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The results and conclusion in this report are based on an investigation conducted over one year. The conditions under which the experiments were carried out and the results 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

Background and objectives

Spider mites remain one of the most serious pests of tomato crops in the UK. To help the tomato industry to achieve their long term aim of pesticide-free crop production, it is necessary to develop a 'biopesticide' that can be used remedially to support the primary control measures based on invertebrate predators.

This project investigates the potential of entomopathogenic fungi to act as biopesticides of spider mites on tomato. Entomopathogenic fungi have been developed as biopesticides for a number of protected crops, both in the UK and USA. They are particularly valuable for the control of sap-feeding pests because they have contact action. Although spider mites are known to be susceptible to entomopathogenic fungi in nature and in experiments, little research has been done on their potential as biopesticides of these pests.

Aims 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 fungal pathogens 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 fungi 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.

Specific targets for Year 1 of the project

The specific aims for the first year of the project were as follows:

1. Review the scientific literature to identify fungi pathogenic to spider mites and related species.
2. Obtain entomopathogenic fungi, used as commercial biopesticides or with reported activity to mites, from culture collections of these fungi.
3. Catalogue and store the fungi.
4. Obtain and culture spider mites that cause both normal and hyper-necrotic damage.
5. Develop laboratory bioassays to measure the effect of fungi on the survival of spider mites.
6. Measure the effect of fungi on the survival of spider mites in the bioassay, using a single dose of fungal spores.

Summary of results

The scientific literature on the fungal pathogens of spider mites was examined. A range of fungi attack mites in nature. Some have been examined as natural control agents of mites on field crops in the southern USA. A few other fungi have been studied as biopesticides of spider mites and related species, mainly in the subtropics. We obtained 40 isolates of fungi from nine species, 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 be infective to mites. We also included fungi used in six proprietary biopesticides.

A laboratory bioassay was developed to measure the effect of spores of entomopathogenic fungi on the survival of two-spotted spider mites. Fixed age cultures of adult female spider mites were sprayed with a suspension of spores, then maintained on a tomato leaf held under controlled conditions of temperature and humidity. This method was used to screen 40 isolates of fungi against spider mites, using a single dose of fungal spores. The fungi exhibited a range of pathogenicities to the mites, and six isolates were chosen for further study in multiple dose bioassays in Year 2. These isolates included five that came in the top ten pathogenicity rankings, four of which were originally isolated from mite or tick hosts. Two of the six isolates are used in proprietary biopesticides.

Future work in the project

Following on from the progress made in Year 1, the work for Year 2 is as follows:

1. Examine virulent isolates, identified in the single dose bioassays, in multiple dose bioassays against the normal form of the spider mite.
2. Examine the scientific literature for information on the compatibility of the species of fungal pathogens, identified above, with arthropod biocontrol agents used in tomato IPM.
3. Identify gaps in the knowledge and, where appropriate, use laboratory bioassays to measure the effect on arthropod biocontrol agents of the fungal pathogens shortlisted above.
4. Select the isolate of fungus that exhibits the most appropriate combination of virulence and compatibility with other biocontrol agents.

Potential benefits to the industry

Representatives of the TGA Technical Committee have expressed their strong support for research that will develop a biological alternative to chemical acaricides for use against spider mites. This will be a significant step forward in the quest for pesticide-free tomato production and will provide UK growers with a considerable, but possibly only temporary, advantage over their European and North African competitors. Nonetheless it will be an important integral part of their long term strategy to build continuous awareness in customers and consumers as to the benefits of UK produce, helping to promote the uniqueness of the "British" product and re-establish true brand loyalty.

The acquisition of this knowledge will also be beneficial to growers of other protected salad crops and growers of ornamental crops who are moving towards IPM.

SCIENCE SECTION

INTRODUCTION

Spider mites as pests of protected tomato crops

A recent survey commissioned by the Tomato Grower's Association (TGA) confirmed that two-spotted spider mite (*Tetranychus urticae*) control had become one of the industry's greatest problems. The survey also revealed that tomato growers expected the quest for "pesticide-free crops" to be one of their greatest challenges, despite having already developed one of the most highly advanced IPM programmes in the world. There is no doubt that these two subjects are inextricably linked.

In recent years, spider mite control in UK tomato crops has been based on the combined use of the predatory mite, *Phytoseiulus persimilis*, and the selective acaricide, fenbutatin oxide (Torq). However, the predators are slow to establish on tomato plants and do not keep pace with the pest's development during hot conditions. Spider mite control has, therefore, been heavily reliant on applications of the acaricide. Unfortunately, fenbutatin oxide is not performing well against all spider mite populations and there are clear indications that some populations are becoming resistant to it (Jacobson *et al*., 1999). An additional acaricide, abamectin (Dynamec), has recently received approval for use on tomato crops in the UK and it may become available to reduce the dependence and resistance selection pressure on fenbutatin oxide (Jacobson *et al.*, 2000a). Abamectin will help alleviate the spider mite problem in the short-term but will not contribute towards the longer-term goal of pesticide-free crop production. There is a requirement, therefore, to develop an effective system of spider mite control without the use of chemical pesticides.

The most effective method for chemical-free control of spider mites is likely to be based on a suite of complementary natural enemies (Jacobson, 1999). This suite must include a 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*., 2000b).

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 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.

The agreed work plan was:

Year 1 (1999/2000)

1.1. Review the scientific literature to identify species and strains of fungi pathogenic to spider mites and related species.
1.2. 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.
1.3. Catalogue and store the fungal isolates using cryopreservation techniques in place at HRI Wellesbourne.
1.4. Obtain and culture spider mites that cause both normal and hyper-necrotic damage, using techniques in place at HRI Stockbridge House.
1.5. 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.
1.6. Measure the virulence of candidate isolates of fungi to the normal form of the spider mite, using a single, fixed dose of fungal conidia.

Year 2 (2000/2001)

2.1. Examine virulent isolates, identified in the single dose bioassays, in multiple dose bioassays against the normal form of the spider mite.
2.2. Examine the scientific literature for information on the compatibility of the species of fungal pathogens, identified above, with arthropod biocontrol agents used in tomato IPM.
2.3. Identify gaps in the knowledge and, where appropriate, use laboratory bioassays to measure the effect on arthropod biocontrol agents of the fungal pathogens shortlisted above.
2.4. Select the isolate of fungus that exhibits the most appropriate combination of virulence and compatibility with other biocontrol agents.

Years 3 & 4 (2001/2002)

3/4.1. Investigate the ability of the selected fungus to control populations of spider mites in small scale, laboratory and glasshouse experiments under different regimes of temperature and humidity.
3/4.2. Study the ability of this fungus to control populations of spider mites on a crop scale.

SUMMARY OF LITERATURE SEARCH

Fungal pathogens of spider mites

Approximately 750 species of fungi in 56 genera are known to be pathogens or parasites of arthropods (Hawksworth *et al*., 1995), and many contribute to the natural regulation of insect and mite species in nature (Tanada & Kaya, 1993; Chandler *et al*., 2000). Most species are in the Entomophthorales and the Mitosporic fungi, and these groups also contain the most virulent entomopathogenic fungi. The infection cycles of the two groups are basically similar (Hajek & St. Leger, 1994), although the Entomophthorales tend to exhibit a wide array of ecomorphological adaptations to their hosts, while the Mitosporic entomopathogens (with the likely exception of *Verticillium lecanii* and *Hirsutella thompsonii*) function more opportunistically (Samson *et al*., 1988). Both groups of fungi have been widely studied as biological control agents of a range of arthropod pests (McCoy *et al*., 1988; Tanada & Kaya, 1993). However, the Mitopsoric entomopathogens tend to be favoured as biopesticides as they can be grown readily in bulk, and can be formulated and applied using conventional techniques. Over 15 biopesticides based on entomopathogenic fungi are available commercially for the control of insect pests (Shah & Goettel, 1999). Most of these products are based on *Beauveria bassiana* (Mitosporic fungi) but commercial biopesticides have also been developed based on other Mitosporic fungi; *Metarhizium anisopliae*, *Paecilomyces fumosoroseus*, *Hirsutella thompsonii*, and *Verticillium lecanii* (Shah & Goettel, 1999). The majority of products have been developed for markets in North and South America. Two products – Mycotal and Vertalec (Koppert BV, Netherlands) – both based on *V. lecanii* and originally developed by the former Glasshouse Crops Research Institute, are sold in the UK for the control of aphids, whiteflies and thrips. Products developed in the USA, and based on *B. bassiana* for the control of a range of glasshouse pests, are expected in the UK in the next few years. Unlike entomopathogenic viruses and bacteria, entomopathogenic fungi do not have to be ingested to infect their hosts, and hence are suitable for the control of sap-feeding pests.

Spider mites have been reported to be susceptible to 11 species of fungi in nature or in experiments. Entomophthoralean fungi reported from spider mites are: *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). The Mitosporic entomopathogenic fungi reported from spider mites are: *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 spp. (Entomophthorales) are important and specific natural regulators of spider mite populations on field crops in warm-temperate regions, such as the southern USA, but they cannot be cultured *in vitro* and therefore are not suitable for development as biopesticides (Chandler *et al*., 2000). Recommendations have been developed to exploit the natural activities of *Neozygites*, however, and it is recognised as a key natural enemy of spider mites in the peanut-maize-soybean agro-ecosystem in the USA.

Hirsutella thompsonii is a specific pathogen of phytophagous mites, particularly members of the Eriophyidae and Tetranychidae, and is an important natural regulator of mites feeding on crops such as citrus, coconut and cassava in the tropics and subtropics (McCoy, 1981;

Lampredo & Luis-Rosas, 1989; Yaninek *et al*., 1996). An isolate of *H. thompsonii* from the eriophyoid *Vasates destructor* gave 60 – 90% control of mites within six days at 27^{O}C when sprayed onto mite infested tomato plants in Cuba (Cabrera & McCoy, 1984). The fungus has also been studied as a biopesticide of the cassava green mite, *Mononychellus tanajoa*, in Kenya, and has reportedly given significant reductions of mite populations in field cage experiments (Odongo *et al*., 1998). A Florida isolate of *H. thompsonii* was developed and registered as the biopesticide Mycar (Abbott Laboratories, USA) in the 1970's / 1980's for the control of the citrus rust mite, *Phyllocoptruta oleivora*, in the USA, and gave initially promising results, but was withdrawn from sale in 1985 (Bergh & McCoy, 1997). Unusually for a Mitosporic entomopathogenic fungus, *Hirsutella thompsonii* grows somewhat slowly in culture, and its failure as Mycar was attributed partly to poor product stability and dependence on high humidity. However, recent advances in formulation technology have made it possible to use fungal biopesticides in environments where humidity has been limiting previously (Milner, 1997). Techniques for the industrial culture of entomopathogenic fungi have also progressed significantly since the 1980's, which may overcome the production difficulties encountered with Mycar.

Beauveria bassiana is reported to infect over 100 species of insects from a range of orders, although isolates of this fungus vary in host range and some exhibit high host specificity (Fargues, 1976; McCoy *et al*., 1988). Naturalis (Troy Biosciences Inc., USA), based on *B. bassiana*, reportedly gave good control of red spider mites on glasshouse roses under normal crop production conditions (Wright & Kennedy, 1996). This product has also been reported to control thrips, whiteflies, aphids, and the cotton boll weevil (Wright, 1993; Wright & Kennedy, 1996). A second *B. bassiana*-based product, BotaniGard (Mycotech, USA), is sold in the USA for the control of a range of glasshouse pests, although it has not been examined against spider mites. *Beauveria bassiana* has 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 *P. fumosoroseus* derived from a homopteran host, in laboratory experiments (Pena *et al*., 1996).

Prospects for the fungal control of spider mites on protected tomatoes

Spider mites are susceptible to entomopathogenic fungi and hence there would appear to be potential to use them for spider mite control on protected tomatoes. To date, very little work has been done in the UK to study the potential of fungi to act as biopesticides of mites. Lewis *et al*. (1981) observed frequent natural infections by *V. lecanii* and *H. thompsonii* in populations of the eriophyoid *Abacarus hystrix* on ryegrass in the UK in some years, but the possibilities of using these fungi for mite biocontrol were not progressed. In preliminary observations at HRI, commercial strains of *B. bassiana* and *V. lecanii* infected populations of spider mites in the laboratory (Jacobson, unpublished; Chandler & Matthews, unpublished). These fungi could have potential to control spider mites on protected tomato crops, together with isolates of *H. thompsonii*. Isolates of entomopathogenic fungi vary widely in host range and other pathogenicity-related characteristics, and hence it is important that a range of fungi are considered for spider mite control to ensure that the final choice has the maximum possible impact on spider mite populations with minimal disruption of other biological components in the IPM programme.

Measuring the susceptibility of spider mites to fungal pathogens

The selection of a fungal isolate to be used as a biopesticide for spider mite control is best done in a structured, hierarchical programme using laboratory bioassays followed by glasshouse experiments. Laboratory bioassays allow the virulences of candidate fungi to be estimated in a controlled setting. Virulent fungi can then be examined further in glasshouse experiments that reflect crop production conditions. It should be noted that bioassays with entomopathogenic fungi can exhibit high levels of residual variation, and considerable effort can be required to develop a workable bioassay. Key factors affecting bioassays include: a) variation in the dose of infectious units received by hosts during inoculation; b) the effect of culture conditions on fungal virulence; c) phenotypic and genotypic variation in the susceptibility of host organisms to infection; d) the interactions between the fungus, the host, the host's food supply, and the abiotic environment.

Given the complexities of bioassay design, and the challenges inherent in handling spider mites (which are delicate and have short life cycles), it is perhaps not surprising that very little information has been published on bioassays of entomopathogenic fungi against spider mites. We have 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.

SCIENTIFIC AND TECHNICAL PROGRESS

Materials and Methods

Fungal isolates
Culture collections of entomopathogenic fungi containing isolates from Acari were identified via the scientific literature and world wide web. Twenty three on-line collections administered by research institutes were identified and searched, plus five collections run by individual researchers at universities. In total, 40 isolates of fungi were obtained for experimentation from four on-line collections, five individual collections, and the HRI culture collection (Table 1). The fungal isolates obtained for experimentation could be broadly classified in three non-mutually exclusive groups: a) isolates derived from Acari; b) isolates derived from nonacarine hosts but reported to kill Acari; c) isolates used in commercial biopesticides. Most emphasis was placed on isolates from phytophagous mites, but we also included three isolates reported to be pathogenic to ticks (*Ixodes* spp. and *Boophilus* spp.) for reference purposes. We included isolates from six commercial biopesticides (Naturalis, BotaniGard, Bioblast, PFR-97, Vertalec, and Mycotal). The isolates were from nine species: *Beauveria bassiana* (7 isolates); *Hirsutella necatrix* (1 isolate); *Hirsutella thompsonii* (7 isolates); *Metarhizium anisopliae* (7 isolates); *Paecilomyces farinsosus* (1 isolate); *Paecilomyces fumosoroseus* (2 isolates); *Tolypocladium inflatum* (1 isolate); *Tolypocladium niveum* (1 isolate); *Verticillium lecanii* (8 isolates). There were also five isolates of the genus *Hirsutella* not identified to species level. Stock cultures of the fungal isolates obtained for the study were catalogued and stored in liquid nitrogen vapour at HRI Wellesbourne (Chandler, 1994).

Production of fungi for bioassays (HRI Wellesbourne)
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 sintered glass thimbles (40 - 100 μm pore). Conidia were enumerated using an improved Neubauer haemacytometer and aliquots (10 ml) were prepared at a concentrations of 1 x 10^7 conidia ml^{-1}.

Synchronised spider mite cultures
Fifteen adult female spider mites (*T. urticae*) were taken from stock cultures on tomato plants (cv 'Spectra'), placed on inverted tomato leaflets (cv 'Spectra') on damp filter paper in each of 40 Petri dishes, and incubated at 21 ± 2^{O}C, 16L:8D for 48 h. Leaflets with spider mite nymphs were then placed on whole 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). The plants were regularly monitored until new adults were found. New adults that had just begun to produce eggs were then selected for experimentation.

Development of a laboratory bioassay to measure the susceptibility of spider mites to entomopathogenic fungi (HRI Stockbridge House)

A series of pilot experiments was done to develop a laboratory bioassay to measure the susceptibility of spider mites to entomopathogenic fungi. The main criteria used to develop the bioassay were as follows:

1. To inoculate spider mites with fungal conidia in a liquid suspension (water plus wetting agent) using a method that causes minimal mortality during treatment and which is controlled and allows a reasonable throughput of treatments.
2. To contain and maintain treated spider mites in an apparatus that enables mites to feed on plant material, allows them to be monitored with low disturbance, and minimises mortality while permitting fungal infection.

The methods examined are summarised in Table 2. In all cases, the cumulative mortalities of mite populations in the various treatments were compared to the known natural mortality rate of spider mite populations in glasshouses (P. Croft, unpublished). Methods that gave a high mortality were rejected. These experiments centred around two key stages to the bioassay process:

1. *Inoculation techniques*. Two techniques were examined for inoculating mites; total immersion in liquid, and spraying using a Potter tower (Potter, 1952). In both cases, the effects of using different volumes and concentrations of wetting agent (Triton X-100) were also investigated.
2. *Techniques to contain mites during inoculation*. Mites sprayed under the Potter tower were maintained on leaf discs, leaf pieces, or glass cover slips, during spraying to prevent them escaping during treatment, then transferred to tomato leaves for the remainder of the bioassay.

Overall, bioassay reference 20 was the most acceptable (see Table 2). This procedure minimised mortality in the untreated controls, minimised loss of mites through escapes from the experimental arena and eliminated secondary acquisition of fungal spores.

Fungal bioassay – final methodology

The final method developed for the bioassay was as follows: 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). Saturating the filter paper with water prevented the mites from leaving the cover slip. The mites were sprayed with 2ml 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 Petri dishes sealed (Parafilm). 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

Table 1: Fungal isolates obtained for screening against red spider mites

HRI code	Accession no	Species	Host/Substrate	Collection site
431.99	T228	*Beauveria bassiana*	unknown mite spp.	Denmark
432.99	Naturalis	*Beauveria bassiana*		
433.99	BotaniGard	*Beauveria bassiana*		
434.99	ARSEF 2869	*Beauveria bassiana*	*Berphratelloides cubensis*	Florida
454.99	I91 636	*Beauveria bassiana*	Unknown mite spp.	
455.99	DAT 049	*Beauveria bassiana*		
460.99		*Beauveria bassiana*	Unknown mite spp.	Israel
436.99	H2	*Hirsutella* sp.	Acari: Tarsonemidae	Poland
437.99	H3	*Hirsutella* sp.	*Eriophyes piri*	Poland
438.99	H4	*Hirsutella* sp.	*Abacarus hystrix*	Poland
440.99	H12	*Hirsutella* sp.	*Stenotarsonemus fragariae*	Poland
457.99	3339C	*Hirsutella* sp.	*Dendrolaelaps* sp.	Poland
49.81	HRI 49.81	*Hirsutella necatrix*	*Abacarus hystrix*	UK
34.79	HRI 34.79	*Hirsutella thompsonii*	*Eriophyes guerreronis*	Ivory Coast
51.81	HRI 51.81	*Hirsutella thompsonii*		
71.82	HRI 71.82	*Hirsutella thompsonii*	*Eriophyes guerreronis*	Jamaica
73.82	HRI 73.82	*Hirsutella thompsonii*	*Phyllocoptruta captrila*	Florida
75.82	HRI 75.82	*Hirsutella thompsonii*	*Colomerus novahebridensis*	New Guinea
77.82	HRI 77.82	*Hirsutella thompsonii*	*Eriophyes guerreronis*	Jamaica
463.99	DoCo AL	*Hirsutella thompsonii*	*Varroa jacobsonii*	Canada
275.86		*Metarhizium anisopliae*	*Cydia pomonella*	Germany
392.93		*Metarhizium anisopliae*	soil	UK
441.99	ARSEF 3297	*Metarhizium anisopliae*	*Boophilus* spp.	Mexico
442.99	ARSEF 4556	*Metarhizium anisopliae*	*Boophilus* spp.	Florida
456.99	DAT F001	*Metarhizium anisopliae*		
444.99	ATCC 38249	*Metarhizium anisopliae*	*Hylobius pales*	
445.99	Bioblast	*Metarhizium anisopliae*		
446.99	CCFC 002085	*Paecilomyces farinosus*	*Mycobates* spp.	Canada
409.96	PFR-97	*Paecilomyces fumosoroseus*		
447.99	KVL 319	*Paecilomyces fumosoroseus*	*Ixodes ricinus*	Denmark
448.99	ARSEF 3278	*Tolypocladium inflatum*	*Mycobates* spp.	Canada
449.99	CCFC 002081	*Tolypocladium niveum*	*Mycobates* spp.	Canada
1.72	Vertalec	*Verticillium lecanii*	*Macrosiphoniella sanborni*	
17.76	HRI 17.76	*Verticillium lecanii*	*Cecidophyopsis* spp.	
19.79	Mycotal	*Verticillium lecanii*	*Trialeurodes vaporariorum*	
30.79	HRI 30.79	*Verticillium lecanii*	*Cecidophyopsis* spp.	
31.79	HRI 31.79	*Verticillium lecanii*	*Cecidophyopsis* spp.	
450.99	IMI 235048	*Verticillium lecanii*	*Cecidophyopsis ribis*	
452.99	CBS 317.70A	*Verticillium lecanii*	*Tetranychus urticae*	
453.99	CCFC 006079	*Verticillium lecanii*	mites on cucumber	Canada

Table 2: Summary of techniques evaluated in bioassay development.

Bioassay ref number	Mite collection method	Surface mites sprayed on:	Immersed or sprayed	Spray volume Per replicate	Tween (%)	Drying time and illuminated period post spraying	Time to mortality assess-ment (days)
1	Pooter	Damp filter paper	Immersed	5ml	0.1	none	1
2					0.05		
3					0.01		
4			Spray		0.1		
5					0.05		
6					0.01		
7	Pooter	Damp filter paper	Spray	5ml	0.01	none	9
8	Pooter	Excised leaf (c.v. Spectra)	Spray	5ml	0.01	none	9
9	Pooter	Leaf disc* on damp filter paper	Spray	5ml	0.01	none	6-8
10		Pooter netting on damp filter paper					
11	Pooter	Leaf disc* floating on water	none	none	n/a	n/a	6
12	Pooter	Leaf disc* floating on water	Spray	5ml	0.01	Drying and light period of 30 minutes	3
13	Pooter	Leaf disc* floating on water	Spray	5ml	0.01	Drying and light period of 60 minutes	5
14						none	
15	Pooter and top up using a fine dissecting needle	Leaf disc with low trichome density*	Spray	2ml	0.01	15 minutes air drying	3
16						15 minutes air drying plus 60 minutes light and heat	
17		Leaf disc with high trichome density*[H]				15 minutes air drying plus 60 minutes light and heat	
18	Dissecting needle	Cover slip	Spray	1ml	0.01	10 minutes air drying	9
19				2ml			
20	Dissecting needle	Cover slip	Spray	2ml	0.01	10 minutes air drying	9

* Leaf disc with relative low trichome density selected from c.v. Spectra.
*[H] Leaf disc with relative high trichome density selected from c.v. Jocker.

with a dissecting needle) were observed daily for 9 d. Cadavers were removed onto damp filter paper within sealed (Parafilm) 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.

Screening fungal isolates against spider mites (HRI Stockbridge House)
Forty isolates of fungi, obtained in the isolate search (Table 1) were screened against *T. urticae* in a replicated, single dose bioassay using the methodology described above. Suspensions of fungal conidia were prepared at a concentration of 1×10^7 ml^{-1} as described previously. The experiment was done according to an alpha design (Patterson & Williams, 1976) (four blocks of 10 isolates constituting a replicate, three replicates in total). Each bioassay run included four controls, except for the first two occasions where two controls were included. Data were analysed using a generalised linear model, and mean fungus-induced mortalities at six days post inoculation (dpi) were predicted following weighted adjustments for block and control effects according to an analysis of variance (in essence, the model predicted the differential between control and fungus-treatments, adjusted for block effects).

Results and discussion

Screening fungal isolates against red spider mites
Mean control mortalities of blocks at 9 dpi ranged from 33.3 % to 14.4% (grand mean 21.1%, sd 4.17). The mortalities of mite populations in the control bioassays were comparable to the mortalities of spider mite populations on tomatoes in glasshouse experiments (P. Croft, unpublished). Predicted mean mortalities due to fungus treatment at 9 dpi (adjusted for controls and block effects) ranged from 31.3% (*B. bassiana* 434.99) to zero (*M. anisopliae* 445.99, *V.lecanii* 30.79) (Table 3). There was no immediate pattern in the ranking of virulences either by fungal species or host of origin. For example, the isolates ranked in the first ten, according to mean percentage mortality at 9 dpi, comprised *B. bassiana* (one isolate), *Hirsutella* sp. (three isolates), *M. anisopliae* (three isolates) and *V. lecanii* (three isolates). The hosts of these isolates included ixodid ticks (two isolates), prostigmatid phytophagous mites (three isolates), Mesostigmatid mites (two isolates), Hymenoptera (one isolate), soil (one isolate) and Homoptera (one isolate). The screening experiment examined six isolates of fungi used in commercial biopesticides (Mycotal, Vertalec, BotaniGard, PFR-97, Naturalis, and Bioblast). Only Mycotal (*V. lecanii*) ranked in the top ten. The rankings of these isolates were as follows: Mycotal (*V. lecanii*) = rank 10; BotaniGard (*B. bassiana*) = rank 16; Naturalis (*B. bassiana*) = rank 18; Vertalec (*V. lecanii*) = rank 36; PFR-97 (*P. fumosoroseus*) = rank 37; Bioblast (*M. anisopliae*) = rank 39. *Hirsutella thompsonii* 463.99, which we believe to be a subculture of the active ingredient of Mycar, passaged through *Varroa jacobsoni*, was ranked 9^{th} in the virulence listing.

The concentration of conidia used in the screening experiment (10^7 ml^{-1}) was based on information from the two previous reports on spider mite bioassays, and was intended to provide a reasonable separation of isolates to facilitate the selection of candidates for further testing in multiple dose experiments. The research has been successful on both counts. Gerson *et al*. (1979) obtained 100% mortality within 5 dpi for populations of *T. cinnabarinus* treated with *H. thompsonii*. The mites were inoculated by confining them for up to 60 h on a mat of sporulating mycelium, which is likely to result in a very high dose of fungal conidia.

Gardner *et al*. (1982) reportedly infected *T. urticae* with single conidia of *H. thompsonii* and observed 95% mortality at 7 dpi, which would appear to suggest that *T. urticae* is highly susceptible to infection by *H. thompsonii*. In the same study, leaf discs sprayed with a suspension of conidia of *H. thompsonii* at 10^4 ml^{-1} caused 40 – 65 % mortality at 7 – 8 dpi of *T. urticae* populations placed on the discs. This method is likely to cause greater mortality than the direct technique employed in our study, because it continually exposes mites to inoculum. A slightly greater level of infection than observed was expected from our study, based on the findings of Gerson *et al*. (1979) and Gardner *et al*. (1982). We suspect that the mites received a low dose of conidia during spraying in our bioassay, because of their small size. Doses will be estimated as part of the multiple dose bioassays to be done in Year 2. The data and the literature also indicate that the secondary acquisition of conidia could be an important route for infection at crop scale. There was a high degree of variation between replicates in the single dose bioassay, however this is not uncommon in experiments of this type and will be taken into consideration for the multiple dose experiments in Year 2. Gardner *et al*. (1982) recorded comparable levels of between-bioassay variation in their studies of *H. thompsonii* against *T. urticae*.

Six isolates have been selected for further study in multiple dose bioassays, based on the virulence ranking and information on their host origin (from which their potential impact on other natural enemies can be tentatively inferred). Four isolates, originating from acarine hosts, were chosen from the top ten ranked fungi. Two isolates from proprietary biopesticides were also chosen. These six isolates are:

1. *Metarhizium anisopliae* 442.99, ex *Boophilus* sp., Florida
2. *Hirsutella* sp. 457.99, ex *Dendrolaelaps* sp., Poland
3. *Verticillium lecanii* 450.99, ex *Cecidophyopsis ribis*, UK
4. *Hirsutella thompsonii* 463.99, ex *Phylocoptruta oleivora* (probably Mycar) via *Varroa jacobsoni*, USA
5. *Verticillium lecanii* 19.79, ex *Trialeurodes vaporariorum* (Mycotal).
6. *Beauveria bassiana* 432.99 (Naturalis).

The most virulent isolate observed in the screening experiment, *B. bassiana* 434.99, was not chosen for further bioassays as it originates from a hymenopteran host, *Bephratelloides cubensis*, raising queries about its compatibility with hymenopteran natural enemies in glasshouses. However, this isolate could be examined as part of the study of the compatibility of fungi with other natural enemies, scheduled for Year 2 of the project, and reincorporated into spider mite assays if it proves to have minimal negative impact on beneficials.

Table 3: Predicted weighted mean % fungus-induced mortalities of red spider mites treated with entomopathogenic fungi (9 dpi)

rank	isolate	species	Host of origin	Area	mortality (%)	SE
1	434.99	*Beauveria bassiana*	*Bephratelloides cubensis*	Florida	31.3	15.94
2	457.99	*Hirsutella* sp.	*Dendrolaelaps* sp.	Poland	30.7	10.27
3	442.99	*Metarhizium anisopliae*	*Boophilus* sp.	Florida	26.9	13.87
4	450.99	*Verticillium lecanii*	*Cecidophyopsis ribis*	UK	25.8	7.19
5	440.99	*Hirsutella* sp.	*Stenotarsonemus fragariae*	Poland	22.2	4.43
6	453.99	*Verticillium lecanii*	mites on cucumber	Canada	21.8	14.36
7	392.93	*Metarhizium anisopliae*	soil	UK	20.9	11.75
8	441.99	*Metarhizium anisopliae*	*Boophilus* sp.	Mexico	20.7	15.41
9	463.99	*Hirsutella thompsonii*	*Varroa jacobsonii*	Canada	18.7	8.84
10	19.79	*Verticillium lecanii* (Mycotal)	*Trialeurodes vaporariorum*		17.5	2.66
11	73.82	*Hirsutella thompsonii*	*Phyllocoptruta captrila*	Florida	17.0	8.53
12	436.99	*Hirsutella* sp.	Acari: Tarsonemidae	Poland	16.7	8.69
13	437.99	*Hirsutella* sp.	*Eriophyes piri*	Poland	16.3	8.57
14	431.99	*Beauveria bassiana*	unknown mite sp	Denmark	15.8	9.46
15	49.81	*Hirsutella necatrix*	*Abacarus hystrix*	UK	15.4	13.38
16	433.99	*Beauveria bassiana* (BotaniGard)			14.5	7.73
17	17.76	*Verticillium lecanii*	*Cecidophyopsis* sp.		14.4	6.02
18	432.99	*Beauveria bassiana* (Naturalis)			14.2	11.32
19	446.99	*Paecilomyces farinosus*	*Mycobates* sp.	Canada	13.6	3.62
20	75.82	*Hirsutella thompsonii*	*Colomerus novahebridensis*	New Guinea	11.7	6.01
21	31.79	*Verticillium lecanii*	*Cecidophyopsis* sp.		11.3	5.66
22	444.99	*Metarhizium anisopliae*	*Hylobius pales*		9.9	7.09
23	449.99	*Tolypocladium niveum*	*Mycobates* sp.	Canada	9.6	8.16
24	455.99	*Beauveria bassiana*			9.3	6.05
25	51.81	*Hirsutella thompsonii*			8.8	1.79
26	452.99	*Verticillium lecanii*	*Tetranychus urticae*		8.8	8.76
27	438.99	*Hirsutella* sp.	*Abacarus hystrix*	Poland	8.2	4.72
28	454.99	*Beauveria bassiana*	Unknown mite sp.		7.9	7.58
29	275.86	*Metarhizium anisopliae*	*Cydia pomonella*	Germany	6.9	6.89
30	71.82	*Hirsutella thompsonii*	*Eriophyes guerreronis*	Jamaica	6.7	5.37
31	447.99	*Paecilo.fumosoroseus*	*Ixodes ricinus*	Denmark	6.6	2.44
32	77.82	*Hirsutella thompsonii*	*Eriophyes guerreronis*	Jamaica	3.6	3.63
33	456.99	*Metarhizium anisopliae*			2.3	1.44
34	448.99	*Tolypocladium inflatum*	*Mycobates* sp.	Canada	2.2	2.16
35	34.79	*Hirsutella thompsonii*	*Eriophyes guerreronis*	Ivory Coast	1.7	0.86
36	1.72	*Verticillium lecanii* (Vertalec)	*Macrosiphoniella sanborni*		1.5	0.80
37	409.96	*Paecilo. fumosoroseus* (PFR-97)			0.8	0.82
38	460.99	*Beauveria bassiana*	Unknown mite sp.	Israel	0.3	0.25
39	445.99	*Metarhizium anisopliae* (Bioblast)			0	0
40	30.79	*Verticillium lecanii*	*Cecidophyopsis* sp.		0	0
control					0	

Figures refer to the predicted mean percentage mortality due to fungus-treatment. Predictions were formed by ignoring the presence of aliased parameters, and were standardised by averaging over the levels of control and block effects as factors (factors were assigned constant weights over levels of other factors). The standard errors are appropriate for interpretation of the predictions as summaries of the data rather than as forecasts of new observations.

CONCLUSIONS

The laboratory bioassay developed in this project allows spider mites to be treated with a single, controlled application of conidia of entomopathogenic fungi and maintained on tomato leaves under conditions of controlled temperature and humidity. The bioassay allows a high throughput of candidate fungal isolates, and we were able to examine more isolates than originally envisaged. *Tetranychus urticae* are susceptible to a range of species of entomopathogenic fungi using this bioassay, although for the fungi examined there was no simple relationship between the species or provenance of an isolate and its virulence to the mites. A number of fungal isolates are more pathogenic to *T. urticae* than isolates used as proprietary biopesticides. Six isolates were chosen for more detailed examination in multiple dose bioassays for Year 2. These included four isolates from acarine hosts which were ranked in the top ten isolates based on virulence to *T. urticae*, plus the two best commercial isolates.

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