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

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PRACTICAL SECTION FOR GROWERS

Commercial benefits of the project

This project investigates the potential of fungal pathogens (known as 'entomopathogens') to provide control of spider mites in tomato crops. The project is strategic in nature and was set up with the support of the British Tomato Growers' Association (TGA) for the purposes of replacing the use of chemical pesticides in UK tomato production. The TGA's motives for pursuing this biological strategy are not to reduce the cost of spider mite control or to increase yield, but to prepare for changing customer demands and thereby ensure that the existing market will be retained.

Two years into the project, the work has successfully identified the product Naturalis-L, based on a specific strain of *Beauveria bassiana* (an entomopathogenic fungus), as a potential remedial treatment for the control of spider mites in tomato crops. Growers will need to await the completion of the work and the approval of use of Naturalis-L in the UK before the results from the project can be fully utilised.

Background and Objectives

To help the tomato industry to achieve their long term aim of pesticide-free crop production, it is necessary to develop a 'biopesticide' to support primary control of spider mites using invertebrate predators. Entomopathogenic fungi have been developed as biopesticides for a number of protected crops, both in the UK and USA. Although spider mites are known to be susceptible to entomopathogenic fungi, little research has been done on their potential as biopesticides of these pests.

The overall aim of this project is to examine the potential of entomopathogenic fungi as biopesticides of spider mites.

Summary of results

<u>1. Identifying suitable fungal pathogens (entomopathogens) for the control of spider mites</u>

In Year 1 of the project, 40 candidate isolates of fungi from nine different species were screened against the two-spotted spider mite in laboratory experiments. The fungi exhibited a wide range of pathogenicities to the mites. Six of the more effective isolates were chosen for further study in Year 2 (*Metarhizium anisopliae* 442.99, *Verticillium lecanii* 450.99,

V. lecanii 19.79, *Hirsutella thompsonii* 463.99, *Hirsutella* sp. 457.99, *Beauveria bassiana* 432.99). *Verticillium lecanii* 19.79 and *B. bassiana* 432.99 are used in the proprietary biopesticides Mycotal and Naturalis-L, respectively. *Hirsutella thompsonii* 463.99 is thought to be derived from the commercial mycopesticide, Mycar, which is no longer in production.

In year 2 of the project, the six isolates of fungi were examined against the two-spotted spider mite in a multiple dose laboratory bioassay to provide an indication of the concentration of spores required to control the mites. This ranged from 8.5×10^{7} ml^{-1} for *M. anisopliae* 442.99 to 1.3×10^{18} ml^{-1} for *Hirsutella* sp. 457.99. *Verticillium lecanii* 19.79 and *Hirsutella* sp. 457.99 returned poor dose responses and were eliminated from further studies.

2. Selecting the most effective fungal pathogen for the control of spider mites

Research workers in the USA have reported reduced effects of entomopathogenic fungi on insects feeding on tomato leaves compared to cucumber leaves. It has been proposed that certain chemicals in tomato leaves are taken in by insects and provide a form of induced resistance to the fungi. Laboratory experiments were conducted in this project to determine whether the efficacy of the fungi against spider mites would vary on different types of tomato (cherry, round, beefsteak and large truss). The results indicated that tomato variety and type had relatively little effect on fungal infectivity. This means that the results of the bioassays done on cultivar Spectra are relevant to other cultivars.

There are different species and colour forms of spider mites found on tomatoes in the UK. Some cause the normal ‘speckling’ on leaves, while others cause much more severe damage known as hyper-necrosis. The initial laboratory experiments had all been done on a strain of two-spotted spider mite that had only been associated with the normal damage symptoms. A further laboratory experiment compared the effects of the four entomopathogenic fungi on this strain of two spotted spider mite with a strain of carmine spider mite that was known to cause hyper-necrosis. There was no significant difference in the susceptibilities of the two strains of spider mites to the entomopathogenic fungi, thus indicating that the results of bioassays done on one strain of spider mites may be extrapolated to others.

An experiment that was not originally included in the work plan explored the significance of the method by which spider mites become infected by entomopathogenic fungi. Batches of spider mites were either sprayed directly and then transferred to untreated leaves, or left

unsprayed and placed on treated leaves, thus comparing the effects of direct application with spores, with 'secondary pick-up' of spores from the leaf surface. The indirect spray technique improved the virulence of *B. bassiana* 432.99 by more than 40%, and increased the virulence of the proprietary biopesticide Naturalis-L by 66%. By contrast, the virulence of *M. anisopliae* 442.99 was similar with both application techniques. This suggests that the spores of some entomopathogenic fungi may be specially adapted to enhance secondary pick-up from the leaf surface. Such fungi should be particularly well suited for use against spider mites on tomato plants.

In glasshouse experiments, all the entomopathogenic fungi tested significantly reduced spider mite populations on mature tomato plants. The most effective treatment was *B. bassiana*, applied as the Naturalis–L product, which reduced the spider mite population by 90% on the upper leaves on the plant.

The formulation of the entomopathogenic fungi is a key factor, since the formulated commercial biopesticide Naturalis-L performed better in our experiments than its unformulated active ingredient.

3. Selecting fungal pathogens that are compatible with integrated pest management (IPM) programmes in tomato production

To integrate entomopathogenic fungi successfully into tomato IPM programmes, it is essential to determine the compatibility of the fungal pathogens with biocontrol agents already in use. A literature review has compiled information about the known effects of such fungi on 12 commonly used biological control agents. Important information is available for *Encarsia formosa* and *Aphidius colemani*. However, more information needs to be sought for *Phytoseiulis persimilis* in particular.

Conclusions

1. Four isolates of entomopathogenic fungi were identified as having potential to control spider mites in tomato crops.
2. In both laboratory and glasshouse studies, the commercial product Naturalis-L (based upon a specific strain of *Beauveria bassiana*) proved to be the most effective fungal pathogen in the control of spider mites. However, Naturalis-L is not currently approved for use in the UK.

3. Tomato variety and type is unlikely to have an effect on the efficacy of fungal pathogens in controlling spider mites.
4. Fungal pathogens should be equally as effective in controlling hyper-necrotic spider mites as well as the normal forms of spider mites found on tomato crops.
5. It is fortunate that indirect spray applications, where the spider mites pick up the fungal spores that are sprayed on to tomato leaves, increases the level of kill for certain fungal pathogens and in particular *Beauveria bassiana* (the fungal strain in Naturalis-L).
6. Information gathered to date on the compatibility of *Beauveria bassiana* with other natural enemies used in integrated pest management programmes for tomato crops, is mainly based on *Encarisia formosa* and *Aphidius colemani*. Information on compatibility with *Phytoseiulus persimilis* is needed in particular.
7. Studies in the final year of the project will evaluate the efficacy of Naturalis-L (based on *Beauveria bassiana*) versus the acaricide, fenbutatin oxide, to act as a remedial treatment for the control of spider mite in an experimental tomato crop. The compatibility of Naturalis-L with *Phytoseiulus persimilis* in a crop situation will also be studied.

Action points for growers

This project is strategic in nature and was set up with the purpose of replacing the use of chemical pesticides in UK tomato production. Two years into the project, the work has successfully identified the product Naturalis-L, based on a specific strain of *Beauveria bassiana* (an entomopathogenic fungus), as a potential remedial treatment for the control of spider mites in tomato crops. Growers will need to await the completion of the work and the approval of use of this product in the UK before the results from the project can be fully utilised.

Anticipated practical and financial benefits

The development of a biopesticide of spider mites would be a significant step forward in the quest for pesticide-free tomato production and will provide UK growers with a considerable marketing advantage. A biopesticide of spider mites will also be beneficial to growers of other protected salad crops and growers of ornamental crops who are moving towards Integrated Pest Management (IPM).

SCIENCE SECTION

INTRODUCTION

Fungal biocontrol of spider mites

Spider mites remain one of the most serious pests of tomato crops in the UK. Control is based on the combined use of the predatory mite, *Phytoseiulus persimilis*, reinforced with sprays of the selective acaricide, fenbutatin oxide (Torq). However, *P. persimilis* is slow to establish on tomato plants and does not keep pace with the pest's development during hot conditions. Therefore control can be heavily reliant on applications of the acaricide. Unfortunately, fenbutatin oxide does not perform well against all pest populations and resistance is developing (Jacobson *et al*., 1999). The use of new acaricides, such as abamectin, could help alleviate this problem in the short-term (Jacobson *et al*., 2000a) but is unlikely to be sustainable given the propensity of spider mites for developing chemical resistance. There is a requirement therefore to develop an effective system for control of the two-spotted spider mite, *Tetranychus urticae*, on tomato without the use of chemical pesticides. The industry also has the long-term aim of pesticide free tomato production to meet underlying public concerns expressed about the environmental impact of pesticides and negative publicity about residues in produce. Non-chemical control of spider mites on tomato would enable the industry to achieve this goal.

On protected crops at least, the most effective method for chemical-free control of *T. urticae* is likely to be based on a suite of complementary natural enemies (Jacobson, 1998). This suite should include a microbial biopesticide that acts as a remedial treatment and replaces the chemical acaricides that are currently used as a second line of defence. This approach has been studied for other pests of protected crops (e.g. control of western flower thrips, *Frankliniella occidentalis*, on cucumbers) and can work well (Jacobson *et al*., 2001).

Entomopathogenic fungi are good candidates for this biopesticide role but there have been very few investigations of their effectiveness against spider mites. *Neozygites floridana* (Zygomycetes, Entomophthorales) is a natural regulator of tetranychid mites in warm-temperate regions, but it cannot be cultured easily *in vitro* and therefore is not yet suitable for development as a microbial biopesticide (Chandler *et al*., 2000). *Hirsutella thompsonii* (Mitosporic fungi, Ascomycetes), a specific pathogen of eriophyoid and tetranychid mites,

killed *T. urticae* and the closely-related carmine spider mite, *Tetranychus cinnabarinus* in laboratory experiments (Gerson *et al*., 1979; Gardner *et al*., 1982). An isolate of this fungus was developed as the proprietary biopesticide Mycar (Abbott Laboratories, USA) for management of the citrus rust mite, *Phyllocoptruta oleivora* (Eriophyidae) in the USA (Bergh & McCoy, 1997) although was not used for *T. urticae* control.

Elsewhere, Naturalis-L (Troy Biosciences Inc., USA), a proprietary biopesticide based on *Beauveria bassiana*, reportedly gave good control of *T. urticae* on glasshouse roses (Wright & Kennedy, 1996). *Beauveria bassiana* has also been studied as a potential biopesticide of the cassava green spider mite in Kenya (Bartowski *et al*., 1988). The tarsonemid broad mite, *Polyphagotarsonemus latus*, was susceptible to *B. bassiana* derived from a hymenopteran host, as well as an isolate of *Paecilomyces fumosoroseus* derived from a homopteran host, in laboratory experiments (Pena *et al*., 1996).

Scientific / technical targets of the project

The aim of this project is to examine the potential of entomopathogenic fungi as biopesticides of spider mites. The objectives of the project are as follows:

1. Identify and obtain species / isolates of entomopathogenic fungi that have potential for the control of spider mites.
2. Quantify the effect of selected fungi on spider mites in laboratory bioassays.
3. Examine the compatibility of selected fungal strains with biological control agents used in tomato integrated pest management (IPM) programmes.
4. Select and evaluate a fungal strain with potential for control of spider mites within IPM programmes in glasshouses, and prepare guide-lines for its use in tomato IPM programmes.

Summary of progress in Year 1 (first Annual Report)

Forty candidate isolates of fungi from nine species were obtained for screening against spider mites. The fungi were obtained from culture collections identified through the internet or scientific literature. Most of these fungi originated from mites or ticks, while others originated from insect hosts but were known from the literature or personal communications to kill mites. Fungi used in seven proprietary biopesticides were also included.

A laboratory bioassay was developed to measure the effect of conidia of entomopathogenic fungi on the survival of *T. urticae*. Fixed age cultures of adult female spider mites were sprayed with a suspension of conidia, then maintained on a tomato leaf held under controlled conditions of temperature and humidity. This method was used to screen the 40 candidate isolates of fungi against *T. urticae*, using a single dose of fungal conidia (1×10^7 ml^{-1}). The bioassay allowed a high throughput of candidate fungal isolates, and we were able to examine more isolates than originally envisaged. The fungi exhibited a range of pathogenicities to the mites, and estimated mean mortalities due to fungus treatment at 6 days post inoculation (adjusted for controls and block effects) ranged from 43.23 to 6.66%. Six isolates were chosen for further study in multiple dose bioassays in Year 2 (see Table 1). These isolates included four that came in the top five pathogenicity rankings, four of which were originally isolated from mite or tick hosts. Two of the six isolates are used in proprietary biopesticides.

While the literature on the susceptibility of spider mites to entomopathogenic fungi is somewhat scant, the information that is available suggests that *T. urticae* should have been more susceptible to infection than observed in our bioassays (Gerson *et al*., 1979; Gardner *et al*., 1982; Bartowski *et al*., 1988; Wright & Kennedy, 1996; Pena *et al*., 1996). This apparent suppression in fungal virulence may have been caused by the action of tomato allelochemicals, which have known antifungal activity, or be an artefact of the bioassay methodology. Both factors were investigated in this second phase of the study.

Background to second Annual Report and adjustment of Milestones

New information from the literature became available in late 2000 indicating that tomato secondary compounds could have a detrimental effect on the virulence of entomopathogenic fungi applied to insect pests feeding on tomato plants (Poprawski *et al*., 2000). Project Milestones were adjusted at a project review meeting on 6 December 2000 and in a Contract Amendment (15 August 2001) to incorporate laboratory experiments to compare the virulences of fungi to *T. urticae* feeding on tomato and bean plants.

This project was originally based at HRI Stockbridge House and HRI Wellesbourne. The project was relocated solely to HRI Wellesbourne on 1st April 2001 following the closure of HRI Stockbridge House (SH). Mr Rob Jacobson, formerly of HRI Stockbridge House and now of Stockbridge Technology Centre, remains as a project manager and consultant to the work.

Project work plan

The amended work plan (15 August 2001) was as follows:

1 *Identify and obtain species / strains of entomopathogenic fungi that have potential for the control of spider mites (04/99 to 12/99)*

1.1 Project Initiation Meeting.

1.2 Review the scientific literature to identify species and strains of fungi pathogenic to spider mites and related species.

1.3. Obtain isolates of entomopathogenic fungi, used as commercial mycopesticides or with reported acarine activity, from known resource collections. HRI also has its own collection of entomopathogenic fungi which includes mite-active strains and commercial mycopesticides. The initial aim was to obtain ten isolates for experimentation in the first instance.

1.4 Catalogue and store the fungal isolates using cryopreservation techniques.

2 *Quantify the effect of selected fungi on spider mites in laboratory bioassays (04/99 to 10/01)*

2.1 Obtain and culture spider mites that cause both normal and hyper-necrotic damage.

2.2 Develop and evaluate protocols for laboratory bioassays to measure the effect of candidate isolates of fungi on the survival of spider mites maintained on vegetation.

2.3. Measure the virulence of candidate isolates of fungi to the normal form of the spider mite, using a single, fixed dose of fungal conidia.

2.4 Examine virulent isolates in multiple dose bioassays against the normal form of the spider mite.

2.5 Measure the virulence of up to six fungal isolates against spider mites feeding on different tomato varieties / types and against bean as a control.

2.6 Examine virulent isolates against the 'hyper-necrotic' form of the spider mite.

3 *Examine the compatibility of selected fungal isolates with biological control agents used in tomato IPM (10/00 to 04/02)*

3.1 Examine the scientific literature for information on the compatibility of species of entomopathogenic fungi, identified in Objective 2, with arthropod biocontrol agents used in tomato IPM (09/01). Liaise with researchers in Canada who have received

funding for the evaluation of a range of entomopathogens against natural enemies of glasshouse crops pests.

3.2 Identify gaps in the knowledge and where appropriate use laboratory bioassays to measure the effect on arthropod biocontrol agents of the entomopathogenic fungi shortlisted in Objective 2 (by 04/02).

4 *Select a fungal isolate with potential for glasshouse control of spider mites within IPM programmes in glasshouses (03/01 to 12/02)*

4.1. Investigate the ability of up to six fungal isolates to control populations of spider mites in small scale glasshouse experiments under different regimes of temperature and humidity (by 11/01)

4.2 Select the isolate of fungus that exhibits the most appropriate combination of virulence and compatibility with other control agents (by 04/02). The ability of the selected fungal isolate to control populations of spider mites will be studied on a crop scale (by 09/02).

4.3 Produce an HDC fact sheet (if relevant)to guide growers in the most appropriate and effective use of the fungal pathogen in IPM programmes in tomato crops (by 12/02).

Multiple dose bioassays of selected isolates of entomopathogenic fungi against the normal form of the two-spotted spider mite

Introduction

In Year 1 of the project, 40 isolates of entomopathogenic fungi were screened against adult female *T. urticae* in single dose laboratory bioassays. Six of these isolates were selected for further study against adult female *T. urticae* in multiple dose laboratory bioassays in Year 2 (Table 1). The isolates were selected on the basis of their virulence to *T. urticae* and information on their host origin (from which their potential impact on other natural enemies can be tentatively inferred). Four of the isolates (*Metarhizium anisopliae* 442.99, *Verticillium lecanii* 450.99, *V. lecanii* 19.79, *Hirsutella* sp. 457.99), were chosen from the top five ranked fungi from Year 1. Two isolates are used in proprietary biopesticides; *V. lecanii* 19.79 is the isolate from the mycopesticide Mycotal, while *B. bassiana* 432.99 is the isolate from the mycopesticide Naturalis-L. *Hirsutella thompsonii* 463.99 we believe is derived from the commercial mycopesticide, Mycar (no longer in production), originating from *Phylocoptruta oleivora* (Acari, Prostigmata) but has been passaged through *Varroa jacobsoni* (Acari, Mesostigmata).

Table 1: Fungal isolates examined against T. urticae in multiple dose bioassays

Species	Isolate	Synonym	Host/Substrate	Origin
Beauveria bassiana	432.99	Naturalis-L	*Anthonomus grandis* (Insecta, Coleoptera)	USA
Metarhizium anisopliae	442.99	ARSEF 4556	*Boophilus* spp. (Acari, Ixodidae)	USA
Verticillium lecanii	450.99	IMI 235048	*Cecidophyopsis ribis* (Acari, Prostigmata)	UK
Verticillium lecanii	19.79	Mycotal	*Trialeurodes vaporariorum* (Insecta, Homoptera)	UK
Hirsutella sp.	457.99	3339C	*Dendrolaelaps* sp. (Acari, Mesostigmata)	Poland
Hirsutella thompsonii	463.99	DoCo AL	*Varroa jacobsonii* (Acari, Mesostigmata	Canada

Materials and Methods

Stock cultures of the fungal isolates were stored in liquid nitrogen vapour (Chandler, 1994). Laboratory cultures of fungal isolates were grown from the cryopreserved stocks on slopes of Sabouraud dextrose agar (SDA) and kept in a refrigerator at c. 4 ^{O}C for up to eight weeks.

For bioassays, subcultures were prepared on SDA from the refrigerated slope cultures and incubated at either 26 ± 1 ^{O}C for 18 – 21 d (*Hirsutella* isolates) or 23 ± 1 ^{O}C for 10 – 14 d (all other isolates) in the dark. Conidia were harvested from the subcultures in sterile 0.01% Triton X-100 and filtered through milk filters (Lantor, Bolton UK). Conidia were enumerated using an improved Neubauer haemacytometer and aliquots were prepared at five concentrations of conidia (3 x 10^6 ml^{-1}, 1 x 10^7 ml^{-1}, 5.6 x 10^7 ml^{-1}, 1 x 10^8 ml^{-1}, 3 x 10^8 ml^{-1}).

Tomato (*Lycopersicon esculentum* 'Spectra') seeds were germinated in rockwool plugs (4 x 4 x 4 cm) containing vermiculite for 14 days prior to being transferred to FP11 pots. Seedlings were maintained in the glasshouse at 25^{O}C and an L:D period of 16:8 h for approximately 5 weeks or until a height of approximately 0.5m was attained. For the production of *T. urticae* cultures, plants were transferred to larger pots (20 cm) containing peat (Levington F2) and staked. Plants were watered *ad libitum.*

Stock cultures of *T. urticae* were reared on tomato cv 'Spectra' plants under glasshouse conditions (20-30^{O}C, 50-70% RH, and at least 16 h of light per day). New colonies of mites were initiated weekly by placing a *T. urticae*-colonised leaf over a 'clean' leaf of a new culture plant. All bioassays were done with fixed age populations of *T. urticae* which were produced as follows: Batches of 15 adult female *T. urticae* were taken from stock cultures, placed on inverted tomato leaflets (cv 'Spectra') from 'clean' plants on damp filter paper in Petri dishes, and incubated at 21 ± 2^{O}C, 16L:8D for 48 h to lay eggs. Adult mites were removed after this period and the leaflets incubated at 21^{O}C, 16:8 L:D for a further 6 d. Leaflets with spider mite nymphs were then placed on whole tomato plants (0.3m in height) and kept under glasshouse conditions (20-30^{O}C, 50-70% RH, and at least 16 h of light per day) for 7 – 9 d. The plants were regularly monitored until new adults were found. New adults that had just begun to produce eggs were then selected for experimentation. Most bioassays in this study required ca. 40 batches of synchronised mites to be prepared.

Groups of ca. 30 fixed-age female *T. urticae* were collected by hand, with the aid of a dissecting needle, from tomato plants. The mites were placed on a roughened glass cover slip (22 x 22 mm) on water saturated filter paper (Whatman, 90 mm diameter) within the base of a Petri dish (90 mm diameter). Mites were sprayed with 2 ml of a suspension of fungal

conidia, using a Potter tower (Potter, 1952) at 50 kPa through an 'intermediate' atomiser. Controls (0.01% Triton X-100) were sprayed prior to the application of fungi to reduce the risk of unintentional infections. Cover slips (with mites) were placed on an inverted tomato leaf on dampened filter paper within a ventilated Petri dish (90 mm diameter, two mesh-covered ventilation panels (0.17 x 0.37mm) per dish) and air dried within a laminar flow cabinet for 10 min. Most of the mites walked off the cover slip during this time but any remaining were transferred by gently tapping the cover slip. The number of mites placed on each leaf was recorded, and the Petri dishes were sealed (Nescofilm). The Petri dishes were maintained at 23^{O}C and a 16 h photoperiod within an airtight perspex cage (Fisher Scientific, 30.5 x 30.5 x 45.7cm) containing a tray of distilled water (ca. 750 ml) at the bottom to maintain a saturated atmosphere. Ventilating the Petri dishes prevented the accumulation of droplets of water on the leaves in which the mites could become trapped.

The numbers of living and dead mites (no movement when touched with a dissecting needle) were observed daily for 7 d. Cadavers were removed onto damp filter paper within sealed (Nescofilm) Petri dishes and examined for the appearance of sporulating mycelium on the integument 7 d after the end of the bioassay. The presence of sporulating mycelium was taken as evidence of fungus-induced mortality, i.e. overt mycosis. The dose of conidia (conidia cm^{-2}) for treatments was estimated by counts of colony forming units from conidia washed from blank microscope coverslips using 0.05% Triton X-100 followed by plating of serially-diluted aliquots onto malt extract agar (Goettel & Inglis, 1997).

The multiple dose bioassay was done according to an alpha design with three replicates of the six isolates. Each replicate was split into three blocks containing four controls and all five concentrations of conidia of two isolates. Each replicate and block was processed at a different time. Isolate *Hirsutella* sp. 457.99 had different doses in different replicates. Dose response was investigated for mite mortality at 6 d post inoculation (dpi). Because control mortality in each block was well estimated and the control effects were clearly reflected in the isolate effects, the data were adjusted to take account of the controls according to (T = t.c) and (D = T– a), where T = adjusted total of alive mites on treated leaves, t = total number of mites per leaf, c = proportion of mites alive on controls at 6 dpi, D = adjusted total of dead mites on treated leaves, a = number of mites alive on treated leaves at 6 dpi. Data were analysed in GenStat (GenStat 5 Committee 1993) using a generalised linear model (probit

analysis) with a logit link function and $\log_{10}$ conidia concentration used to fit a dose response model.

Results and Discussion

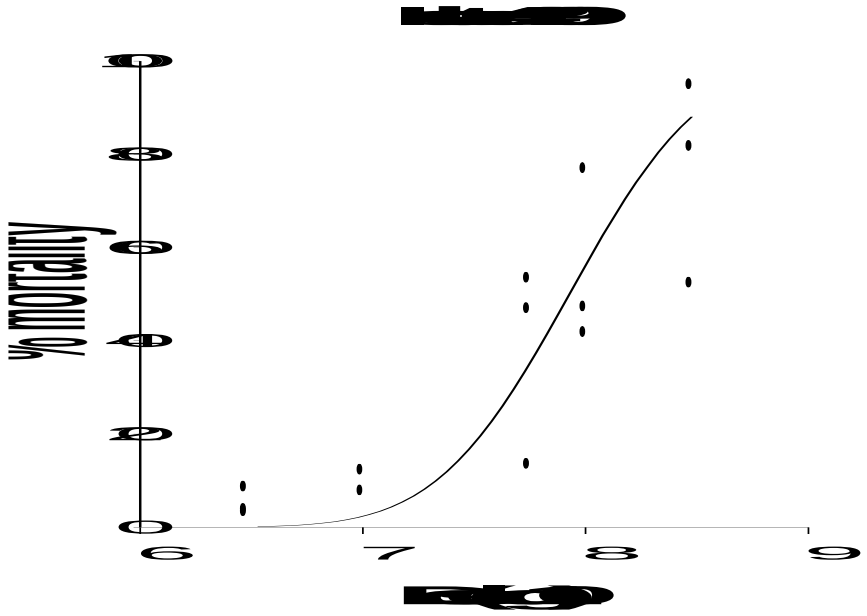
Treatment behaviour was as expected with the controls having the lowest mortality at all times. Mean deviances were relatively high for most isolates (Table 2). There was a straight line relationship between $\log_{10}$ conidia concentration and $\log_{10}$ conidia dose (conidia cm^{-2}) with a slope of unity. We estimate that a treatment concentration of 10^8 ml^{-1} deposits ca. 800 conidia on a mite (reduction factor = 10^5) but a proportion of this is likely to be lost subsequently by droplet run-off or through conidia failing to bind to the integument. Compared to bigger, insect hosts, the dose received by *T. urticae* under the Potter tower is probably small, which may have lead to the variability in response observed in this experiment.

Table 2 : Estimated median lethal concentrations (LC_{50}) of five fungal isolates to adult female T.urticae

Species		LC_{50}	mean deviance
M. anisopliae	442.99	8.46×10^7	2.32
B. bassiana	432.99	6.01×10^8	3.81
H. thompsonii	463.99	1.46×10^9	1.61
V. lecanii	450.99	2.46×10^9	1.63
V. lecanii	19.79	1.20×10^{11}	2.06
Hirsutella sp.	457.99	1.26×10^{18}	3.72

The estimated median lethal concentrations of conidia for the five fungal isolates at 6 dpi ranged from 8.5×10^7 ml^{-1} to 1.3×10^{18} ml^{-1} (Table 2, Figure 1). Isolates *V. lecanii* 19.79 and *Hirsutella* sp. 457.99 returned poor dose responses and should not be considered further for *T. urticae* biocontrol. The median lethal concentrations of the other isolates are high in comparison to the LC_{50}s of some insect hosts (for example, the LC_{50}s of fungi that kill aphids are often in the range $10^5 – 10^6$ ml^{-1}). This could be a result of the small target size proffered by mites during spraying, as discussed, but may also be caused by fungal inhibition by tomato secondary metabolites.

Figure 1: dose response of M. anisopliae *442.99 against adult female* T. urticae



Virulence of selected fungal isolates against two-spotted spider mites feeding on different tomato varieties / types

Introduction

Poprawski *et al*. (2000) provided evidence of an interaction between entomopathogenic fungi and tomato plants that had a detrimental effect on the virulence of the fungus. The virulences of *Beauveria bassiana* and *Paecilomyces fumosoroseus* were measured in a laboratory bioassay against third instar nymphs of the glasshouse whitefly *Trialeurodes vaporariorum* reared on cucumber and tomato plants. Nymphs were highly susceptible to both fungi on cucumber plants. In contrast, insects reared on tomato were significantly less susceptible to infection. The authors hypothesised that the glycoalkaloid tomatine may have been involved in antimicrobiosis on tomato leaves. Tomatine was mixed into Noble agar at concentrations of 20, 50, 100, 500 & 1000 ppm (mg ml^{-1}) and its effect on conidia germination of the fungi was measured. Germination of both fungi was affected little at 20, 50 & 100 ppm. Germination of *B. bassiana* was reduced by ca. 20% and 40% at 500 & 1000 ppm tomatine respectively. Germination of *P. fumosoroseus* was completely inhibited at 500 & 1000 ppm tomatine respectively. Inhibition of the *in vitro* germination of *P. fumosoroseus* and *B. bassiana* by tomatine has previously been reported by Lacey & Mercadier (1998) and Costa & Gaugler (1989). However the effect of tomato allelochemicals on entomopathogenic fungi may vary according to the variety of tomato and the species / isolate of fungus. For example, Bolckmans *et al*. (1995), Sosnowska & Piatkowski (1996), and van de Veire & Degheele (1996) have all reported good control of *T. vaporariorum* by *P. fumosoroseus* on glasshouse grown tomato. Sequestered tomatine by whitefly nymphs would partly explain the insect's defence against the fungi observed by Poprawski *et al*. (2000). Poprawski *et al* (2000) suggest that *if* tomatine affected the pathogenicity of *B. bassiana*, it would have its effect after germination and host penetration.

In this study, a laboratory experiment was done to measure the virulence of two isolates of entomopathogenic fungi (*M. anisopliae* 442.99 and *B. bassiana* 432.99) to adult female *T. urticae* reared on four different varieties of tomato, with bean as a positive control plant. The tomato types were as follows: classic round (*L. esculentum* cv 'Spectra'); cherry (cv 'Favorita'); beefsteak (cv 'Quest'); and large truss (cv 'Clotilde'). The fungal isolates were selected from the virulence predictions from the multiple dose bioassays.

Materials and Methods

Four varieties of tomato (*L. esculentum* cv 'Spectra', 'Favorita', 'Quest', and 'Clotilde') plus bean (*Phaseolus vulgaris* cv 'Dwarf Tendergreen) were raised from seed (Levington F2 compost) in the glasshouse (25^{O}C, 16:8 h photoperiod) for approximately 5 weeks or until a height of 0.5m. Synchronised cultures of *T. urticae* were reared on each plant type as described previously. Suspensions of fungal conidia (1×10^{8} ml^{-1}) were prepared as described previously. Fixed age female *T. urticae* reared on each plant type were inoculated with suspensions of fungal conidia as described previously and incubated on leaves of their corresponding plant (23^{O}C, 16:8 L:D). Mite mortality was assessed as described previously. Fungal isolates *M. anisopliae* 442.99 and *B. bassiana* 432.99 were bioassayed as a pair on seven occasions at one concentration of conidia (1×10^{8} conidia.ml^{-1}). All treatments were done on each occasion. Replication was balanced to provide the optimum number of bioassay repetitions for a reliable separation of treatment effects, given the between-assay heterogeneity observed in the multiple dose virulence bioassay. There were 20 treatments per occasion (five plant types x two fungus isolates + 2 negative control batches per plant type (treated with 0.01% Triton X-100)). Each treatment comprised a replicate of a single batch of 20-30 mites.

Data were adjusted to take account of control mortality as described in the previous experiment. Adjusted data from the replicate experiments were combined and percentage mortalities calculated at six days post inoculation (dpi). Data were compared by ANOVA in GenStat (percentage mortalities angular transformed).

Results and Discussion

Control mortalities at 6 dpi ranged from 16.2% (Tomato – var. Clotilde) to 68.1% (Bean – var. Dwarf Tendergreen) (Table 3). The average control mortality on tomato at 6 dpi was 22.6 %, which is comparable to that from previous experiments. However, the control mortality on bean was significantly higher than on tomato ($p = 0.05$) (Table 3). The results from bean should be interpreted with some caution because of the high control mortality (data analysis indicated an interaction of fungus-induced and natural mortalities). The high control mortality on bean was unexpected - the most likely explanation is that the bean leaves deteriorated during the course of the bioassay (note however that a certain amount of leaf senescence may be favourable to mite performance if it decreases the C:N ratio).

Spider mites on tomato variety / type Clotilde survived significantly better (p = 0.05) than on tomato variety / type Quest but there were no differences in survival on the other tomato varieties (Table 3). When adjusted for fungus - induced mortality, the results indicated that tomato is unlikely to have an effect on fungus infection. Fungus induced mortalities ranged from 0 to 50% (Table 4). There was no significant difference in fungus – induced mortality between *M. anisopliae* 442.99 and *B. bassiana* 432.99 on bean (Table 4). For *B. bassiana* 432.99, there was no significant difference in fungus-induced mortality between bean and tomato varieties / types Clotilde, Quest and Spectra. However there was a significant reduction in fungus-induced mortality on tomato variety / type Favorita compared to bean. For *M. anisopliae* 442.99, there was no significant difference in fungus-induced mortality between bean and any of the tomato varieties / types. These results contrast with the findings of Poprawski *et al*. (2000) for *T. vaporariorum* on tomato, which could be due to: (a) variation in response to tomato allelochemicals between different isolates of fungi; (b) low allelochemical production in the tomato varieties studied here; (c) varying ability of *T. urticae* and *T. vaporariorum* to sequester allelochemicals. The findings indicate that bioassays done on cv Spectra may be extrapolated to other tomato cultivars.

Table 3 : Mean percentage mortality of adult female T. urticae *treated with* B. bassiana *432.99 and* M. anisopliae *442.99 (1x 10^8 ml^{-1}, 6 dpi) (transformed data in parentheses).*

	control	***B. bassiana* 432.99**	***M. anisopliae* 442.99**
Bean			
Dwarf Tendergreen	68.1 (56.7)	79.3 (65.7)	81.5 (67.6)
Tomato			
Clotilde	16.2 (20.0)	35.5 (36.4)	46.3 (40.9)
Favorita	23.1 (26.9)	25.7 (29.6)	41.6 (39.9)
Quest	29.7 (32.0)	40.8 (37.4)	56.0 (48.5)
Spectra	21.4 (25.8)	36.4 (36.0)	45.2 (42.1)

LSD (p=0.05; df=114) Isolate – Variety = (13.22); Control – Isolate = (11.45); Control – Variety = (9.35).

Table 4 : Predicted adjusted mean percentage fungus-induced mortality of adult female T. urticae *treated with* B. bassiana *432.99 and* M. anisopliae *442.99 (1x* 10^8 ml^{-1}*, 6 dpi)(transformed data in parentheses).*

	***B. bassiana* 432.99**	***M. anisopliae* 442.99**
Bean		
Dwarf Tendergreen	29.6 (37.0)	50.0 (47.1)
Tomato		
Clotilde	23.5 (27.5)	36.5 (37.8)
Favorita	-2.3 (13.7)	15.3 (30.9)
Quest	18.3 (23.6)	38.6 (36.7)
Spectra	16.8 (21.0)	27.7 (29.6)

LSD (p=0.05; df=54) = (17.86)

Effect of inoculation method on the susceptibility of two - spotted spider mites to entomopathogenic fungi

Introduction

The findings of the previous experiment indicated tentatively that tomato variety / type did not adversely affect fungal virulence and was unlikely to account totally for the lower than expected mortalities observed thus far in the bioassays. We suspected that the mites received a low dose of conidia during inoculation compared to insect hosts because of their small size, leading to a lower than expected response in bioassays. Greater kill may have been obtained with a different inoculation technique. Prior to this study, we found only two published methodologies for measuring the susceptibility of spider mites to fungi by laboratory bioassay. Gerson *et al*. (1979) inoculated *Tetranychus cinnabarinus* with *H. thompsonii* by caging mites for 30 – 60 h on a mat of sporulating mycelium. Mites were then maintained on bean leaves at high humidity and observed daily. Although high levels of infection were observed, this method of inoculation is crude, providing little in the way of dose uniformity, and nowadays is not recommended (Goettel & Inglis, 1997). Gardner *et al*. (1982) inoculated spider mites with single conidia of *H. thompsonii* using an undescribed micromanipulation technique, then incubated the mites on leaf discs of shamrock floated on water to prevent the mites escaping. Although mites were infected with this technique, it is labour intensive, prone to handling errors, and not suitable for screening large numbers of fungal isolates. Gardner *et al*. (1982) also infected *T. urticae* with *H. thompsonii* by spraying leaf discs with a suspension of conidia, allowing them to dry, then incubating populations of mites on the leaf discs for the remainder of the assay. This method allows a high throughput, but mites are constantly exposed to inoculum and the method relies on secondary acquisition for infection, hence dose cannot be controlled with precision. However, secondary acquisition of conidia may be an important route of infection in glasshouses and this method could be a powerful experimental tool.

In this experiment, which was not included in the original work plan, we investigated secondary acquisition of conidia as a method of bioassaying entomopathogenic fungi against *T. urticae* on tomato in comparison to the direct spray technique.

Materials and Methods

Fixed age populations of the 'normal' form of the two-spotted spider mite (*T. urticae*) were reared on tomato cv 'Spectra' using the methods described previously. Suspensions of fungal conidia (1 x 10^8 ml^{-1}) were prepared as described previously from four isolates of fungi: *B. bassiana* 432.99, *M. anisopliae* 442.99, *V. lecanii* 450.99, and *H. thompsonii* 463.99. The commercial mycopesticide 'Naturalis-L' (Troy Biosciences Inc. Phoenix AZ USA) – which contains *B. bassiana* 432.99 as its active ingredient – was included as an additional treatment. Naturalis-L was applied at the manufacturer's recommended rate (1.0 x 10^5 conidia ml^{-1}). All bioassays were done using tomato cv 'Spectra'.

Fixed age female *T. urticae* were inoculated with fungal conidia using two methods. In the 'direct spray' method, groups of approximately 30 mites were sprayed with 2 ml of a suspension of conidia using a Potter tower (Potter, 1952) as described previously. In the 'indirect spray' method, groups of approximately 30 mites were placed onto glass coverslips placed on water saturated filter paper within the base of a Petri dish (90 mm diameter). Tomato leaves were then sprayed with 2 ml of suspension of fungal conidia using a Potter tower at 50 kPa (controls treated with 0.01% Triton X-100) and placed on damp filter paper within a Petri dish (90 mm diameter triple vented). Each group of mites was then transferred, by gently tapping the coverslip, onto the lower surface of each leaf and left to dry for 1 h. All Petri dishes were then sealed with Nescofilm and maintained at 23^{O}C and a 16 h photoperiod within an airtight perspex cage as described previously. Mortality was assessed daily for 7 d. All fungal isolates were bioassayed concurrently and the experiment replicated on six occasions.

Data were adjusted to take account of control mortality as described in the previous experiment. Data from replicate experiments were combined and percentage mortalities calculated at six days post inoculation (dpi). Data was compared by ANOVA in GenStat (percentage mortalities angular transformed).

Results and Discussion

Average control mortalities were 25 % to 33 % for the indirect and direct inoculation methods respectively (Table 5). There was no significant difference between control mortalities for direct and indirect inoculation ($p = 0.05$). For completeness data on mean percentage mortality (i.e mortality due to all causes) in the fungus treatments is included in

Table 5. However, as described above, in this report the fungus treatments have been compared using predictions of fungus-induced mortality (Table 6). Fungus-induced mortality due to direct sprays ranged from 9 % (*H. thompsonii* 463.99) to 33 % (*M. anisopliae* 442.99), while fungus-induced mortality due to indirect sprays ranged from 31 % (*M. anisopliae* 442.99) to 93% (Naturalis-L) (Table 6). There was no significant difference in fungus-induced mortalities between the isolates in direct spray treatments (Table 6). However Naturalis-L caused significantly ($p = 0.05$) more mortality than *M. anisopliae* 442.99, *V. lecanii* 450.99 and *H. thompsonii* 463.99 in indirect spray assays (Table 6).

The indirect spray technique also significantly increased ($p = 0.05$) mortality caused by *B. bassiana* 432.99 and Naturalis-L. Mortality induced by *B. bassiana* 432.99 increased from 20% to 63% (Table 6) while mortality induced by Naturalis-L increased from 27 % to 93%. The indirect spray technique probably increases the exposure of mites to fungal inoculum. However, the fact that only *B. bassiana* responded to the technique may indicate an adaptation by this fungus to enhance the secondary acquisition of conidia. Fungi with this characteristic should be well suited for use against spider mites on tomato plants. This finding could also have important implications for how isolates are selected in other mycopesticide research programmes.

There are indications too that formulation improved the secondary acquisition of conidia (i.e. Naturalis-L caused more mortality than *B. bassiana* 432.99 in the indirect spray experiment, despite being applied at a lower concentration). It is likely that formulation could similarly improve the effectiveness of other fungal isolates.

Table 5 : Mean percentage mortality of adult female T. urticae *inoculated directly or indirectly with entomopathogenic fungi (1x 10^8 ml^{-1}, 6 dpi) (transformed data in parentheses).*

	Direct spray	**Indirect spray**
Control	32.8 (34.4)	25.1 (29.8)
B. bassiana 432.99	46.2 (42.8)	72.2 (63.7)
M. anisopliae 442.99	54.4 (47.6)	48.0 (43.4)
V. lecanii 450.99	51.8 (46.1)	55.7 (48.4)
H. thompsonii 463.99	37.6 (37.7)	54.9 (47.9)
Naturalis-L	52.1 (46.7)	95.2 (79.2)

LSD (p=0.05; df=62) Isolate – Inoculation method = (13.25); Control – Isolate = (11.48); Control – Inoculation method = (9.37).

Table 6 : Predicted adjusted mean percentage fungus-induced mortality of adult female T. urticae *inoculated directly or indirectly with entomopathogenic fungi (1x 10^8 ml^{-1}, 6 dpi) (transformed data in parentheses).*

	Direct spray	**Indirect spray**
B. bassiana 432.99	19.7 (24.7)	62.9 (57.2)
M. anisopliae 442.99	33.0 (34.5)	31.1 (33.0)
V. lecanii 450.99	26.5 (28.1)	40.9 (39.2)
H. thompsonii 463.99	8.5 (15.8)	41.0 (37.8)
Naturalis-L	27.0 (30.7)	93.0 (77.3)

LSD (p=0.05; df=41) = (22.40).

Susceptibility to entomopathogenic fungi of 'hyper-necrotic' spider mites

Introduction

A laboratory experiment was done to measure the susceptibilities to entomopathogenic fungi of adult female *T. urticae* (the 'normal' form of the two-spotted spider mite) and a population of carmine spider mites (*Tetranychus cinnabarinus*, previously identified by Zhi-Qiang at the Natural History Museum) which cause hypernecrotic damage in tomato crops.

Materials and Methods

Fixed age populations of the *T. urticae* and *T. cinnabarinus* were reared on tomato cv 'Spectra' using the methods described previously. Suspensions of fungal conidia (1×10^8 ml^{-1}) were prepared as described previously from four isolates of fungi: *B. bassiana* 432.99, *M. anisopliae* 442.99, *V. lecanii* 450.99, and *H. thompsonii* 463.99. Batches of 20 – 30 fixed age female spider mites were inoculated with suspensions of conidia (1×10^8 ml^{-1}) using the secondary acquisition method described previously. Mite mortality was assessed as described previously. All fungal isolates were bioassayed concurrently and the experiment replicated on six occasions. There were two control batches (0.01% Triton X-100) for both *T. urticae* and *T. cinnabarinus* for each occasion. Data were adjusted to take account of control mortality and replicates combined as described previously. ANOVAs were done on percentage mortalities at 6 dpi (angular transformed) as described previously.

Results and Discussion

Average control mortalities for normal and hypernecrotic mites were 25 % and 45% respectively (Table 7). There was a significant difference in control mortalities ($p = 0.05$) indicating that the normal mites survived better in the bioassay system than hypernecrotic mites. Fungus – induced mortalities of *T. urticae* ranged from 26% (*M. anisopliae* 442.99) to 100% (Naturalis-L) (Table 8). Fungus – induced mortalities of *T. cinnabarinus* ranged from 11 % (*H. thompsonii* 463.99) to 99% (Naturalis-L). Naturalis-L caused significantly more mortality than the other fungus treatments (Table 8). *Beauveria bassiana* 432.99 also caused significantly more mortality ($p=0.05$) than *M. anisopliae* 442.99, *V. lecanii* 450.99 and *H. thompsonii* 463.99. However there was no significant difference in the susceptibilities of *T. urticae* and *T. cinnabarinus* to the fungal isolates (Table 8). The results imply that the findings of other experiments with *T. urticae* will also be applicable to *T. cinnabarinus*. The results of this research may also be applicable to other species of tetranychid mites.

Table 7 : Mean percentage mortality of adult female 'normal and 'hyper-necrotic' mites inoculated indirectly with entomopathogenic fungi (1x 10^8 ml^{-1}, 6 dpi) (transformed data in parentheses).

	'normal' two-spotted mite	**'hyper-necrotic' mites**
Control	25 (27.8)	45.1 (41.3)
B. bassiana 432.99	81.9 (67.6)	96.4 (81.1)
M. anisopliae 442.99	47.0 (43.2)	75.6 (64.2)
V. lecanii 450.99	54.1 (47.7)	63.5 (53.3)
H. thompsonii 463.99	49.8 (44.6)	53.7 (47.4)
Naturalis-L	100 (90.0)	99.3 (88.0)

LSD (p=0.05; df=67) Isolate – Mite type = (14.76); Control – Isolate = (12.79); Control – Mite type = (10.44).

Table 8 : Predicted adjusted mean percentage fungus-induced mortality of adult female 'normal and 'hyper-necrotic' mites inoculated indirectly with entomopathogenic fungi (1x 10^8 ml^{-1}, 6 dpi) (transformed data in parentheses).

	'normal' two-spotted mite	**'hyper-necrotic' mites**
B. bassiana 432.99	75.6 (63.8)	94.7 (79.2)
M. anisopliae 442.99	25.6 (29.9)	31.5 (50.0)
V. lecanii 450.99	35.8 (35.6)	21.8 (30.1)
H. thompsonii 463.99	32.5 (33.5)	10.6 (24.3)
Naturalis-L	100 (90.0)	98.8 (87.5)

LSD (p=0.05; df=45) = (20.58).

Compatibility of selected fungal isolates with biological control agents used in tomato IPM

Introduction

To integrate entomopathogenic fungi successfully into tomato IPM programmes, it is essential to determine the compatibility of the fungal pathogens with the arthropod biocontrol agents already in use. Integrated pest control is more advanced in UK tomato crops than in other areas of horticulture or agriculture, with approximately 17 different species of arthropod biocontrol agents being used (Thomas & Garthwaite, 1997). An assessment was done of published accounts of the compatibility of Mitosporic entomopathogenic fungi with potential to control spider mites (Chandler *et al*., 2000) with arthropod biocontrol agents used in tomato IPM, together with a data base search of host activity of entomopathogenic fungi from 28 on-line culture collections. In total, only seven reports could be identified which examined the compatibility of entomopathogenic fungi with arthropod biocontrol agents used in tomato IPM. The findings are summarised in Table 9. There were two reports of natural infections, viz.: (a) *Hirsutella thompsonii* infecting *Amblyseius peregrinus*; (b) *Conidiobolus obscurus* infecting *Phytoseiulus persimilis*. There were four reports of incompatibility between isolates of entomopathogenic fungi and arthropod biocontrol agents used in tomato IPM: (a) *B. bassiana* infecting *Encarsia formosa;* (b) *B. bassiana* infecting *Phytoseiulus* spp.; (c) *C. obscurus* infecting *Phytoseiulus persimilis;* (d) *M. anisopliae* infecting *P. persimilis.* However, there were also reports of isolates of *B. bassiana*, *Beauveria brongniartii* and *V. lecanii* having no effect on populations of *E. formosa, P. persimilis, Aphidius colemani, Trichogramma* spp. and *Amblyseius cucumeris.*

Contact was established with Dr Ken Fry of the Alberta Research Council (Vegreville, Alberta, Canada) who is due to initiate studies of the compatibility of entomopathogenic fungi with other biocontrol agents as part of the Natural Sciences and Engineering Research Council of Canada's Biocontrol Network initiative. It was initially thought that Dr Fry's research encompassed a full range of entomopathogenic fungi and arthropod natural enemies used in protected crops and would be of value to this research project. We have now established that his research is more limited in scope and will cover sublethal effects of *B. bassiana* on a selected number of predators / parasitoids of aphids and / or whiteflies.

Table 9 : Biological control agents used in tomato crops in Great Britain and their compatibility with entomopathogenic fungi

Species	Usage area (m^2)	*Compatible entomopathogenic fungi*	*Incompatible entomopathogenic fungi*
Biological control agents			
Encarsia Formosa	59 386 684	*Beauveria bassiana (*Sterk *et al*, 1999; Wright & Kennedy, 1996) *Beauveria brongniarti (*Sterk *et al*, 1999 *Verticillium lecanii* (Sterk *et al*, 1999)	*B. bassiana* (Naturalis-L) (Jacobson, 2001)
Phytoseiulus persimilis	17 694 105	*B. bassiana (*Sterk *et al*, 1999) *B. brongniarti* (Sterk *et al*, 1999) *V. lecanii* (Sterk *et al*, 1999)	*B. bassiana* (Naturalis-L) (Chandler *et al*, 2001) *Conidiobolus obscurus* (Nyiira, 1982; Petrova & Petrov, 1976 in van der Geest, 1985) *Metarhizium anisopliae* (Chandler *et al*, 2001)
Diglyphus isaea	10 586 080	-	-
Dacnusa sibirica	6 726 511	-	-
Therodiplosis persicae	2 483 725	-	-
Aphidius colemani	1 591 823	*B. bassiana* (Murphy *et al*, 1999; Sterk *et al*, 1999; Jacobson, 2000b; Jacobson *et al* 2001) *B. brongniarti (*Sterk *et al*, 1999) *V. lecanii* (Sterk *et al*, 1999)	- - -
Macrolophus caliginosus	1 103 357	-	-
Bacillus thuringiensis	829 061	-	-
Amblyseius spp.	639 163	-	*Hirsutella thompsonii* (McCoy & Selhime, 1974 in McCoy, 1981)
Trichogramma spp.	342 039	*B. bassiana* (Sterk *et al*, 1999) *B. brongniarti (*Sterk *et al*, 1999) *V. lecanii* (Sterk *et al*, 1999)	- - -
Anagrus spp.	105 699	-	-
Other biological control agents[1]	99 049	*B. bassiana* (Naturalis-L) (Jacobson 2001; Jacobson *et al*, 2001)	-
Dacnusa sibirica/ Diglyphus isaea	34 307	-	-
Aphidoletes aphidimyza	6 737	-	-
Pollinators			
Bombus terrestris	1 866 905	-	-

[1] Other biological control agents includes *Amblyseius cucumeris* and *Typhlodromus* sp.
Information taken from: Thomas M.R. & Garthwaite D.G. (1997) Pesticide usage survey report 136: protected crops (edible and ornamental) 1995, Maff, London.

Effect of entomopathogenic fungi on populations of two - spotted spider mite on tomato (glasshouse experiment)

Introduction

The aim of this experiment was to evaluate the effect of entomopathogenic fungi on populations of *T. urticae* feeding on a glasshouse tomato crop.

Materials and Methods

The experiment comprised six treatments: untreated control, *M. anisopliae* 442.99, *H. thompsonii* 463.99, *V. lecanii* 450.99, *B. bassiana* 432.99, and the proprietary mycopesticide Naturalis-L (active ingredient *B. bassiana* 432.99, 2.5 x 10^8 conidia ml^{-1} product, obtained from Troy Biosciences Inc, Phoenix, USA). Naturalis-L was stored at 5^OC before use and spray mixtures were prepared according to the manufacturer's instructions at the manufacturer's recommended rate, equivalent to a concentration of 1.0 x 10^5 conidia ml^{-1}. The concentration of viable conidia in the Naturalis-L spray mixture was estimated from counts of colony forming units (cfu) recorded from 1 ml aliquots sampled before spraying, serially diluted and cultured on SDA. Estimates (cfu's) were in keeping with the product concentrations stated by the manufacturer. Populations of adult female *T. urticae* (mixed ages) were reared as described previously.

The experiment was done according to an incomplete Latin square design. Treatments were applied in each of four single rows of the crop (i.e. one side of a double row) separated by guards. Each row contained all six treatments in a randomised block design (i.e. four blocks of six treatments per block), one treatment per plant. Each treated plant was separated by two guards. Each treatment was applied to three marked leaves at the top (ca. 2 m from the ground), middle (1.5 m) and bottom of the plant (i.e. a total of 12 leaves per treatment)

The experiment was done twice, on the 4th July 2001 and the 4th September 2001. On the first occasion, adult female spider mites were released onto a leaflet of the marked leaves, 10 per leaf, using a detachable pooter tip system. This was followed by a further release of spider mites 1 wk later. Fungal treatments were applied 1 wk after the second release of spider mites. Suspensions of conidia were sprayed to run-off onto the leaflet on which the mites were released. Sprays were done with a hand held sprayer. The floor of the house was hosed lightly with water to increase humidity and simulate a whole crop spray. Spraying was done in the late afternoon. Leaf sprays of conidia suspensions were done once every 7 d for three weeks (i.e. total of three sprays). Seven days after the final spray, leaves were removed and numbers of spider mites per leaf were counted.

On the second occasion, the experiment was done as described with the following exceptions: (a) 30 mites were released per leaf; (b) the treatments were applied higher in the crop; (c) only two sprays were applied. Data were combined and total counts (alive +eggs) calculated. The transformation $\log_{10}$ (total + 0.375) was applied to the data before ANOVA in GenStat.

Results and Discussion

On the first occasion, mite numbers were low overall (Table 10). There was a significantly greater number of mites and eggs on leaflets on the top and middle parts of the plant (mean 5.22 mites and eggs per leaflet c.f. 2.15 mites and eggs per leaflet in the bottom positions). This was probably due to a better leaf condition on the upper parts of the plant. Treatments *H. thompsonii* 463.99 and Naturalis-L significantly (p = 0.05) reduced the number of mites and eggs found in the top leaflet position.

Table 10 : Mean (backtransformed) total number of adult female T. urticae *and eggs found per leaflet after treatment with entomopathogenic fungi, under glasshouse conditions; Occasion 1- 4th July 2001(transformed data in parentheses)*

	Top	**Middle**	**Bottom**
Control	5.22 (0.718)	3.84 (0.584)	2.15 (0.332)
B. bassiana 432.99	1.93 (0.286)	4.73 (0.675)	1.92 (0.284)
M. anisopliae 442.99	8.49 (0.929)	3.18 (0.502)	1.89 (0.277)
V. lecanii 450.99	2.67 (0.426)	3.99 (0.601)	1.04 (0.018)
H. thompsonii 463.99	1.11 (0.046)	1.98 (0.297)	1.29 (0.112)
Naturalis-L	1.17 (0.070)	1.02 (0.005)	1.15 (0.061)
LSD (p<0.05, one-tailed)	(0.5831)	(0.7142)	(0.5256)

Better mite establishment was observed on the second occasion (Table 11). There was a trend for increasing numbers of mites higher up the plant (102.56 mites and eggs per leaflet on the top position as opposed to 6.41 mites and eggs per leaflet on the bottom position). Treatments *M. anisopliae* 442.99, *V. lecanii* 450.99, *H. thompsonii* 463.99 and Naturalis-L significantly (p = 0.05) reduced the number of mites and eggs per leaflet on the top position. Naturalis-L reduced mite populations by over 90% on the top position. All treatments significantly reduced the number of mites and eggs per leaflet on the middle and bottom positions. Overall, *Beauveria bassiana* 432.99 and Naturalis-L significantly reduced the number of mites and eggs more than the other treatments.

Table 11 : Mean (backtransformed) total number of adult female T. urticae *and eggs found per leaflet after treatment with entomopathogenic fungi, under glasshouse conditions; Occasion 2- 4th September 2001 (transformed data in parentheses).*

	Top	**Middle**	**Bottom**
Control	102.56 (2.011)	9.33 (0.970)	6.41 (0.807)
B. bassiana 432.99	32.43 (1.511)	3.37 (0.528)	0.68 (-0.166)
M. anisopliae 442.99	15.38 (1.187)	3.43 (0.536)	2.22 (0.346)
V. lecanii 450.99	12.36 (1.092)	2.31 (0.363)	1.49 (0.173)
H. thompsonii 463.99	7.69 (0.886)	3.71 (0.570)	2.19 (0.340)
Naturalis-L	6.00 (0.778)	1.69 (0.229)	1.30 (0.115)
LSD ($p<0.05$, one-tailed)	(0.5564)	(0.4029)	(0.2138)

OVERALL CONCLUSIONS

The experiments done in this phase of the project indicate that entomopathogenic fungi have potential as biocontrol agents of spider mites on tomato. Our research considered a series of interactions between the pathogen, mite and host plant which could influence the degree of fungus-induced mortality in mite populations. The results thus far indicate that tomato variety and type has relatively little effect on fungal infectivity. Moreover there was no discernible difference in the susceptibilities of normal and hypernecrotic mites to entomopathogenic fungi. However inoculation method could affect fungal virulence depending on the fungal species. In particular, using an indirect spray technique improved the virulence of *B. bassiana* 432.99 by more than 40%, and increased the virulence of Naturalis-L by 66%. In glasshouse experiments, all fungal treatments significantly reduced mite populations. Naturalis-L appeared to be the most effective, reducing mite populations by up to 90%.

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REFERENCES

Bartowski, J., Odindo, M.O. & Otieno, W.A. (1988). Some fungal pathogens of the cassava green spider mites, *Mononychellus* spp. (Tetranychidae) in Kenya. *Insect Science and its Application* **9**, 457-459.

Bergh, J.C. & McCoy, C.W. (1997). Aerial dispersal of citrus rust mite (Acari: Eriophyidae) from Florida citrus groves. *Environmental Entomology* **26**, 256-264.

Bolckmans, K., Sterk, G., Eyal, J., Sels, B. & Stepman, W. (1995). PreFeRal (*Paecilomyces fumosoroseus* strain Apopka 97), a new microbial insecticide for the biological control of whiteflies in greenhouses. *Meded. Fac. Landbouwk. Toegep. Biol. Wet. Univ. Gent* **1995**: 707 – 711.

Chandler, D.C. (1994). Cryopreservation of fungal spores using porous beads. *Mycological Research* **98**, 525-526.

Chandler, D., Davidson, G., Pell, J. K., Ball, B. V., Shaw, K. V. & Sunderland, K. D. (2000). Fungal biocontrol of Acari. *Biocontrol Science and Technology*, **10**, 357-384.

Chandler, D.C., Davidson, G., Shaw, K.E., Ball, B.V., Pell, J.K. & Sunderland, K. (2001). Screening pathogens for biocontrol of *Varroa destructor*. Report of contract work undertaken for DEFRA, March 2001, 6pp.

Costa, S. D. & Gaugler, R. R. (1989). Sensitivity of *Beauveria bassiana* to solanine and tomatine: Plant defensive chemicals inhibit an insect pathogen. *Journal of Chemical Ecology*, **15**, 687 – 706.

Gardner, W.A., Oetting, R.D. & Storey, G.K. (1982). Susceptibility of the two-spotted spider mite, *Tetranychus urticae* Koch, to the fungal pathogen *Hirsutella thompsonii* Fisher. *Florida Entomologist* **65**, 458-465.

Gentat 5 Committee (2000). Genstat for Windows, Release 4.2. Fifth Edition. VSN International Ltd., Oxford.

Gerson, U., Kenneth, R. & Muttah, T.I. (1979). *Hirsutella thompsonii*, a fungal pathogen of mites. II Host-pathogen interaction. *Annals of Applied Biology* **91**, 29-40.

Goettel, M.S. & Inglis, G.D. (1997). Ch. V-3 Fungi: Hyphomycetes. *In* "Manual of Techniques in Insect Pathology". (Lacey, Ed.), pp.213-248, Academic Press.

Jacobson, R.J. (1998). Spider mite control: Options and opportunities. *In Abstracts, Tomato Conference, Wellesbourne, England, 1 October 1998* (ed. F. Sheppard), p14.

Jacobson, R. J., Croft, P. & Fenlon, J. (1999). Resistance to fenbutatin oxide in populations of *Tetranychus urticae* Koch (Acari: Tetranychidae) in UK protected crops. *Crop Protection.* **18**, 47-52.

Jacobson, R., Croft, P. And Sampson, C. (2000a). Optimising the use of abamectin in cucumber and tomato research Programmes. *HDC Fact Sheet*, 18/00. 4pp.

Jacobson, R.J., Croft, P.C. & Fenlon, J.S. (2000b). Cucumbers: The development of remedial treatments for the control of western flower thrips within IPM programmes. Report of contract work undertaken for HDC, April 2000, 26pp.

Jacobson, R. J., Chandler, D., Fenlon, J. & Russell, K. M. (2001). Compatibility of *Beauveria bassiana* (Balsamo) Vuillemin with *Amblyseius cucumeris* Oudemans (Acarina: Phytoseiidae) to control *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) on cucumber plants. *Biocontrol Science and Technology,* **11**, 381 - 400.

Jacobson, R.J. (2001). Control of capsid bugs within IPM programmes in protected crops. Report of contract work undertaken for HDC, October 2001, 38pp.

Lacey, L. A. & Mercadier, G. (1998). The effect of selected allelochemicals on germination of conidia and blastospores and mycelial growth of the entomopathogenic fungus *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes). *Mycopathologia,* **142**, 17 – 25.

McCoy, C.W. (1981). Pest control by the fungus *Hirsutella thompsonii*, *In Microbial Control of Pests and Plant Diseases, 1970-1980* (Burges, H.D., Ed.), Academic Press, London, UK, pp. 499-512.

Murphy, B., Von Damm-Kattari, D. & Parrella, M. (1999). Interaction between fungal pathogens and natural enemies: Implication for combined biocontrol of greenhouse pests. Integrated Control in Glasshouses, IOBC Bulletin **22 (1)**, 181-184.

Nyiira, Z.M. (1982). Cassava green mite: its distribution and possible control, *In Root crops in Africa: Proceedings of a workshop held in Kigali, Rwanda, 23-27 November 1980.* International Research Centre, Ottawa, Canada, pp. 65-67.

Pena, J.E.,Osborne, L.S. & Duncan, R.E. (1996). Potential of fungi as biocontrol agents of *Polyphagotarsonemus latus* (Acari: Tarsonemidae). *Entomophaga* **41**, 27-36.

Poprawski, T. J., Greenberg, S. M. & Ciomperlink, M. A. (2000). Effect of host plant on *Beauveria bassiana*– and *Paecilomyces fumosoroseus*-induced mortality of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). *Environmental Entomology* **29**, 1048-1053.

Potter, C. (1952). An improved laboratory apparatus for applying direct sprays and surface films, with data on the electrostatic charge on atomized spray fluids. *Annals of Applied Biology* **29**, 1-29.

Sosnowska, D. & Piatkowski, J. (1996). Efficacy of entomopathogenic fungus *Paecilomyces fumosoroseus* against whitefly (*Trialeurodes vaporariorum*) in greenhouse tomato cultures. *IOBC / WPRS Bulletin*, **19**, 179 – 182.

Sterk, G., Hassan, S.A., Baillod, M., Bakker, F., Bigler, F., Blumer, S., Bogenschutz, H., Boller, E., Bromand, B., Brun, J., Calis, J.N.M., Coremans-Pelseneer, J., Duso, C., Garrido, A., Grove, A., Heimbach, U., Hokkanen, H., Jacas, J., Lewis, G., Moreth, L., Polgar, L., Roversti, L., Samsoe-Petersen, L., Sauphanor, B., Schaub, L., Staubli, A., Tuset, J.J., Vainio, A., Van de Veire, M., Viggiani, G., Vinuela, E. & Vogt, H. (1999). Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS- Working Group 'Pesticides and Beneficial Organisms'. *Biocontrol*, **44(1)**, 99-117.

Thomas, M.R. & Garthwaite, D.G. (1997). Pesticide usage survey report 136: protected crops (edible and ornamental) 1995, Maff, London.

Van der Geest, L.P.S. (1985). Pathogens of spider mites, *In Spider Mites, Their Biology, Natural Enemies and Control*, Vol. 1B (Helle, W. & Sabelis, M.W., Eds), Elsevier, Amsterdam, The Netherlands, pp. 247-258.

Van de Veire, M. & Degheele, D. (1996). Toxicity of the fungal pathogen *Paecilomyces fumosoroseus* strain Apopka 97 to the greenhouse whitefly *Trialeurodes vaporariorum* and the parasitoid *Encarsia formosa*, and first results of a control experiment in glasshouse tomatoes. *IOBC / WPRS Bulletin*, **19**, 191 – 194.

Wright, J.E. & Kennedy, F.G. (1996). A new biological product for control of majorgreenhouse pests. *Proceedings of the Brighton Crop Protection Conference-Pests and Diseases 1996* **3**, 885-892.

