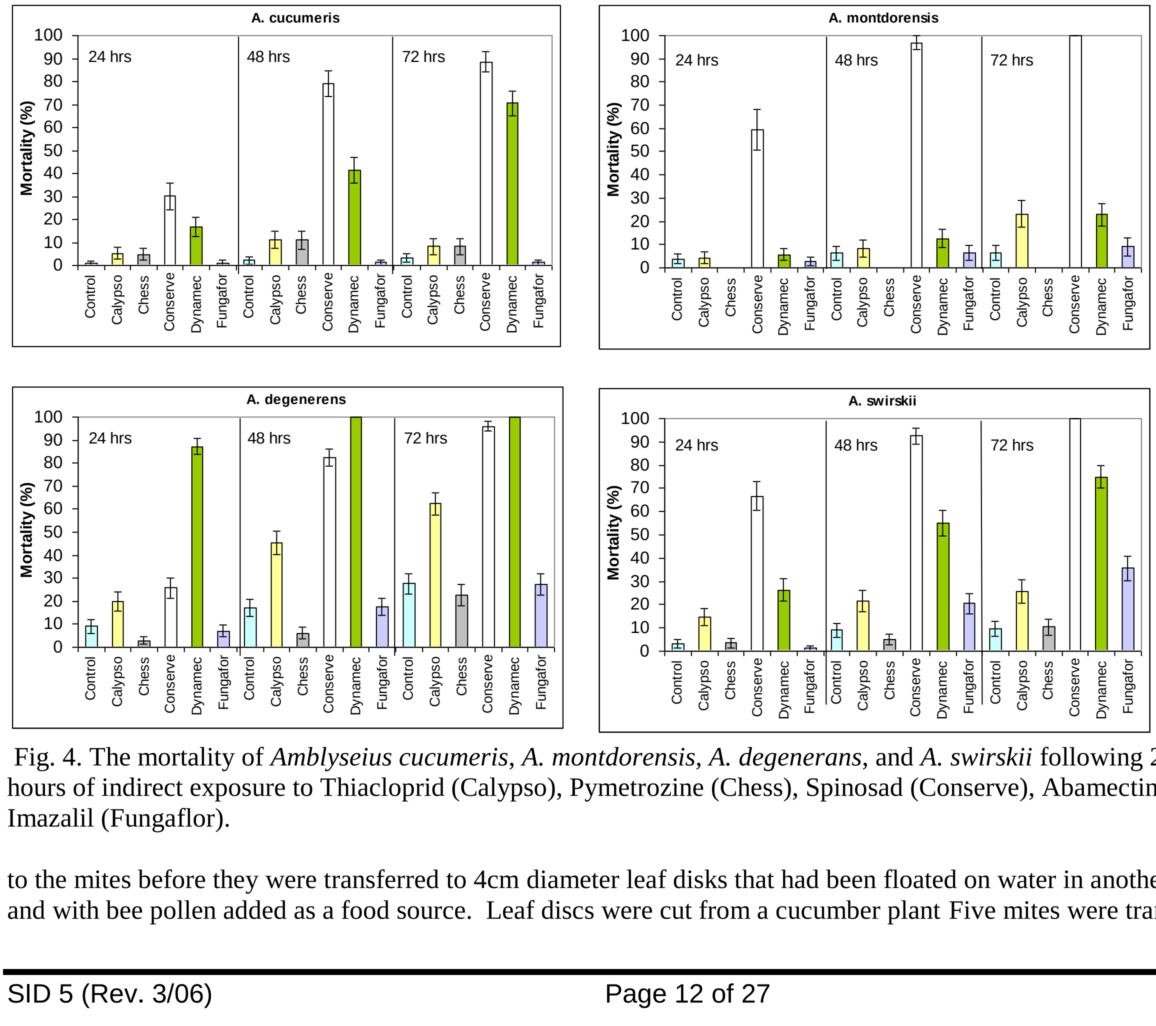


Fig. 4. The mortality of *Amblyseius cucumeris*, *A. montdorensis*, *A. degenerans*, and *A. swirskii* following 24, 48 and 72 hours of indirect exposure to Thiacloprid (Calypso), Pymetrozine (Chess), Spinosad (Conserve), Abamectin (Dynamec) and Imazalil (Fungaflor).

of five glass slide cover slips housed within a 9cm diameter Petri dish. The selected pesticide was then topically applied to the mites before they were transferred to 4cm diameter leaf discs cut from a cucumber plant (Cucumis sativus L. cv. Telegraph improved) (*Cucumis sativus* L. cv. Telegraph Improved) using a sharpened cork borer. using a sharpened cork which borer, which were floated on water in another 9cm petri dish containing bee pollen as a food source.Five mites were transferred to each of five glass slide cover slips housed within a 9cm diameter Petri dish. The selected pesticide was then topically applied to the mites before they were transferred to 4cm diameter leaf discs cut from a cucumber plant (Cucumis sativus L. cv. Telegraph improved)TheThe Petri dish lids were sealed and they were arranged on a laboratory tray that in turn was sealed in a clear plastic bag, and incubated in an CE cabinet at 21 ± 2°C± 2°C, 65% r.h. 16:8 hr L:D. Mortality of mites (defined as there being no movement following physical stimulation) was assessed after 24 and 48 hours. There were four controls (one for each species of mite) consisting of an identical experimental procedure to those used for treatments, with the exception that mites were sprayed with water. This procedure was repeated 20 times for each mite species.

Experiment 2 - Exposure to chemical residues: Cucumber plants were sprayed to run off using a hand held spray applicator then air dried for 24 hours in a fume cupboard. Leaf discs were cut from the plants using a similar procedure to that described above for direct exposure experiments, before being "floated" on water in 9cm Petri dishes. Five predatory mites were confined on each leaf disk, the lids were sealed and the Petri dishes placed on a tray, sealed in a clear plastic bag, and incubated in a CE cabinet at 21 ± 2°C, 65% r.h. 16:8 hr L:D. Mortality of mites was assessed after 24, 48 and 72 hrs using the criteria noted above. Control treatments followed the same procedure as treatments except that leaves were sprayed with water only. The procedure was repeated 20 times for each mite species and each pesticide.

Data analysis: The data were analysed by logistic regression (generalized linear model) assuming a binomial distribution and with a logit link function using Genstat 10.2 (VSN International Ltd).

The standard errors of the differences (s.e.d.) between predictions were used for pair-wise comparisons between the control (untreated) responses and the treatment responses. This amounted to 20 comparisons per experiment and the decision level for a statistically significant difference was changed from $p=0.05$ to $p=0.0025$ as a Bonferroni correction to allow for these multiple comparisons ($0.05/20 = 0.0025$).

Results:
At both 24 and 48 hours after direct application of Spinosad (Conserve), Abamectin (Dynamec) and Imazalil (Fungaflor), mortality of all four mite species was significantly ($p<0.0025$) higher than mortality following control treatments (Fig AB). Similarly mortality of *A. degenerans* was significantly ($p<0.0025$) higher than that recorded in controls following exposure to Thiacloprid (Calypso), although there was no significant difference in mortality of the other three mite species.

Thus the results indicate that all four species of predatory mite showed good survival following direct exposure to Pymetrozine (Chess), and *A. cucumeris*, *A. montdorensis* and *A. swirskii* were compatible with the use of Thiacloprid (Calypso).

After 24 hours of indirect exposure to Spinosad (Conserve) mortality of *A. cucumeris*, *A. montdorensis*, *and A. swirskii* was significantly ($p<0.0025$) higher than mortality following control treatments (Fig AC). Similarly, mortality of *A. degenerans* and *A. swirskii* was higher than in controls following exposure to Abamectin (Dynamec).

After 48 hours exposure to pesticide residues mortality was significantly ($p<0.0025$) higher than in controls in all mite species in Spinosad (Conserve) treatments, in three mite species (*A. cucumeris*, *A. degenerans*, *A. swirskii*) in Abamectin (Dynamec) treatments, and in *A. degenerans* in thiacloprid (Calypso) treatments.

A similar result emerged after 72 hours of exposure to pesticides with the exception that significantly ($p<0.0025$) higher mortality than recorded for *A. swirskii* exposed to Imazalil (Fungaflor) treatments.

Conclusion:
The results indicate that all four species of predatory mite showed good survival following both direct and indirect (residue) exposure to Pymetrozine (Chess), and that *A. cucumeris*, *A. montdorensis* and *A. swirskii* were compatible with the use of Thiacloprid (Calypso).

Exposure of *A. montdorensis* to Abamectin (Dynamec) residues resulted in low mortality, but potential use of the insecticide is limited by significant mortality resulting from direct exposure that would require re-introduction following treatment. Similarly use of the fungicide Imazalil (Fungaflor) is limited by significant mortality following direct exposure of all four predatory mites, although low impact of dry residues on *A. cucumeris*, *A. montdorensis* and *A. swirskii* indicate that these biocontrol agents may be introduced (or re-introduced) following control of fungal diseases if required.

Use of multiple species of predatory mite in parallel (Milestone 5 and 9):
The results of experiments investigating the feeding activity of predatory mites showed that when individual mites were offered various life stages of *T. palmi*, more first and second instar larvae were consumed than adults All four mite species investigated indicated that each showed potential as a biocontrol agent for the thrips, and as a suitable range of conventional pesticides were found to be compatible with all species, the option of utilising more than one predatory mite species in the

IPM strategy developed under this project remains viable. To investigate this option further, rates of consumption of *T. palmi* larvae when more than one species of predatory mite are foraging in the same arena were investigated.

Experimental details:
Experiments were conducted in the experimental arenas described by Tashiro (1967). Different combinations of predatory mites were confined (at 21 °C, 16:8 L:D) in each arena with 10 second instar thrips larvae except the 4 species treatment that were offered 20 thrips. Due to limitations of the availability of *T. palmi*, advantage was taken of the provision made in the SID3 document to use the model thrips species, *Thrips nigropilosus* as prey. Treatments included: 1 *A. montdorensis* & 1*A. degenerans*; 1 *A. cucumeris* & 1 *A. degenerans*; 1 *A. cucumeris* & 1 *A. swirskii*; 1 *A. montdorensis* & 1 *A. swirskii*; 1 *A. cucumeris* & 1 *A. montdorensis* and finally 1 of each species of predatory mite. Consumption of prey was assessed after a five day exposure period, when the number of thrips dead or missing was recorded. Controls consisted of 20 thrips of the same life stage maintained under identical conditions, but with the predatory mites absent. There were 15 replicates of each treatment

The number of thrips either dead or missing out of a total of those recorded as alive, dead or missing, was analysed as a binomial Generalised Linear Model (GLM), with a logit link, using Genstat 10. The model included the two species of thrips, the 3 life stages, the 4 predatory mite species and control, and interactions between them.

Results and conclusions:
Analysis of the data collected indicated that there was a significant difference in the percentage of dead or missing thrips between treatments (F=15.95; d.f. = 6,98; p<0.001). More dead or missing thrips were recorded following all treatments than in the control. A significantly greater percentage of dead or missing thrips were recorded following all treatments than in the control The *A. swirskii & A. cucumeris* treatment was followed by a significantly lower mortality than the other treatments apart from that involving *A. montdorensis & A. swirskii*. The *A. montdorensis & A. swirskii* treatment was followed by significantly lower mortality than *A. montdorensis & A. cucumeris* and the "All four mite species" treatment. Thrips mortality following exposure to several treatments *(A. cucumeris & A. degenerans, A. montdorensis & A. degenerans, A. montdorensis & A. cucumeris* and "All 4 mite species") were not significantly different from each other.

<u>Mutual compatability between predatory mites (Milestone 5 and 9)</u>**:**
Results of experiments investigating the use of multiple species of predatory mites in parallel indicated significant reduction in numbers of thrips numbers when cohorts were exposed to various combination of predator species. No indication of significant differential predation of mites between species was noted by experimenters, supporting the conclusion that the selected mite species could be used in parallel within the IPM system under development. To provide further support for this conclusion trials in which mites of different species were confined together without prey.

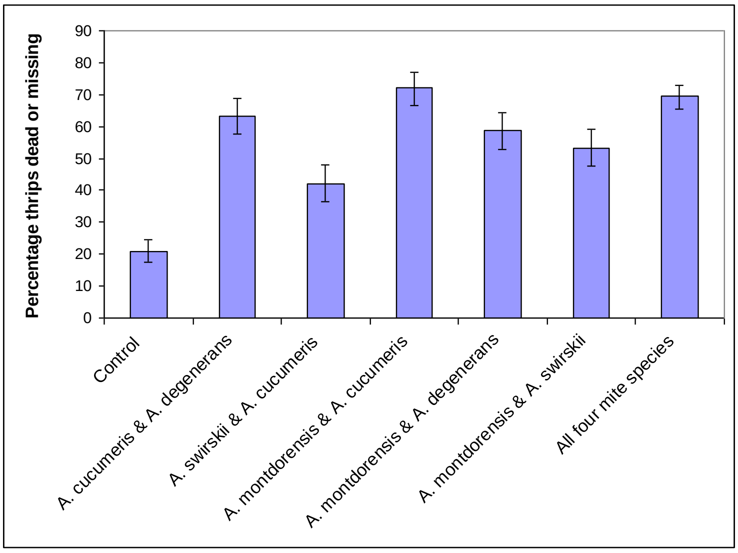


Fig 5. The percentage of *Thrips nigropilosus* recorded as dead or missing following exposure to different combinations of four species of predatory mites.

Experimental procedure:
Eight predtory mites (four of each of two species were placed on cucumber leaf discs contained in an experimental arena (Tashiro (1967), Following incubation in a controlled environment cabinet at 21 ± 2°C, 65% relative humidity (r.h.), and 16:8 Light:Dark (L:D). The number of mites of each species that were dead or missing, was recorded after two days. The experiment was replicated 15 times. Each feeding trial for each species of mite will be replicated 15 times.

The procedure was repeated for the following combinations of predatory mites, *A. degenerans/A. montdorensis; A. cucumeris/A. degenerans; A. cucumeris/A. swirskii*; *A. montdorensis/A. swirskii; A. cucumeris*/*A. montdorensis*; *A. cucumeris/A. swirskii*; *A. montdorensis/A. swirskii; A. cucumeris*/*A. montdorensis*.

Results and conclusions:
Following each trial no evidence was recorded of an effect of predatory mites of different species having a significant effect on each other, supporting the findings of earlier feeding trials.

B(ii) Categories addressing soil dwelling stages of the pest: Impact of* Atheta coriaria, Hypoaspis miles and Steinernema carpocapsae *on* T. palmi *pupae (Milestone 9 and 11)

A range of biocontrol agents were required for a module of the IPM system that addresses the control of life stages of *T. palmi* likely to be found in the surface layers of the soil. Three candidate agents (*Atheta coriaria*, *Hypoaspis miles* and *Steinernema carpocapsae*) were identified by the literature search but further data on the potential feeding rates/impact of these agents on *T. palmi* was required. Accordingly, experiments were conducted to investigate feeding rates of the agents when offered *T. palmi* or the model species *T. nigropilosus* in laboratory experiments.

Experimental details:
To investigate feeding rates of the predators *A. coriaria* and *H. miles* on *T. palmi* and *T. nigropilosus* pupae, trials were conducted using standard Petri dish experimental arenas. A single predator was confined on compost (at 21 °C, 16:8 L:D) in each arena with 10 pupae. Trials consisted of 10 replicates for each predator and were assessed after a five day exposure period, when the number of live and dead pupae and the number of pupae missing was recorded. Predator free controls consisted of 10 pupae maintained under identical conditions, but without the predator.

The activity of the entomopathogenic nematode *S. carpocapsae* was investigated using the method of Smith *et al.* (2005). Suspensions of *S. carpocapsae* were applied to compost in 15 cm Petri dishes at the commercially recommended dose rate using a Hoselock Polyspray 2 hand held sprayer (Cuthbertson *et al*., 2005; North *et al*., 2006)). Ten thrips pupae were then placed onto the soil surface, the lid replaced and sealed with parafilm and incubated at 21 °C, 16:8 L:D. Pupal mortality was assessed after 48 hours.

The number of thrips either dead or missing out of a total of those recorded as alive, dead or missing, was analysed as a binomial Generalised Linear Model (GLM), with a logit link, using Genstat 10. The model included the two species of thrips, the biological agents and control, and interactions between them. Results from experiments involving entomopathogenic nematodes were compared to the water control; those from experiments involving macro biocontrol agents were compared to the predator free control.

Results and Conclusions:
The accumulated analysis of deviance showed a statistically significant interaction between Treatment and Thrips (F=3.77, df=4,90, p=0.007). For the two main effects, Treatment (F=12.14, df=4,90, p<0.001) was significant but Thrips (F= 1.49, df=1,90, p=0.226) was not. The only difference between thrips for any treatment is for the nematodes, where significantly higher mortality was recorded for *T. palmi* than for *T. nigropilosus*. When offered *T. palmi* as prey no significant difference in thrips mortality was recorded following exposure to the three BCA's, but in each case thrips mortality was significantly higher than in the control. When *T. nigropilosus* was offered as prey, exposure to *Atheta coriaria* was followed by significantly higher thrips mortality than was recorded in all other treatments.

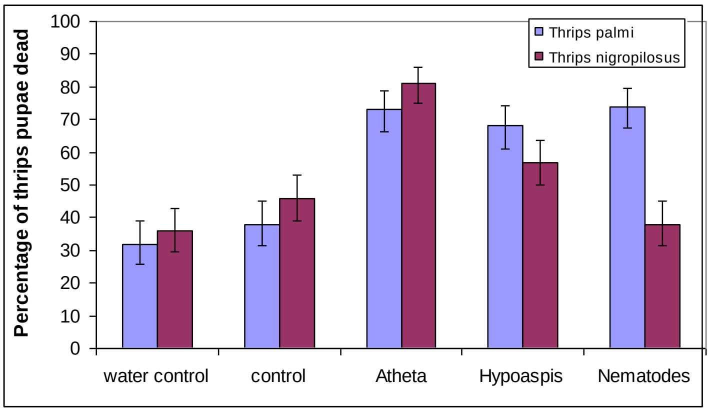


Fig 6. The percentage of dead *T. palmi* or *T. nigropilosus* recorded following exposure to *A. coriaria*, *H. miles* and *S. carpocapsae*.

Mutual compatibility of Atheta coriaria, Hypoaspis miles *and* Steinernema carpocapsae (milestone 9, 11 and 12)

The results of feeding experiments indicate that both *Atheta coriaria and Hypoaspis miles* have the potential to be utilised as a component of the IPM system under development. Experiments were therefore conducted to investigate the potential for utilising these soil dwelling biocontrol agents in parallel.

Experimental details:

Experiments on the compatibility of adult stages were conducted within soil-filled 15cm Petri dishes into which were placed a single adult *A. coriaria* and 10 adult *H. miles*. The lid of the dish was replaced, sealed with parafilm and incubated at 21°C, 65% r.h. 16: 8h L:D. The control followed the same procedure except that arenas contained only *A. coriaria* or *H. miles*. After 48 hours the number of *H. miles* alive in each arena was recorded. The experiment was repeated with a single *H. miles* and 10 *A. coriaria.*

Investigations of interactions between adult and larval life stages of *Atheta coriaria* and *Hypoaspis miles* also used a similar procedure except that a single *A. coriaria* adult and 5 *H. miles* larvae (or a single adult *H. miles* and 5 larval *A. coriaria)* were placed in each arena. Controls consisted of arenas containing only *H. miles* or only A. *A. coriaria* larvae (N=10). After 48 hours larval survival was recorded.

To investigate interactions with *S. carpocapsae*, the nematode was first applied (at the recommended rate) using a Hoselock Polyspray 2 hand held sprayer to soil-filled 15cm Petri dishes after which either a single adult *A. coriaria* and *H. miles* were added. The lid of the dish was replaced and sealed with parafilm. Mortality of *A. coriaria* and *H. miles* was assessed after 48 hours incubation under the conditions noted above. Controls used the same procedure except that the arenas were sprayed with water only (wet control) or remained unsprayed (dry control).

The data were analysed by logistic regression (generalized linear model) assuming a binomial distribution and with a logit link function using Genstat 10.2 (VSN International Ltd).

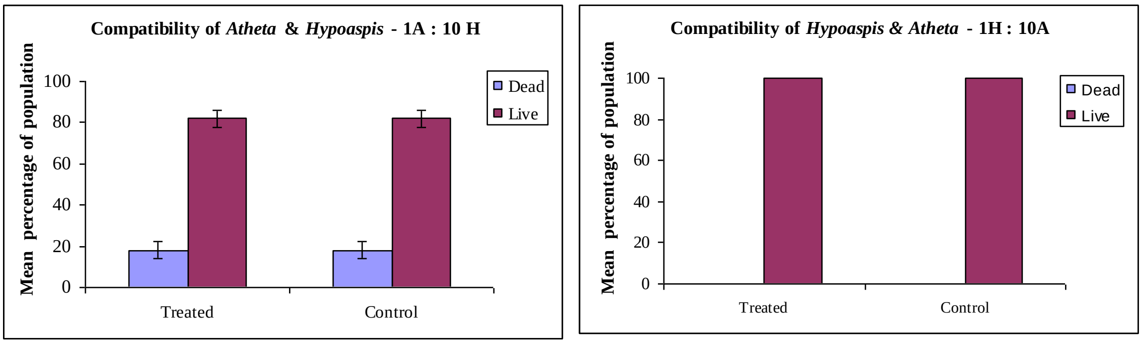


Fig 7 Survival of adult *H. miles* and *A. coriaria* when confined in experimental arenas (a) 1 adult *A. coriaria* and 10 adult *H. miles* and (b) 1 adult *H. miles* and 10 adult *A. coriaria*

Results and conclusions:

No interactions were recorded between adult *A. coriaria* and adult *H. miles* (Fig. 7) with survival of both species when exposed to each other being similar to that in control treatments. However, exposure to adult *H. miles* reduced survival of larval *A. coriaria* (Fig. 8). Exposure of *H. miles* larvae to a single *A. coriaria* adult resulted in no significant difference in the number of dead larvae recorded when compared to the control (F=0.2, d.f. = 1,38, p=0.661). However, significantly more *A. coriaria* larvae were recorded than in the control when they were exposed to a single *H. miles* adult (F=19.41, d.f. = 1,38, p<0.001). Thus the parallel use of these two predators in an IPM strategy could result in significant impact on the population establishment of *A. coriaria* used for prophylactic control of soil dwelling stages of *T. palmi* (category 2) of the IPM strategy.

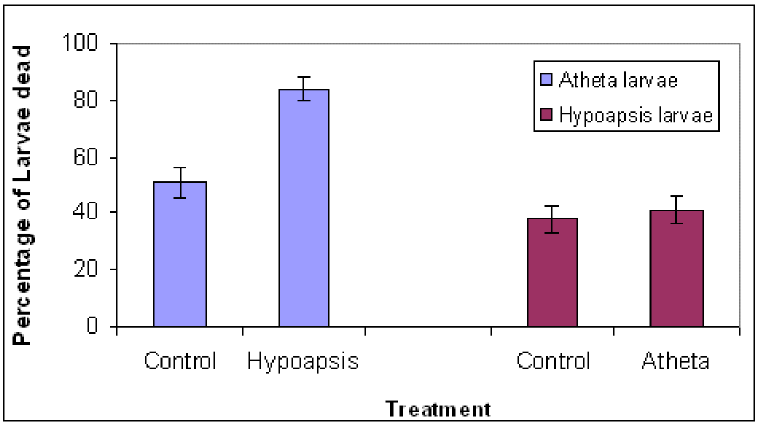


Fig. 8. Mortality of *A. coriaria* or *H. miles* larvae when confined in experimental arenas with a single adult of the second species of predator.

Exposure to arena soil to which *S. carpocapsae* had been applied had no adverse effects on adult *A. coriaria* or adult *H. miles* with survival rates being similar to the control treatments in both cases (Fig. 9).

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. A full report of the work is too extensive for the space allowed in this SID5 form, so on the advice of the with the project officer, is provided in Appendix II. The study showed that male *Thrips palmi* produce a chemical that is not produced by females or 2nd instar larvae. Chemical analysis has shown that the compound is an ester of a monoterpene alcohol and an unsaturated 5 carbon fatty acid, i.e. it is a monoterpene pentenoate with a molecular weight of 236. This appears to be analogous to the situation in *Frankliniella occidentalis* where males produce the aggregation pheromone neryl (*S*)-2-methylbutanoate. This compound is also an ester of a monoterpene alcohol (nerol) and a saturated 5 carbon fatty acid (methylbutanoic acid), i.e. it is a monoterpene pentanoate with a molecular weight of 238.

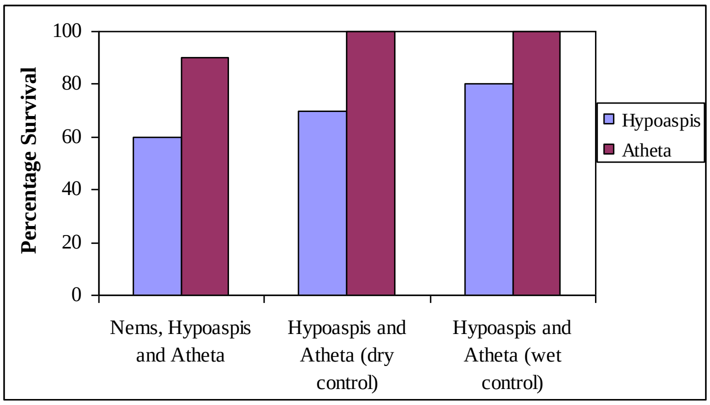


Fig. 9. Survival of adult *H. miles* and *A. coriaria* when exposed to the entomopathogenic nematode *S. carpocapsae* in experimental arenas.

<u>Use of multiple species of soil dwelling natural enemies in parallel (Milestone 9, 11 and 12)</u>:
The results of feeding experiments indicate that exposure to both *A. coriaria* and *H. miles* were followed by reduced pupal survival of both *T. palmi* and *T. nigropilosus* in laboratory experiments. Similarly, exposure to *S. carpocapsae* was followed by significantly reduced pupal survival of T. palmi. However, little information was available on the potential for using A. coriaria and H. miles in parallel within an IPM system. Experiments were conducted to investigate whether mutual interference may limit the efficiency of these two predatory species as control agents for thrips. As any use of *S. carpocapsae* in the IPM system was likely to be separated from use of *A. coriaria* or *H. miles* in time or space, assessments of impact of combined treatments involving the entomopathogenic nematode were not undertaken.

Experimental details:
Due to a limited *T. palmi* supply from the source in Japan, this experiment series was conducted using the model species *T. nigropilosus*. To investigate interactions between the soil dwelling predators *A. coriaria* and *H. miles* when offered *T. nigropilosus* pupae, trials were conducted using standard Petri dish experimental arenas. A single individual of either *A. coriaria* or *H. miles* was confined on compost (21 °C, 16:8 L:D) in each arena with 10 pupae. In a third set of arenas, pupae were exposed to both predatory species. Trials consisted of 10 replicates for each predator and were assessed after a five day exposure period, when the number of live, dead and missing (all body parts consumed) pupae was recorded. Controls followed the same procedure except no predators were released into arenas. To allow comparison with the effect of exposure of thrips pupae to S. carpocapsae, the entomopathogenic nematode was first applied (at the recommended commercial rate) using a Hoselock Polyspray 2 hand held sprayer to soil filled-15cm Petri dishes after which 10 pupae were added. The lid of the dish was replaced and sealed with parafilm and incubated under standard conditions until mortality was assessed.

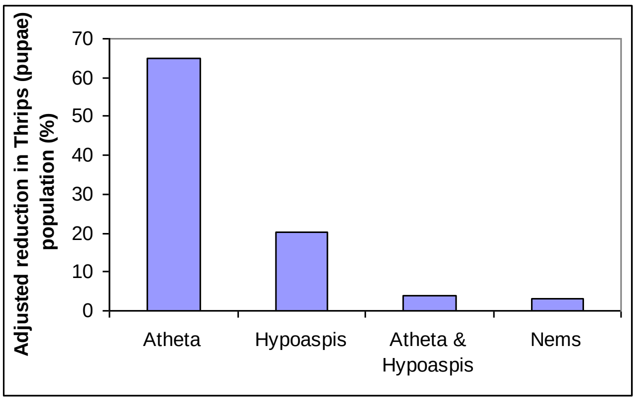


Fig. 10. Adjusted (Abbots formula) reduction in the survival of *T.nigropilosus* pupae following single or combined exposure to *A. coriaria*, *H. miles* and *S. carpocapsae*.

Abbot's formula (1-((treated alive/treated N)*(control alive/control N)))*100) was applied to the data generated to adjust for mortality not associated with the treatment (i.e the natural mortality in the untreated control), enabling comparison of the mean reduction in population for each treatment.

Results and conclusions:
Exposure of pupae to *A. coriaria* alone resulted in 64.81% mortality of pupae under the experimental conditions, significantly higher than that recorded for *H. miles* (20.37%). However, when thrips pupae were exposed to a combination of *A. coriaria* and *H. miles* mortality was significantly lower (3.70%) than when exposed to either species on their own. Mortality of predators was not apparent (supporting earlier results indicating high levels of survival when adults were confined with eachother), suggesting another mechanism such as behavioural interference may explain the result. Thus further evidence was obtained of the need for caution if parallel use of these two predatory species within the IPM system under development is considered.

Low mortality of *T. nigropilosus* pupae (3.13%) was recorded following exposure to *S. carpocapsae* reflecting the results of feeding experiments conducted using this thrips species.

C) CONFIRMATION OF AGENT SELECTION FOR USE IN THE MODULAR IPM PROGRAMME (MILESTONE 8 AND 10)

Following the generation of new data, the selection of control agents for use in the IPM protocols under development was reviewed and confirmed. The different constraints and morphological characteristics of newly planted and established crops resulted in the decision being taken to write separate protocols for addressing *T. palmi* infestations found in each (see Section E). Each was structured to enable both integrated use of conventional pesticides and biological agents, and (by selecting only biological agents under the various actions noted) a chemical-free option. This meets the requirements of milestone 13.

Newly planted crops:
The conventional pesticides selected for investigation have all been confirmed for either use in conjunction with biological agents during the crop growth period or as part of a final clean up treatment after outbreaks have been brought under control. The protocols recommend use of appropriate pesticides for each action based on their specific characteristics.

Prophylactic control on flowers/foliage:
Amblyseius cucumeris: Used to protect against juvenile instars of thrips hatching from eggs.
Amblyseius swirskii: Recommended for use during the summer months to protect against juvenile instars of thrips hatching from eggs.
Amblyseius montdorensis: Use in summer to provide additional protection to the tops of crops.

Prophylactic control on the ground:
Atheta coriaria: Used *to* control *T. palmi* when they drop to the floor to pupate.
Hypoaspis miles: Used to control *T. palmi* when they drop to the floor to pupate
Steinernema carpocapsae: A possible alternative in organic soil grown crops but not included as a main recommendation in other crops due to commercial husbandry considerations.

Remedial control on flowers/foliage:
Lecanicillium muscarium: To assist other biological agents by slowing down pest populations. If *Beauvaria bassiana* becomes available for use in the UK then a review of published data may indicate that it could be considered as an additional agent for *T. palmi* control.

Remedial control:
Atheta coriaria: Use as a component of "clean up" treatments
Steinernema carpocapsae: Use as a component of "clean up" treatments

Established crops: Prophylactic control on flowers/foliage:
Amblyseius cucumeris: Use to provide continued protection against juvenile thrips on leaves.
Amblyseius montdorensis: Use to provide additional protection in the tops of plants.
Amblyseius degenerans: Possible alternative to *A. montdorensis* but as it is reported to be most successful in pollen rich crops it was not included in protocols for cucumber.

Prophylactic control on the ground:
Atheta coriaria: Used *to* control *T. palmi* when they drop to the floor to pupate.
Hypoaspis miles: Used *to* control *T. palmi* when they drop to the floor to pupate

Remedial control on flowers/foliage:
Lecanicillium muscarium: To assist other biological agents by slowing down pest populations. The above comments concerning *Beauvaria bassiana* may also be relevant for established crops.

Leaf area size and the relevance to release rates of biological agents for thrips species (milestone 13)

Release of predatory mites for biological control of pests of cucumbers in commercial production usually occurs during the first seven days of growth in the production house. Following this period, the rapid growth of plants results in the leaf area being too large (Fig. 11) for mites introduced at an economically viable rate to search effectively, and thus pest survival remains high. Even during the 7-day application window, release rates that are too low will result in less effective control for similar reasons. As environmental conditions result in leaves from plants grown in commercial productions systems being larger than those utilised in the laboratory, the results of the laboratory experiments conducted under this project need to be converted into effective release rates for use in production houses. To provide a basis for translating the results of laboratory experiments to release rates of predatory mites for the control of *T. palmi* in commercial glasshouses, leaf area measurements were required to calculate predator density.

Experimental details:
Estimates of the leaf areas of plants used in laboratory experiments were taken from plants on day 14 of experimental runs (21 days after sowing: the point at which control agents were released onto the plants) and again on day 28 of experimental runs. All the leaves were removed from each of nine cucumber plants (cv. Shakira). Leaf area (laminar only) was then measured using a leaf area machine (LI-3100 Area Meter) to provide an estimate for the abaxial surfaces of the leaves. The mean total leaf area per plant was then calculated.

Measurements were also taken from plants grown in commercial production systems. All the leaves were removed from each of five cucumber plants (cv. Shakira). Leaf area (laminar only) was then measured by tracing each leaf onto squared paper and measuring the total area of the paper to provide an estimate for the abaxial surfaces of the leaves. The mean total leaf area per plant was then calculated. Measurements were taken from plants at the point of delivery from the propagators and on a second set of plants after 7-day growth within the production house (the period during which mites would normally be released). The point of delivery was 21 days after sowing.

Fig. 11. Leaf area of cucumber plants in a commercial production house (A) 7 days post delivery (B) 14-days post delivery

Results: At the point of delivery to the production house a mean leaf area of 970 cm^2 per plant was recorded from plants grown under commercial conditions (Table 1). This rose to a mean of 3960 cm^2 per cucumber plant after seven days growth in the production house. In both data sets plants displayed a strong uniformity in total leaf area, an important objective of growers and justifying the level of replication.

Table 1: The mean total leaf area (± standard error) per plant (cucumber, cv. Shakira) from plants grown under commercial and laboratory conditions. Plants from laboratory experiments: Measurements taken at the point of release of biocontrol agents (Leaf area 1) and end (Leaf area 2) of the experiment. Plants from commercial production: Measurements taken at the point of delivery to propagators (Leaf area 1) and 7 days post delivery (Leaf area 1). All measurements represent abaxial surface only.

Source	N	Leaf Area 1 (cm^2)	Leaf Area 2 (cm^2)
Laboratory Experiment	9	561.5 ± 97.3	1160.7 ± 25.85
Commercial Production	5	970.0 ± 29.8	3960.0 ± 202.4

Conclusions: The results indicate that laboratory grown plants at the point at which predatory mites were introduced in experiments had less than 60% of the leaf area recorded on plants in commercial production houses at the point predators would be released in the IPM strategies being developed under this project. Subsequent growth resulted in the leaf areas of production house plants increasing at a faster rate than those of the experimental plants. Thus after 7 days experimental plants had approximately 25% of the leaf area of equivalent plants in commercial glasshouses. Hence application rates of predatory mites used in the IPM strategies should be adjusted to ensure that densities per cm^2 of leaf area in commercial houses are similar to those used in experiments at the time of release. Refinement of release rates should be an objective of field testing of the protocols when suitable outbreaks occur and quarantine restrictions permit the work to be undertaken.

D) EXAMPLES OF DATA OBTAINED FROM THE PUBLISHED LITERATURE AND SOME UNPUBLISHED SOURCES TO SUPPORT IPM PROTOCOL DEVELOPMENT (MILESTONE 13)

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 precludes a comprehensive description being appropriate in the limted space available in this report. Appendix III illustrates the kind of the data/information obtained from these sources.

E) DRAFT PROTOCOLS FOR MODULAR-BASED CONTROL STRATEGIES ON CUCUMBER (MILESTONE 13)

The draft protocols are too long to be accommodated in the space available in this SID5 form and on the advice of the project officer, are provided in Appendix IV.

F) LABORATORY TESTS OF CUCUMBER BASED PROTOCOLS (MILESTONE 15)

Based on published literature and experimental work conducted under this project, protocols for control of outbreaks of *Thrips palmi* on cucumber have been developed. Comprehensive field tests of these protocols will require access to outbreaks of the pest in UK glasshouses as plant health restrictions preclude the articificial infestation of cucumber crops with *T. palmi* for experimental purposes. Accordingly the Steering Group for this project recommended and agreed the use of a model (indigenous) thrips species (*Thrips nigropilosus*) in laboratory experiments designed to test components of the IPM protocols. This experiment series investigates: a) The potential of each of the predatory mites to control thrips populations on whole cucumber plants without the use of other control agents and b) potential of the predatory mites to control thrips populations on whole cucumber plants in sequential application with chemical pesticides.

Experimental details:
Thrips nigropilosus used in the experiments were cultured following the technique of McDonald *et al*. (1999). Predatory mites *Thrips nigropilosus* used in the experiments were cultured following the technique of McDonald *et al*. (1999). Predatory mites (*Amblyseius cucumeris*, *A.* degenerans, *A. montdorensis, A.swirskii*) and pesticides were obtained from commercial suppliers.

Whole plant population control experiment:
Cucumber plants at the 2-3 true leaf stage were infested with 20 adult thrips, sealed individually inside breathable plastic bread bags and incubated at 21 ± 2°C, 65% relative humidity (r.h.), and 16:8 Light:Dark (L:D) for 2 weeks. Following this period, predatory mites (one of the selected species) were released into the bag at the recommended commercial rate before it

was re-sealed and the plants returned for incubation under the same conditions for a further 2 week period. After incubation plants were removed and the leaves and stems washed in 70% ethanol. The total of number of thrips present was recorded.

The above procedure was repeated except that after the first two week incubation period a chemical pesticide was applied at the recommended commercial rate. Three days later, predatory mites were released onto the plants, also at the commercial rate. Thrips numbers were assessed using the approach described above, following a further two weeks incubation. The experiment was repeated for each of the four predatory mites and two pesticides (spinosad as Conserve (0.8ml/L, *a.i.* 12% w/w, Fargro Ltd., UK); Imazalil as Fungaflor 100EC (1.0ml/L, *a.i.* 10% w/w, Certis, Amesbury, UK)).

Each trial was replicated 12 times, with controls following identical procedures with the exception that no predatory mites were applied (resulting in three controls). The experiment was repeated for each of the selected predatory mites.

The number of thrips found on the plants, were analysed as a Generalised Linear Model (GLM), utilising a poisson distribution, using Genstat 10. Post-hoc pair-wise comparisons are not available with GLM but a comparison of two means can be made using the tabulated standard errors of the differences for t-tests.

Results and Conclusions:

Analysis of the numbers of adult and juvenile thrips: The accumulated analysis of deviance showed a statistically significant interaction between Treatment and Predatory Mite ($F=12.74$, $df=8,164$, $p<0.001$) and the probabilities associated with these two as main effects were <0.001 and <0.001 respectively.

Within each chemical treatment, each mite species had significantly fewer thrips remaining on the plant when compared to the control, and each chemical treatment had significantly fewer thrips than the control treatment with no chemical ($p<0.05$), indicating a depression of population increases by the mites and confirming the insecticidal activity of both the fungicide and insecticide. Where no chemical treatment was applied, *A. degenerans* was followed by significantly higher numbers of thrips after the incubation period than the other three mite species, confirming the results of earlier feeding trials that indicated a lower potential impact of *A. degenerans* under the experimental conditions (particularly temperature) employed.
Where Fungaflor was applied, release of *A. cucumeris* and *A. montdorensis* was followed by significantly fewer thrips being recorded compared to *A. degenerans* and *A. swirskii*, as would be expected from the 72 hr mortality records in the indirect exposure compatibility trials reported above.

There were no significant differences between thrips numbers recorded on plants on which the different mite species were released following application of Conserve. A high depression of thrips numbers followed application of Conserve alone, and further reductions in thrips numbers were recorded following the release of each of the mite species.

For *A. cucumeris* and *A. degenerans* there was no significant difference between final thrips numbers in the Fungaflor and no pesticide treatments, but significantly higher numbers of thrips were recorded following both the Fungaflor and Control treatments than following the Conserve treatment. For *A. montdorensis* and *A. swirskii* the number of thrips recorded following the Fungaflor treatment was significantly higher than following the no pesticide treatment, which was in turn significantly higher than following the Conserve treatment

Thus highly significant depressions of thrips populations followed the use of all four species of predatory mites and the insecticide, and predatory mites could be used successfully in the presence of a dry residue of the product as recommended in the protocols. Use of the fungicide to control diseases whilst utilising the IPM protocols for *T. palmi* control will require care, and may result in a need to release further predatory mites after application. If an alternative fungicide more compatible with predatory mites becomes available, further testing to confirm its use in conjunction with the IPM protocols will be required.

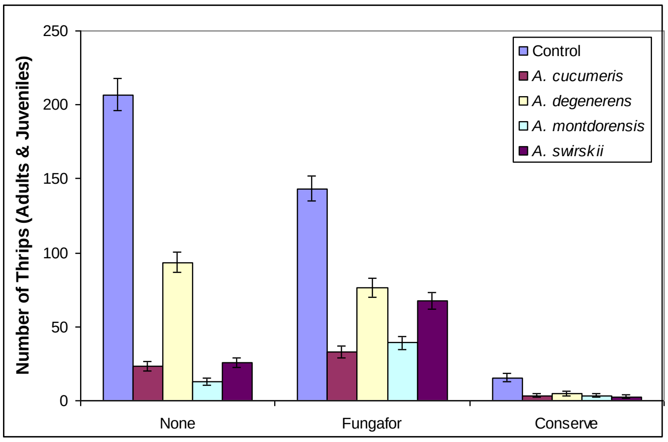


Fig. 12. The number of adult and juvenile thrips recorded in tests of the IPM protocols on whole cucumber plants. None = experiments investigating the impact of predatory mites on the number of adult and juvenile thrips (two weeks after the

release of mites) in the absence of pesticides; Fungaflor = experiments in which mites were released after a Fungaflor treatment has been applied; Conserve = experiments in which mites were released after a Conserve treatment has been applied. In each case controls received the pesticide treatment (if any applied) but no mites were released.

Analysis of the numbers of adult and juvenile thrips separately:
On the plants treated with Conserve, no significant differences were found between the final number of adult thrips recorded in the pesticide only control treatments or those in which any of the predatory mite species were released. However, when only the final number of juvenile thrips were analysed, there were significantly lower numbers recorded following release of all four mite species when compared to the insecticide only control. This suggests that at these low population levels the predatory mites were able to keep the juvenile numbers at exceptionally low levels under the experimental conditions.

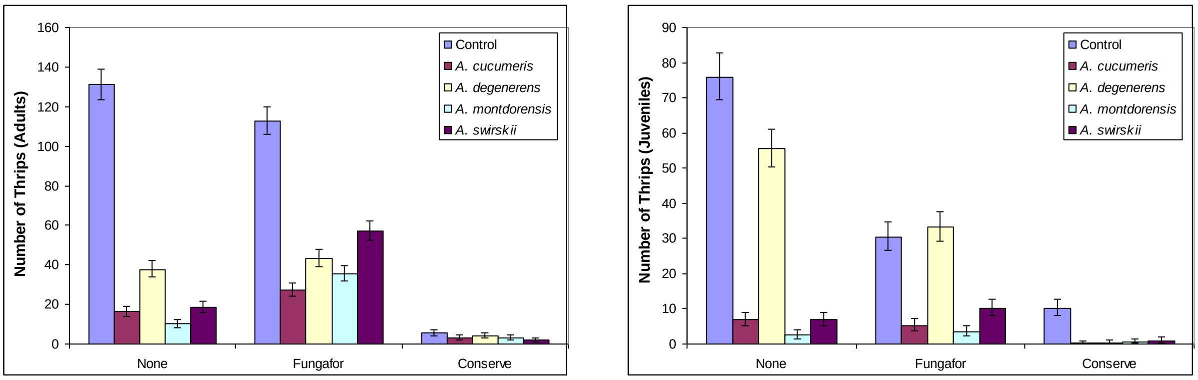


Fig. 13. The number of adult or juvenile thrips recorded in tests of the IPM protocols on whole cucumber plants. None = experiments investigating the impact of predatory mites on the number of adult and juvenile thrips (two weeks after the release of mites) in the absence of pesticides; Fungaflor = experiments in which mites were released after a Fungaflor treatment has been applied; Conserve = experiments in which mites were released after a Conserve treatment has been applied. In each case controls received the pesticide treatment (if any applied) but no mites were released.

G) POTENTIAL FOR EXTRAPOLATION TO OTHER CROP PLANTS WITH DIFFERENT LEAF ARCHITECTURE/STRUCTURE (Milestone 14)

Transportation and spread of quarantine pests on plants and plant products in global trade, coupled with increasing concerns about the widespread use of chemical pesticides for containment and eradication of outbreaks when they occur, and potential moves within the EU from risk to hazard based assessment for registration purposes (potentially resulting in a severely reduced range of active ingredients available) has led to the need to develop IPM strategies to provide alternatives to the use of chemical-only strategies. Such strategies can be time consuming and expensive to develop and research is usually constrained to their development for use on a single major crop. However, many of these strategies have potential for use (sometimes with changes to key components) on other crop plants, and supplementary work to identify and assess such potential is often valuable. Although cucumber crops were the main target agreed in the SID3 for this project, a small component was planned to consider whether the protocols established might be developed for application to another crop type in order to provide a basis for decisions on further research under other projects.

With regard to extrapolation between crops of the conventional pesticide components of the IPM strategies, a complex of factors collectively effect the relative efficacy against the target pest, which have recently been reviewed (Glass *et al.*, submitted) and therefore will not be discussed in detail here. However, as an example, studies have been done to evaluate the retention of pesticide spray on different types of leaf surfaces and crop canopies, and the role played by surface tension of the spray liquid and the droplet size used to deliver the pesticide. De Rutter *et al.* (1990) found that dynamic surface tension of the liquid was critical for droplet retention, but the nature of the cuticular surface was much more important than the properties of the liquid or the orientation of the leaf. Stevens et al. (1993) reported that dynamic surface tension was more important than droplet size and velocity in determining retention on pea leaves. Modern agricultural and horticultural practices tend to use lower volumes of carrier (water) to deliver the pesticide to the crop target. In the past, spraying to run-off was common practise, as it achieved good leaf coverage, but was also wasteful, with pesticide lost to the ground, with subsequent environmental consequences. The use of lower carrier volumes requires a better understanding of droplet impaction and retention which is beginning to emerge (Glass *et al.*, submitted) to achieve adequate leaf coverage at the critical positions within the crop canopy, supporting the level of optimisation of volume in application utilised in the cucumber protocols developed under this project. Similarly, a range of crop specific characteristics has been shown to affect the efficacy of biological agents for the control of various quarantine pests (e.g. Head *et al*., 2004), including various leaf surface characteristics such as density and size of leaf hairs, waxy surfaces, etc (MacVean *et al*., 1982). The above factors (and others) result in potential difficulties for direct extrapolation of the IPM technique developed on cucumbers to other canopy/leaf types, therefore screening tests to establish if further work is needed are required.

To investigate whether the IPM protocols developed for cucumber crops have the potential (with targeted research and amendment) to be used for the control of *Thrips* spp. on other crops with different leaf architecture/structure, experiments were conducted to test their efficacy on aubergine.

Experimental details:
A similar experimental approach was utilised to that described for whole plant tests of the IPM protocols developed in this study that were conducted on cucumber and which is described in section F. Tests on aubergine and cucumber were conducted using the insecticide Conserve and two predatory mites, *Amblyseius cucumeris* and *A. swirskii*. *Thrips nigropilosus* (cultured using the method indicated in section F) was used as the target pest.

Results and Conclusions:
The number of thrips found on the plants, were analysed as a Generalised Linear Model (GLM), utilising a poisson distribution, using Genstat 10. Post-hoc pair-wise comparisons are not available with GLM but a comparison of two means can be made using the tabulated standard errors of the differences for t-tests.

Comparison of Aubergine and Cucumber: There was a significant interaction between plant and predatory mite (F=68.47, df=2,131, p<0.001) and between treatment and mite (F=11.1, df=1,131, p=0.001). The main effects of both treatment and mite were significant (p<0.001), but plant was not (p=0.078).

On aubergine plants, when no pesticide treatment was applied, significantly fewer thrips were recorded after the incubation period following release of both predatory mite species than in the control. Significantly fewer thrips were recorded following the release of *A. cucumeris* than after exposure to *A. swirskii*. When the mites were released following the application of the insecticide Conserve, only treatments involving *A. cucumeris* were followed by significantly fewer thrips at the end of the incubation period than in controls.

Comparing the results from aubergine with those from cucumber the number of thrips on plants receiving no treatments (either predatory mites or insecticide) were significantly higher on cucumber than on aubergine. Without insecticide, treatments involving both mite species were followed by significantly higher numbers of thrips on aubergine than on cucumber. When the insecticide (Conserve) was used in the absence of mites there was no significant difference between the number of thrips recorded on aubergine or cucumber. Similarly there were no significances between thrips numbers on the two plants following release of *A. cucumeris*, but thrips numbers were significantly higher on aubergine compared to cucumber following release of *A. swirskii*.

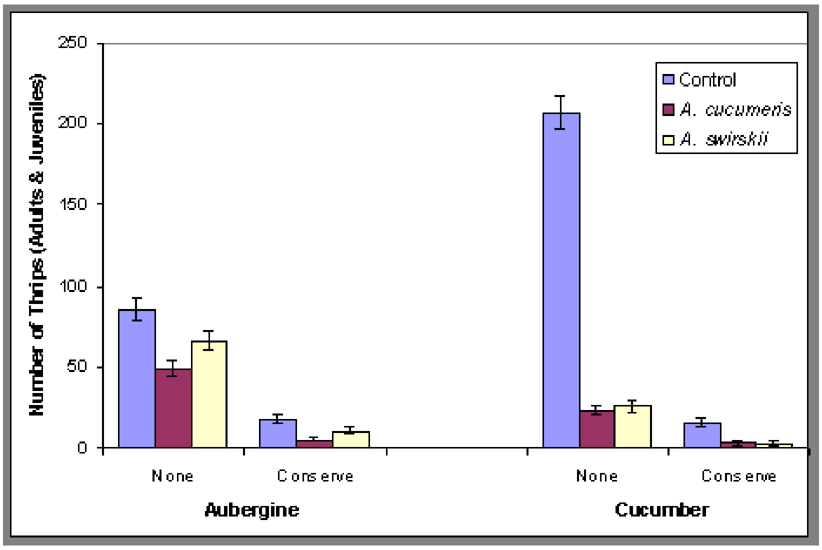


Fig. 14. The number of adult and juvenile thrips recorded in tests of the IPM protocols on whole aubergine or cucumber plants. None = experiments investigating the impact of predatory mites on the number of thrips (two weeks after the release of mites) in the absence of pesticides; Conserve = experiments in which mites were released after a Conserve treatment has been applied. In each case controls received the pesticide treatment (if any applied) but no mites were released.

Analysing adult counts alone there was a significant interaction between plant and mite (F=39.77, df=2,131, p<0.001) and between plant and treatment (F=31.05, df=1,131, p=0.001). The main effects of both treatment and mite are significant (p<0.001), but Plant is not (p=0.796). The results indicate that the differences between performance of the various control agents/product on the two host plants noted when adults and juveniles counts were combined (see above) were reflected when only adult counts were analysed except that in the absence of Conserve on aubergine no significant difference was found between the numbers of thrips recorded following exposure to the two predatory mites (in both cases numbers of live thrips were significantly lower than in controls).

Analysing juvenile counts alone there was a significant interaction between plant and mite (F=52.21, df=2,131, p<0.001) and between plant and treatment (F=4.64, df=1,131, p=0.033). The main effects of both treatment and mite were significant (p<0.001), and for juveniles there was a clear effect of Plant (p<0.001).

Once again the differences between performance of the various control agents/product on the two host plants noted when adults and juveniles counts were combined (see above) were reflected when only juvenile counts were analysed except that no differences between mite species were recorded after conserve but there was a significant difference following application of conserve in the absence of predatory mites (fewer on aubergine).

Thus tests indicated that significant control of *Thrips* spp. can be achieved using similar methods on both aubergine and cucumber, indicating that there is potential for the IPM method developed under this project for cucumber to be transferred to other crop plants. However, significant differences in the efficacy of individual components on the two plants (e.g. predatory mites were less effective on aubergines) suggest that direct extrapolation from cucumber to other crops may carry risks, and that targeted work to tailor the protocols for the new crops will be required. Such work may involve, for example, selecting appropriate control agents in relation to leaf structure, crop architecture and a range of other physical and biological characteristics of the crop and conducting confirmatory tests of their efficacy. Thus the principles of a modular IPM strategy that have been established under this project are transferable to a range of crop types, but the specific components will currently require further testing in each case.

H) COST-BENEFIT ANALYSIS OF THE MODULAR-BASED CONTROL STRATEGIES ON CUCUMBER (MILESTONE 16)

The cost-benefit analysis conducted is too extensive to be accommodated in the space available in this SID5 form and is provided in Appendix V.

I) POLICY IMPLICATIONS OF THE WORK

Thrips palmi Karny is listed in the EC Plant Health Directive (2000/29/EC) in Annex IAI, as it is recognized as a harmful organism which is not known to occur in the EU, and therefore its introduction and spread within all EU member states is banned. Thus, *T. palmi* is liable to eradicatory action wherever it is found in the EU.

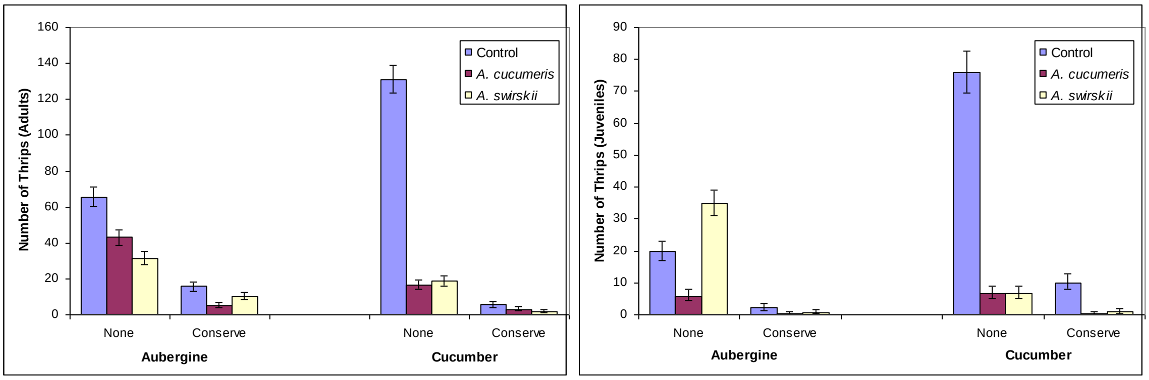


Fig. 15. The number of adult or juvenile thrips recorded in tests of the IPM protocols on whole aubergine or cucumber plants. None = experiments investigating the impact of predatory mites on the number of thrips (two weeks after the release of mites) in the absence of pesticides; Conserve = experiments in which mites were released after a Conserve treatment has been applied. In each case controls received the pesticide treatment (if any applied) but no mites were released.

The occurrence of the pest in 2000-01 in a UK outbreak in a chrysanthemum crop resulted in a variety of chemical insecticides being registered and available for use in this situation (Cannon et al., 2007b), but some of these have since been revoked or rescinded. If an outbreak of *T. palmi* occurred in an nursery growing edible crops, the chemicals options available would be very slim, and in the absence of effective biological control agents, could result in the most drastic and expensive outcome, i.e. crop destruction.

Cox *et al.* (2006) concluded that sustainable, biologically based pest control programmes against *T. palmi* will need to depend on a suite of natural enemies with complementary life styles. In situations where the pest is established – such as Japan – biological control systems are in general less well-developed than in Western Europe, and furthermore, utilise native biological control agents (BCAs) which are both unavailable and unregistered in the UK. As such they are classified as non-native organisms; they would require extensive testing and regulatory approval via the UK Advisory Committee on Releases to the Environment (ACRE) before they could be released).

The current research has reviewed, selected and tested a suite of biological control agents which can be used as components of an IPM system, targeting pest life stages that occur on both the aerial parts of the plant and soil dwelling stages. All are currently registered for use in the UK. They provide a basis for both prophylactic and eradicatory techniques that can be applied under a range of conditions. As it is unlikely that biological agents alone will be successful in exerting sufficient control to meet Plant Health requirements for eradication, compatibility tests have also been conducted that have defined how they can be used in conjunction with conventional (chemical) insecticides registered for use in cucumber crops. Pesticides tested not only include those used for the control of thrips species but in addition , recognizing the need to simultaneously control other pest species such as aphids and fungi, a commonly used aphicide and fungicide.

The outputs of the research project have been used to write a modular protocol for the use of the biological agents and conventional insecticides in a combined IPM protocol for the control of *T. palmi* in cucumber crops. Having been developed in advance of an incursion, it has enabled contingency plans to be put in place by the Plant Health Action Management team, which would minimize any potential impacts of this pest on the Industry. It has provided the basis for more effective, and safer controls methods, which would allow eradication and or containment of the pest whilst harvesting and cropping continued, thus minimizing losses and limiting expenses incurred by control measures, until such time that the pest was eradicated or a crop break could occur, when clean up measures could be carried out.

This protocol could be extended, with further research, to other quarantine thrips species and other glasshouse situations (e.g. other crops, large plants in publicly accessible glasshouses) to provide a sound basis from which to address incursions into the UK of quarantine organisms, thus improving further the protection of UK industry and trade.

J) PUBLICATIONS EMERGING FROM THE PROJECT AND OTHER TECHNOLOGY TRANSFER

Examples of technology transfer activities are listed in Appendix VI.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

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