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The results and conclusions in this report are based on an investigation conducted over three years. 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.

CONTENTS

PRACTICAL SECTION FOR GROWERS

Headline

This project investigated the potential of fungal pathogens (known as 'entomopathogens') to control spider mites in tomato crops. The product Naturalis-L, which is based on a specific strain of *Beauveria bassiana* (an entomopathogenic fungus), was identified as a new type of remedial treatment for spider mite control. Growers will need to await the approval of use of Naturalis-L in the UK before the results from the project can be fully utilised.

Background and expected deliverables

The project was set up with the support of the British Tomato Growers' Association (TGA) for the purposes of replacing chemical pesticides in UK tomato production. To help the tomato industry to achieve this, it is necessary to develop a 'biopesticide' to support primary control of spider mites using invertebrate predators. 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.

The overall aim of this project was to examine the potential of entomopathogenic fungi as biopesticides of spider mites. The deliverables were:

- An evaluation of entomopathogenic fungi as biopesticides of spider mites on tomato.
- A comparison of fungal biopesticides with chemical acaricides as a second line of defence against spider mites.

Summary of results

1. Identifying suitable fungal pathogens (entomopathogens) for the control of spider mites

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 8.1×10^7 ml^{-1} for *M. anisopliae* 442.99 to 1.1×10^9 ml^{-1} for *Hirsutella* sp. 463.99. *Verticillium lecanii* 19.79 and *Hirsutella* sp. 457.99 showed low pathogenicity in this bioassay and were eliminated from further study.

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 commonly grown 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. 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.

Two glasshouse experiments were done in the project. In the first experiment, five entomopathogenic fungi, applied as sprays, 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 97 % on the upper leaves on the plant. In the second glasshouse experiment, Naturalis-L was used as a second line of defence (i.e. as a supplementary treatment) to the predatory mite *Phytoseiulus persimilis*, and compared against the selective acaricide, Torq (fenbutatin oxide). Naturalis-L was very effective as a remedial treatment, and out performed Torq. A single spray of Naturalis-L caused a 98 % reduction in numbers of *T. urticae* adults, nymphs and eggs. In contrast, a single spray of fenbutatin oxide caused an 80 % reduction in the numbers of *T. urticae* nymphs, but did not significantly reduce the numbers of adults or eggs. Lower but equivalent numbers of *P. persimilis* were found with both the Naturalis-L and Torq treatments. We suspect that these lower numbers were caused by a lack of spider mite prey in the Naturalis-L and Torq treatments causing the *P. persimilis* to migrate, rather than the Naturalis-L and Torq killing the *P. persimilis*. It was clear that any depression of *P. persimilis* numbers was no worse with Naturalis-L than with Torq, but more experimentation may be necessary to understand the cause of this observation. There was no evidence that Naturalis-L was deleterious to bumblebees used in the crop.

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. Information was compiled in a literature review about the effects of entomopathogenic fungi on 12 biological control agents. Important information is available

for *Encarsia formosa* and *Aphidius colemani* (see reports for HDC projects PC 123 and PC 129). There is no strong evidence that *Beauveria bassiana* is pathogenic to *Phytoseiulis persimilis* in the glasshouse, but more work is needed in this area.

Conclusions

1. Four fungal isolates were identified with potential to control spider mites in tomato.
2. The commercial product Naturalis-L (based upon a specific strain of *Beauveria bassiana*) gave the most effective control of spider mites. However, Naturalis-L is not currently approved for use in the UK.
3. Naturalis-L out performed Torq as a second line of defence against spider mites in the glasshouse. Naturalis-L controlled spider mite adults, nymphs and eggs, while Torq was only active against the nymphs.
4. There is no strong evidence that *Beauveria bassiana* is pathogenic to *Phytoseiulis persimilis* in the glasshouse, but more work could be done in this area.
5. Tomato variety and type is unlikely to have an effect on the efficacy of fungal pathogens in controlling spider mites.
6. 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.
7. 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 *Beauveria bassiana* (the fungal strain in Naturalis-L).

Financial benefits

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

Action points for growers

This project is strategic in nature and was set up to replace the use of chemical pesticides in UK tomato production. The work has 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 approval of use of this product in the UK before the results from the project can be fully utilised.

SCIENCE SECTION

INTRODUCTION

Fungal biocontrol of spider mites

Spider mites (*Tetranychus* spp.) are major pests of crops world-wide, principally because they have evolved resistance to many chemical pesticides (Walter & Proctor, 1999; Cranham & Helle, 1985). In response to pesticide resistance, farmers and growers have increased their use of biological control, which is done by conserving natural enemies and / or by applying predatory phytoseiid mites. However, this is often not effective on its own, and supplementary acaricide sprays are used routinely.

In the UK, spider mites are a particular problem on tomato crops, and control here is based on applications of the predatory mite, *Phytoseiulus persimilis* reinforced with sprays of a selective acaricide, fenbutatin oxide. However, *P. persimilis* is slow to establish on tomato plants and does not keep pace with the pest's development during hot weather, and this has resulted in excessive dependence on fenbutatin oxide. As a consequence, the numbers of chemical sprays used against spider mites on tomato crops are greater than the total applied against all other pests, despite the use of *P. persimilis*. Unfortunately, fenbutatin oxide does not perform well against all spider mite 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*., 2000) but is unlikely to be sustainable given the propensity of spider mites to develop chemical resistance. It will also not help the many tomato growers who have a long term goal of pesticide-free production in response to consumer demands (see Tomato Growers' Association R&D Strategy). There is a requirement, therefore, to develop an effective method of *T. urticae* control on tomato that does not involve chemicals. This is most likely to be done with a suite of natural enemies that complement one another's activities at different times during crop and pest development (Jacobson, 1999). In particular, it should include a fast-acting microbial biopesticide to replace the chemical acaricides that are currently used as a second line of defence. The use of a microbial biopesticide in this way has worked well for other pests, for example control of western flower thrips, *Frankliniella occidentalis*, on cucumbers using entomopathogenic fungi and the predatory mite *Neoseiulus* (*Amblyseius*) *cucumeris* (Jacobson *et al*., 2001).

The most promising microbial control agents of spider mites are entomopathogenic fungi, which invade their hosts by growing through the cuticle. They are able, therefore, to infect sap-feeding pests, such as spider mites, which are unlikely to acquire a pathogen *per os*. Eleven species of fungi have been reported to kill *Tetranychus* species in nature or experiments. Six species of entomophthoralean fungi (Zygomycetes, Entomophthorales) have been reported: *Basidiobolus* sp.; *Conidiobolus obscurus*; *Conidiobolus thromboides*; *Neozygites floridana*; *Neozygites tetranychis*; and *Zoophthora radicans* (Kenneth *et al*., 1971; van der Geest, 1985; Smitley *et al*., 1986; Dick & Buschmann, 1995). Five species of anamorphic fungi have also been reported: *Aspergillus depauperatus*; *Beauveria bassiana*; *Hirsutella thompsonii*; *Paecilomyces terricola*; and *Verticillium lecanii* (van der Geest, 1985; Weiser, 1968; Wright & Kennedy, 1996; Gardner *et al*., 1982; Gillespie *et al*., 1982; Andreeva & Shternshis, 1995). *Neozygites floridana* has been investigated as a natural regulator of tetranychid mites in warm-temperate regions, (Smitley *et al*., 1986; Dick *et al*., 1992) but it cannot be cultured easily *in vitro* and therefore is probably not yet suitable for development as a microbial biopesticide (Chandler *et al*., 2000). *Hirsutella thompsonii* is a specific pathogen of eriophyoid and tetranychid mites and it is active against the two-spotted spider mite, *Tetranychus urticae* and the closely-related carmine spider mite, *Tetranychus cinnabarinus* (Gerson *et al*., 1979; Gardner *et al*., 1982). Elsewhere, the *B. bassiana* – based biopesticide Naturalis (Troy Biosciences Inc., USA), is reported to have significantly reduced populations of *T. urticae* on raspberry leaves (DeFrancesco *et al*., 1999), roses (Wright & Kennedy, 1996), cotton (Hinz & Wright, 1997) and goutweed (Abbey & Pundt, 1998).

In this paper, we report on a series of laboratory and glasshouse experiments to investigate the susceptibility of *T. urticae* to entomopathogenic fungi on tomato. Complementary experiments were also done with *T. cinnabarinus*. The aims of the project are set out below.

Scientific / technical targets of the project

The aim of this project was to examine the potential of entomopathogenic fungi as biopesticides of spider mites. The objectives of the project were 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 IPM.
4. Select and evaluate a fungal strain with potential for control of spider mites within IPM programmes in glasshouses, and prepare guidelines for its use in tomato IPM programmes.

Summary of progress up to the reporting year (1999 to 2001)

Obtaining candidate isolates of fungi

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.

Measuring the susceptibility of T. urticae *to entomopathogenic fungi in laboratory bioassays*

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, but only three isolates caused significantly greater mortality than the control: these were *Metarhizium anisopliae* 442.99, *Hirsutella* spp. 457.99, and *Verticillium lecanii* 450.99. These isolates were assessed in a multiple-dose bioassay, together with three isolates cultured from commercial biopesticides as follows: *Beauveria bassiana* 432.99 (cultured from 'Naturalis-L', Troy Biosciences USA); *Hirsutella thompsonii* 463.99 (cultured from 'Mycar', Abbott Laboratories, USA); and *V. lecanii* 19.79 (used in 'Mycotal' Koppert BV, the Netherlands). *Beauveria bassiana* 432.99, *H. thompsonii* 463.99, *M. anisopliae* 442.99, and *V. lecanii* 450.99 were all pathogenic to *T. urticae* in this bioassay. LC_{50} values for these isolates ranged from 8.11×10^7 ml^{-1} (*M. anisopliae* 442.99) to 1.13×10^9 ml^{-1} (*H. thompsonii* 463.99).

The low levels of pathogenicity shown by most of the isolates in the screening bioassay was unexpected, because the literature suggests that *T. urticae* should have been more susceptible to infection (Gerson *et al*., 1979; Gardner *et al*., 1982; Bartowski *et al*., 1988; Wright & Kennedy, 1996; Pena *et al*., 1996). The low levels of mortality could have been caused by the action of tomato allelochemicals, which have known antifungal activity, or be a result of the bioassay method. Both factors were investigated in the following sections.

Measuring the virulence of selected fungal isolates against T. urticae *feeding on different tomato varieties / types*

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'). Fixed age female *T. urticae* reared on each plant type were inoculated with suspensions of fungal conidia and incubated on leaves of their corresponding plant (23^{O}C, 16:8 L:D). The fungal isolates were bioassayed as a pair on seven occasions at one concentration of conidia (1×10^{8} conidia.ml^{-1}). Spider mites on tomato variety / type Clotilde survived significantly better than on tomato variety / type Quest but there were no differences in survival on the other tomato varieties. When adjusted for fungus - induced mortality, the results indicated that tomato is unlikely to have an effect on fungus infection. The findings indicated also that bioassays done on cv Spectra can be extrapolated to other tomato types.

The effect of inoculation method on the susceptibility of T. urticae *to entomopathogenic fungi in laboratory bioassays*

An experiment was done – which was not included in the original work plan - to investigate whether *T. urticae* mortality in a laboratory bioassay was affected by the method used to apply the fungus. The direct application bioassay used previously in the project was compared with an indirect application bioassay, in which tomato leaves were sprayed with a suspension of fungal conidia, left to dry, and then fixed age adult female *T. urticae* placed on them in Petri dishes and maintained under controlled conditions of temperature and humidity. Four isolates of fungi were used: *B. bassiana* 432.99, *H. thompsonii* 463.99, *M. anisopliae* 442.99, and *V. lecanii* 450.99. Naturalis-L (active ingredient *B. bassiana* JW-1 / HRI 432.99) was included as an additional treatment, and was applied at the manufacturer's recommended

concentration (1 x 10^5 conidia ml^{-1}). There was no significant difference in percentage mortality between the fungal isolates when the direct method was used. In contrast, Naturalis-L and *B. bassiana* 432.99 caused significantly more mortality than the other isolates when the indirect application method was used. The indirect application method significantly increased the percentage mortality of *T. urticae* treated with *B. bassiana* 432.99 and Naturalis-L compared to the direct application method. Mortality of *T. urticae* treated with *B. bassiana* 432.99 increased from 46 % to 72 % at 6 days post inoculation, while mortality with Naturalis-L increased from 52 % to 95 %.

The susceptibility of T. cinnabarinus *to entomopathogenic fungi*

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 (*T. cinnabarinus*, previously identified by Zhi-Qiang at the Natural History Museum) which cause hyper-necrotic damage in tomato crops. Fixed age populations of the *T. urticae* and *T. cinnabarinus* were reared on tomato cv 'Spectra' and were treated with suspensions of fungal conidia from four isolates (*B. bassiana* 432.99, *M. anisopliae* 442.99, *V. lecanii* 450.99, and *H. thompsonii* 463.99) plus Naturalis-L, using the indirect application bioassay described above. Naturalis-L caused significantly more mortality than the other fungus treatments, and *B. bassiana* 432.99 caused significantly more mortality 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. The results imply that the findings of other experiments with *T. urticae* will also be applicable to *T. cinnabarinus*.

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

An assessment was done of published accounts of the compatibility of entomopathogenic fungi with potential to control spider mites 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. Seven reports were identified which examined the compatibility of entomopathogenic fungi with arthropod biocontrol agents used in tomato IPM. 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.* These conflicting reports probably arise as a result of researchers using different fungal isolates and different methods in their bioassays. Naturalis-L is reported by its manufacturers to have no significant effects on a range of predators / parasitoids, including species of *Geocoris*, *Encarsia*, *Eretmocerus* and *Chrysoperla* (Wright & Knauf, 1994).

Effect of entomopathogenic fungi on populations of T. urticae *on tomato in the glasshouse*
A glasshouse experiment was done to measure the effect of entomopathogenic fungi on *T. urticae* populations in a long season tomato crop (*L. esculentum* cv Espero) grown hydroponically in rockwool slabs according to the V system. Irrigation with nutrient solution, CO_2 dosing, and plant husbandry (training, tying in, deleafing, layering, and fruit picking) were done according to standard commercial practice (Van de Vooren et al., 1986; Adams & Valdés, 2002). The experiment comprised six treatments: (1) untreated control; (2) *M. anisopliae* 442.99; (3) *H. thompsonii* 463.99; (4) *V. lecanii* 450.99; (5) *B. bassiana* 432.99; and (6) Naturalis-L. Suspensions of fungal conidia were applied at a concentration of 1×10^8 ml^{-1} while Naturalis-L was used at the manufacturer's recommended rate (1×10^5 conidia ml^{-1}, equivalent to 0.5g l^{-1} water). A population of *T. urticae* was established on marked leaves at three positions in the crop (top, middle and bottom), and two sprays of the fungi were applied, seven days apart. Seven days after the second spray, leaves were removed and the numbers of motile *T. urticae* (nymphs + adults) and eggs per leaf were counted. There was a trend for increasing numbers of motile *T. urticae* higher up the plant. At the top sample position, four of the treatments significantly reduced the numbers of motile *T. urticae* and eggs per leaf compared to the untreated control: *H. thompsonii* 463.99, *M. anisopliae* 442.99, *V. lecanii* 450.99, and Naturalis-L. Naturalis-L reduced the motile *T. urticae* population by 97 % at the top sample position. At the middle sample position, all the treatments significantly reduced the numbers of motile *T. urticae* compared to the control, and *V. lecanii* 450.99 and Naturalis-L significantly reduced the numbers of *T. urticae* eggs. Finally, at the bottom sample position, all the treatments significantly reduced the numbers of motile *T. urticae* compared to the control, and four treatments significantly reduced the numbers of *T. urticae* eggs: *B. bassiana* 432.99, *H. thompsonii* 463.99, *M. anisopliae* 442.99, and Naturalis-L. Overall, Naturalis-L had the greatest effect on the *T. urticae* population.

Effect of Naturalis-L as a second line of defence to the predatory mite *Phytoseiulus persimilis*, in comparison to the selective acaricide, fenbutatin oxide (glasshouse experiment).

Introduction

The aim of this experiment was to examine the efficacy of Naturalis-L as a second line of defence to the predatory mite *Phytoseiulus persimilis*, in comparison to the selective acaricide, fenbutatin oxide (Torq), on populations of *T. urticae* feeding on a glasshouse tomato crop.

Materials and Methods

Crop raising

The experiment was done in a long season tomato crop (*L. esculentum* cv Espero) planted on 20 March 2002. The crop was grown, hydroponically in rockwool slabs, in two adjacent compartments of a 1456 m^2 glasshouse. Each compartment measured 10 x 9.3 x 4.2 m. The compartments were maintained at a minimum temperature of 17 °C and vented at 19 - 26°C depending on the age of the crop. The floor of each compartment was covered with white plastic to reflect light and minimise the risk from soil borne plant pathogens. The crop in each compartment consisted of six double rows of plants. Each double row contained 24 plants grown in six, 120 cm rockwool slabs (i.e. four plants per slab) and trained in a V system. Irrigation with nutrient solution, CO_2 dosing, and plant husbandry (training, tying in, deleafing, layering, and fruit picking) were done according to standard commercial practice (Van de Vooren *et al*., 1986; Adams & Valdés, 2002). Pollination was done with bumblebees (BCP Ltd, Ashford, Kent, UK). No additional chemical insecticides or acaricides were applied during the experiments, although a fungicide treatment of Thiovit (Novartis, Cambridge, UK) was applied to control powdery mildew on 13 May 2002.

Naturalis-L

The commercial biopesticide Naturalis-L (Troy Biosciences Inc., Phoenix, USA), which contains the JW-1 isolate of *B. bassiana* as its active ingredient (ARSEF 3097 = ATCC 7404 = FCI 7744 = Horticulture Research International (HRI) isolate 432.99), was stored at 5°C

before use and prepared according to the manufacturer's instructions. The concentration of conidia in Naturalis-L = 2.5 x 10^8 ml^{-1} product.

Phytoseiulus persimilis cultures

Phytoseiulus persimilis cultures were obtained from BCP Ltd. (Ashford, Kent, UK). The cultures were supplied in 30 ml dispenser tubes containing c. 2000 individuals in approximately 25ml of vermiculite carrier. The cultures were stored at 10°C for a maximum of 24h prior to use. *Phytoseiulus persimilis* were dispensed onto tomato leaves by tapping the tube once over each leaf. The number of mites dispensed in this way was estimated to be 4-6 mites per leaf. This estimate was in keeping with that stated by the manufacturer. The manufacturer's recommended release rate was five *P. persimilis* per m^2 for low infestation and 20 per m^2 for heavier infestations.

Fenbutatin oxide

The selective acaricide fenbutatin oxide was obtained as the commercial product Torq (Fargro Ltd., Littlehampton, Sussex, UK). It was stored according to the manufacturer's recommendations and was applied using a 500 ml hand held sprayer (Cherwell, Southam, UK) at the manufacturer's recommended concentration (0.5 g l^{-1})

Glasshouse experiment

Four treatments were used in the experiment: (1) untreated control; (2) *P. persimilis*; (3) *P. persimilis* + fenbutatin oxide; and (4) *P. persimilis* + Naturalis-L. Fenbutatin oxide and Naturalis-L were prepared at the manufacturer's recommended rates (0.5 g l^{-1} and 1 x 10^5 conidia ml^{-1} respectively). Low numbers of *P. persimilis* were introduced into the crop to simulate a situation in which control with *P. persimilis* was failing, and hence sprays of fenbutatin oxide / Naturalis-L were needed as a second line of defence.

The experiment was done according to a randomised block design within two adjacent glasshouse compartments. The treatments were applied in each of four single rows of the crop (i.e. one side of a double row) separated by guard rows. Each row contained two treatments. There was a block of six plants per treatment. Each block was separated by two guard plants. Each treatment was applied to one marked leaf at the top (3 m from the ground) of each of the six plants. There was a total of 24 leaves per treatment.

The experiment was done on the 5 July 2002. Adult female *T. urticae* were released onto one leaflet of each marked leaf, 25 per leaf, using *T. urticae* - colonised leaf material from the stock culture. This was followed by a further release of *T. urticae* 14 d later. *Phytoseiulus persimilis* were introduced 7 d after the second release of *T. urticae*, at a rate of 4 – 6 per leaf, as described previously. Fenbutatin oxide and Naturalis-L were sprayed to run-off onto marked leaves, 4 d after the release of *P. persimilis*. Sprays were applied with a 500 ml hand held sprayer (Cherwell, Southam, UK). 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. Seven days after the application of fenbutatin oxide and Naturalis-L, leaves were removed and numbers of *T. urticae* eggs, nymphs and adults, and numbers of *P. persimilis*, were counted. Data from the blocks were combined and total numbers of *T. urticae* eggs, nymphs and adults, and total numbers of *P. persimilis*, were calculated. The transformation $\log_{10}$ (total + 0.375) was applied to the data before ANOVA (Genstat 2000).

Leaves sprayed with Naturalis-L were collected 0, 1, 2, 3 and 10 days after application of the fungus. Ten leaf discs (1.5 cm diameter) were cut from the leaves and added to 5ml of sterile 0.05% Triton X-100 and shaken on an orbital shaker for 2 mins. Suspensions were diluted and aliquots (100 µl) plated onto 30% SDA and incubated in darkness, at 23^{O}C. After four days the number of colony forming units were counted.

Dead bumblebees were collected from the glasshouse compartments and incubated on damp filter paper at 23°C for up to 7 d and inspected for the presence of sporulating mycelium of *B. bassiana* on cadavers.

Results

Application of *P. persimilis* on its own did not significantly ($p < 0.05$) reduce numbers of *T. urticae* adults, nymphs or eggs (Table 1). Application of *P. persimilis* + fenbutatin oxide significantly reduced ($p < 0.05$) the numbers of *T. urticae* nymphs (80% reduction), but did not significantly ($p < 0.05$) reduce the numbers of adults or eggs compared to the untreated control or the *P. persimilis* treatment. In contrast, application of *P. persimilis* + Naturalis-L significantly ($p < 0.05$) reduced numbers of *T. urticae* adults, nymphs and eggs compared to all other treatments (98 % reduction in all three cases). Significantly ($p < 0.05$) fewer numbers of *P. persimilis* were recorded from both the *P. persimilis* + fenbutatin oxide

treatment, and the *P. persimilis* + Naturalis-L treatment, than the *P. persimilis* - only treatment. Unfortunately, we were unable to provide data on the survival of Naturalis-L on tomato leaves. Leaf washings from Day 0 plated onto SDA produced a lawn of *B. bassiana*. When the washings from Day 0 were diluted more and plated out again, there was a large amount of contamination from bacteria and fungi (*Aspergillus* and *Penicillium*) which overgrew the *B. bassiana* colonies. Leaf washings from the other sample days were also heavily contaminated which prevented colony counting. There was no evidence that Naturalis-L was the cause of mortality in any of the dead bumblebees collected in the glasshouse compartments.

Table 1. Mean (backtransformed, n = 24) number of *T. urticae* adults, nymphs and eggs per leaf following treatment with *Phytoseiulus persimilis* +/- fenbutatin oxide or Naturalis-L. Log $_{10}$ transformed data in parentheses.

	Adults	**Nymphs**	**Eggs**	***Phytoseiulus persimilis***
Control	38.2 (1.59)	484.9 (2.69)	537.9 (2.26)	-
P. persimilis	39.9 (1.60)	210.5 (2.32)	96.7 (1.99)	2.9 (0.52)
P. persimilis + fenbutatin oxide	40.5 (1.61)	101.5 (2.01)	115.5 (2.06)	0.8 (0.06)
P. persimilis + Naturalis-L	0.8 (0.69)	11.5 (1.08)	9.1 (0.98)	0.5 (-0.06)
LSD ($p < 0.05$, one-tailed)	(0.656)	(0.5288)	(0.756)	(0.440)

General discussion of project results

The experiments done in this 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 hyper-necrotic 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 the first of

two glasshouse experiments, five fungal treatments (*M. anisopliae* 442.99; *H. thompsonii* 463.99; *V. lecanii* 450.99; *B. bassiana* 432.99; and Naturalis-L) significantly reduced *T. urticae* populations. Naturalis-L was the most effective overall, reducing mite populations by up to 97 %. In the second glasshouse experiment, Naturalis-L was effective as a second line of defence to *P. persimilis*, and out performed fenbutatin oxide. A supplementary spray of Naturalis-L caused a 98 % reduction in numbers of *T. urticae* adults, nymphs and eggs. In contrast, a supplementary spray of fenbutatin oxide caused an 80 % reduction in the numbers of *T. urticae* nymphs, but did not significantly reduce the numbers of adults or eggs.

Spider mites are serious pests of crops because of the damage they cause and their capacity to develop resistance to chemical pesticides, but little work has been done on microbial control as an alternative to chemical treatments. This is the first study in which a range of species and isolates of entomopathogenic fungi have been compared against tetranychid mites. Three of the fungal species used in this study have been reported previously to kill tetranychid mites: *B. bassiana, H. thompsonii,* and *V. lecanii* (Wright & Kennedy, 1996; Gardner *et al*., 1982; Gillespie *et al*., 1982). *Metarhizium anisopliae* 442.99 was the most virulent isolate identified in our laboratory bioassays, but this species has not been reported before as a pathogen of tetranychid mites. In addition, sporulating cadavers were observed in the bioassays with four other species of fungi which have not been reported before as pathogens of tetranychid mites: *Paecilomyces farinosus*, *Paecilomyces fumosoroseus*, *Tolypocladium niveum*, and *Tolypocladium inflatum*.

Overall, only a small proportion of *T. urticae* cadavers supported sporulating mycelium in the bioassays, which probably reflects the low levels of mortality that were also observed. It is unlikely that the mites were too small to support sufficient fungal biomass for sporulation, because the entomophthoralean fungus *N. floridana* is known to sporulate profusely on *T. urticae* cadavers (Dick *et al*., 1992). *Hirsutella thompsonii* and *V. lecanii* both cause natural epizootics in populations of eriophyoid mites (McCoy, 1981; Lewis *et al*., 1981) which suggests that these fungi are also able to sporulate well on acarine hosts. Research is required to investigate this further, because poor sporulation on cadavers in tomato crops is likely to limit the persistence of fungal infection in *T. urticae* populations.

The simplest explanation for the low levels of mortality observed in the first bioassays is that most of the fungal isolates examined were weak pathogens of *T. urticae*. However, the results of these bioassays were unexpected to us, because some of the species / isolates had been reported elsewhere to cause high levels of mortality in tetranychid mites. For example, Naturalis-L (used in the first bioassays as a laboratory culture, *B. bassiana* 432.99) had been reported to cause significant *T. urticae* mortality in laboratory, glasshouse and field studies (Wright & Kennedy, 1996; Abbey & Pundt, 1997; Hinz & Wright, 1997; DeFrancesco *et al*., 1999). *Hirsutella thompsonii* is a specific pathogen of eriophyoid and tetranychid mites, and is known to kill *T. urticae*, *Tetranychus cinnabarinus* and *Tetranychus turkestani* (Gardner *et al*., 1982; Gerson *et al*., 1979; McCoy 1978, cited in Samson *et al*., 1988). Gardner *et al*. (1982) obtained 40 – 65 % mortality of *T. urticae* using *H. thompsonii* sprayed onto leaf discs of shamrock at a concentration of only 1 x 10^4 ml^{-1}. They were also able to infect *T. urticae* with single conidia of *H. thompsonii* applied with a micromanipulation technique. Similarly, the *V. lecanii* - based biopesticide Mycotal, which uses the HRI 19.79 isolate as its active ingredient, was reported to have caused 93% control of *T. urticae* after 4 days incubation in a laboratory bioassay based on cucumber leaves (Gillespie *et al*., 1982).

It can be inferred from these reports that additional factors could have contributed to the low levels of mortality observed in the first bioassays. We found that tomato variety / type had no effect on fungal virulence (although this does not rule out the possibility that tomato allelochemicals can inhibit infection for some fungal isolates – see below). However the method used to apply fungal conidia to *T. urticae* affected the efficacy of at least one isolate, because using an indirect application method for the bioassay significantly increased the mortality of *T. urticae* treated with Naturalis-L and *B. bassiana* 432.99 (which was cultured from Naturalis-L). However, it had no effect on the three other isolates examined; *H. thompsonii* 463.99, *V. lecanii* 450.99, and *M. anisopliae* 442.99. The reason why application method only affected the efficacy of *B. bassiana* 432.99 / Naturalis-L is not known. The ability of conidia to attach to the host cuticle is strongly correlated with virulence (Altre *et al*., 1999), and so it is possible that more conidia of this fungus attached to *T. urticae* using the indirect application technique compared to the direct technique. However, application method can also influence the pattern of deposition of conidia on the cuticle, and this can have a greater effect on fungal efficacy than the total number of conidia deposited. For example, Fernandez *et al*. (2001) found that when larvae of the Colorado potato beetle, *Leptinotarsa decemlineata*, were exposed to *B. bassiana*–treated foliage, they acquired more

conidia, but showed lower mortality, than larvae sprayed with a conidia suspension. This was because conidia acquired from the foliage attached predominantly to the ventral surface of the larvae, which was a poor site for infection.

We chose a direct application bioassay for the first bioassays because it enables a precise dose of conidia to be presented, which is thought to be important for reducing variability and enabling results to be repeated (Goettel & Inglis, 1997; Butt & Goettel, 2000). In this study, however, the direct application bioassay had shortcomings. It could have caused some of the inconsistencies between the single- and multiple-dose bioassays, and it could have led to mistakes in isolate selection. Further work is required to determine the following : (1) the mechanism by which the efficacy of *B. bassiana* 432.99 was increased in the indirect application bioassay; (2) if the response is unique to *B. bassiana* 432.99 or is exhibited by other isolates / species; (3) if the acquisition of conidia from foliage is an important method for infecting *T. urticae* in the glasshouse. If the last point is true, then screening isolates against *T. urticae* should be done using the indirect application method as well as / instead of the direct application method.

Another explanation for the low levels of mortality in the bioassays is that tomato allelochemicals may have inhibited fungal infection. In particular, the glycoalkaloid tomatine has a general antifungal activity and is known to inhibit entomopathogenic fungi (Costa & Gaugler, 1989; Gallardo *et al*., 1990; Lacey & Mercadier, 1998; all cited in Poprawski *et al*., 2000), but its effects may vary with fungal isolate, host species and tomato variety (Poprawski *et al*., 2000). Poprawski *et al*. (2000) reported that third instar nymphs of glasshouse whitefly, *Trialeurodes vaporariorum*, were significantly less susceptible to *B. bassiana* and *P. fumosoroseus* in bioassays on tomato plants than on cucumber plants. In contrast, Vidal *et al*. (1998) reported good control of silverleaf whitefly, *Bemisia argentofolii*, by *P. fumosoroseus* on cucumber, cabbage and three varieties of tomato, with no effect of plant species or tomato variety on fungal infectivity. Likewise, Tang & Hou (1998) observed no difference in the virulence of *Nomuraea rileyi* applied to maize, tomato, soybean and chrysanthemum and fed to larvae of the corn earworm, *Helicoverpa armigera*. Our brief was to investigate entomopathogenic fungi against *T. urticae* for use by tomato growers, and hence tomato plant material had to be used in the experiments irrespective of whether it was detrimental to fungal infectivity or not. We found that tomato variety / type had no significant effect on fungal virulence, and good control was observed with some isolates in

the glasshouse (see below), and hence we have no evidence that tomato was detrimental to fungal efficacy. It is possible that the fungal isolates that killed *T. urticae* in our study were tolerant of tomato allelochemicals. Because (1) tomatine has been reported to inhibit entomopathogenic fungi, and (2) most of the isolates caused low mortality in this study, more detailed investigation of the effect of tomato allelochemicals on fungal control of *T. urticae* is warranted in future.

The fungal isolates examined in the glasshouse studies gave better control of *T. urticae* than expected from the laboratory bioassays. However, with the exception of Naturalis-L, they were applied at a high concentration (1×10^8 ml^{-1}), which is unlikely to be economic in a commercial product. The best results were observed with Naturalis-L, which reduced *T. urticae* populations by up to 97 % on its own, and caused up to 98 % reduction within 7 d when applied with *P. persimilis*. In the second glasshouse experiment, Naturalis-L out performed fenbutatin oxide as a second line of defence to *P. persimilis*. Application of *P. persimilis* + fenbutatin oxide significantly reduced numbers of *T. urticae* nymphs, but did not significantly reduce numbers of adults or eggs. In contrast, application of *P. persimilis* + Naturalis-L significantly reduced numbers of *T. urticae* adults, nymphs and eggs (98 % reduction in all cases). Both the *P. persimilis* + fenbutatin oxide treatment, and the *P. persimilis* + Naturalis-L treatment, recorded significantly fewer numbers of *P. persimilis* than the *P. persimilis* -only treatment. It is not known whether Naturalis-L and fenbutatin oxide caused this by killing *P. persimilis*, or whether the lack of *T. urticae* prey caused *P. persimilis* to migrate.

A key finding in this project was that Naturalis-L had a markedly greater efficacy than the laboratory isolate cultured from it, *B. bassiana* 432.99. In the experiment to compare the direct and the indirect application bioassays, Naturalis-L was applied at the manufacturer's recommended concentration of 1×10^5 ml^{-1}, and in the direct application bioassay caused the same mortality as *B. bassiana* 432.99 applied at 1×10^8 ml^{-1}. In the indirect application bioassay, Naturalis-L (1×10^5 ml^{-1}) caused significantly greater mortality than *B. bassiana* 432.99 (1×10^8 ml^{-1}). Similarly, in Glasshouse Experiment 1, Naturalis-L (1×10^5 ml^{-1}) gave better overall control of *T. urticae* than *B. bassiana* 432.99 (1×10^8 ml^{-1}). We assume that the difference in efficacy was due to better inoculum quality and / or commercial formulation in Naturalis-L. This may be another explanation for the low levels of mortality observed in Laboratory Experiments 1 and 2. Formulation is known to increase the efficacy of microbial

biopesticides by improving application and coverage, activity, and persistence on the leaf surface (Jones *et al*., 1997). Nonetheless, we were surprised at the size of the difference between Naturalis-L and *B. bassiana* 432.99. We examined other isolates cultured from commercial products in Laboratory Experiments 1 and 2, but none had high virulence. Laboratory cultures were used deliberately, to be able to compare their intrinsic virulences, but this may have caused us to overlook potentially useful commercial products. We may also have obtained greater activity against *T. urticae* with our 'non-commercial' isolates if we had been able to culture and formulate them to commercial standards.

Overall conclusions

Our study indicates that Naturalis-L has considerable potential for the control of *T. urticae* on tomato crops, and it is a good example of an entomopathogenic fungus performing better than the standard chemical treatment for a pest. Investigation of Naturalis-L against *T. urticae* on a range of other crops is also warranted. An important issue now is to determine its compatibility with *P. persimilis* in more detail. Naturalis-L is reported by its manufacturers to have no significant effects on a range of predators / parasitoids, including species of *Geocoris*, *Encarsia*, *Eretmocerus* and *Chrysoperla* (Wright & Knauf, 1994). In our glasshouse experiments, Naturalis-L caused a reduction in the numbers of *P. persimilis*, but as stated previously, we do not know if the fungus was pathogenic or whether the absence of prey caused the predator to migrate. Even if Naturalis-L has some activity against *P. persimilis*, this may not prevent its use as a supplementary treatment provided that (1) it does not persist in the crop, and (2) *P. persimilis* can be introduced quickly once the fungus has reduced *T. urticae* populations to below the economic threshold. Finally, if the compatibility of Naturalis-L with other natural enemies turns out to be a problem, there are opportunities to investigate other commercial fungal biopesticides against *T. urticae*, which may have narrower host ranges.

Technology transfer

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