FINAL REPORT

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The results and conclusions in this report are based on a series of experiments that were conducted in HDC funded and other projects. The conditions under which the experiments were carried out and the results have been reported with detail and accuracy in the original reports. 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.

Authentication

I declare that this work was done under my supervision according to the procedures described herein and that this report represents a true and accurate record of the results obtained.

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PRACTICAL SECTION FOR GROWERS

COMMERCIAL BENEFITS OF THE PROJECT

This information will assist the HDC Protected Crops Panel in developing a co-ordinated R&D programme for integrated pest management in protected crops and in particular the evaluation of the entomopathogenic fungus, *Beauveria bassiana*.

INTRODUCTION

Beauveria bassiana has been evaluated for the control of a number of important pests in HDC funded projects. These projects have also included studies to evaluate the compatibility of the pathogen with some invertebrate biological control agents and with fungicides commonly used against diseases in protected salad crops. A wealth of such information has now been reported in HDC Reports, abstracts from horticultural conferences and articles in the trade press. A review of work conducted on *Beauveria bassiana* was commissioned by the HDC Protected Crops Panel in order to summarise the results to date and to help prioritise items for R&D funding in the future.

Beauveria bassiana is a naturally occurring fungal pathogen of insects and mites that is common in soils throughout the world and indigenous to the UK. As a species, it has a very broad host range but there are many strains with differing host specificities. When a *B. bassiana* spore comes into contact with a suitable host, a germ tube is produced that penetrates the insect's cuticle allowing the fungus to feed and grow within the insect and eventually kill it. Under suitable conditions, germination usually occurs in 1-2 days, the host is killed in 3-5 days and then the fungus sporulates on the surface of the dead body. The spores are released to infect other hosts. Two companies have commercialised the pathogen in the USA and both are currently pursuing product registrations in the UK. These products are Naturalis L (JW-1 strain) and Botanigard WP (GHA strain). In all products, spores of the fungus are formulated to mix readily in water and may be applied through standard spray equipment.

The authors of this report advocate the use of microbial insecticides, such as *B. bassiana*, as a second line of defence to support preventative pest control measures based on invertebrate natural enemies. This concept has been most fully developed for the control of *F. occidentalis* on cucumber crops but is considered for other crop/pest combinations.

There are several factors that could limit the success of *B. bassiana*:

- The strains of *B. bassiana* included in the commercial products were originally selected for activity against whiteflies and are not necessarily the most effective strains against the other major horticultural pests.
- Immature insects moult at regular intervals to allow growth and may escape infection by shedding ungerminated fungal spores on the old skin. In such circumstances, repeated applications of the microbial insecticide may improve results.
- Entomopathogenic fungi are generally most successful under conditions of moderate temperature and high relative humidity and this has led some research workers to attempt to improve their efficacy by artificially elevating humidity in

the glasshouse. However, the authors suggest that the microclimate on the surface of the insect is probably close to ideal for spore germination and therefore advocate good spray coverage to ensure that the pathogen comes into contact with as many insects as possible.

OBJECTIVES

1. To summarise relevant data produced in HDC funded projects PC 123, PC 129, PC 132, PC 136, PC 139, PC 161 and PC 163 and present in the following categories:
   - Efficacy of *B. bassiana* against a range of horticultural pests.
   - Relative efficacy of methods of applying *B. bassiana*.
   - Compatibility of *B. bassiana* with some of the most important invertebrate biological control agents.
   - Compatibility of *B. bassiana* with fungicides commonly used against diseases in protected crops.
2. To determine by bioassay, the potential of *B.bassiana* against larvae of sciarid flies and shore flies.
3. To identify gaps in the knowledge and recommend subjects for further research.

SUMMARY OF RESULTS AND CONCLUSIONS

Efficacy studies

Western flower thrips (*Frankliniella occidentallis*) [HDC project PC 129] – A second line of defence has been sought to support the primary biological control agent, *Amblyseius cucumeris*, on cucumber crops. In one experiment, two HV sprays of *B. bassiana*, applied with a six day interval, reduced numbers of immature WFT by 75% compared to untreated controls. There was no indication of any harmful effects to *A. cucumeris* that were exposed to the sprays on leaves or in culture packs. In a second experiment, a sequence of three HV sprays, each containing greater spore concentrations than previously tested, reduced numbers of immature WFT by 83% of untreated controls. In both experiments, great care was taken to ensure that all parts of the plants (particularly undersides of all leaves) were coated with spray deposits. However, spray techniques currently used in commercial cucumber crops are unlikely to provide such complete cover and growers must improve their spray practice before they can expect to match the control achieved in these experiments. [Please see report for project PC 136 (2000) for further information on HV spray application in cucumber crops].

Capsid bugs (*Lygus rugulipennis* and *Liocoris tripustulatus*) [HDC project PC 123] - Bioassays demonstrated that *B. bassiana* could infect both species of capsid bugs but subsequent studies in experimental glasshouses were limited to *L. rugulipennis* on cucumbers. A single HV application of *B. bassiana* spores reduced numbers of adult capsids by 60% compared to untreated controls. A sequence of three HV sprays, each of which contained larger numbers of spores than used previously, reduced numbers of capsids by 78% of untreated controls.

Lettuce aphids [HDC project PC 132] - A series of three experiments evaluated the relative efficacy *Verticillium lecanii* and *B. bassiana* against four species of aphids (*Nasonovia ribisnigri*, *Myzus persicae*, *Aulocorthum solani* and *Macrosiphum euphorbiae*) on young lettuce plants. The results were disappointing with neither product providing satisfactory control of any of the aphid species at up to ten times the recommended application rates. The poor control has been at least partly attributed to the growth habit of the lettuce plants, which makes it very difficult to obtain good spray cover on the undersides of the lower leaves. These studies have been terminated.

***Macrolophus caliginosus* [HDC project PC 139] -** An IPM compatible means of managing populations of this predatory bug is required for tomato crops. In laboratory bioassays, *V. lecanii* and *B. bassiana* reduced numbers of *M. caliginosus* by 54% and 26% respectively compared to a background mortality of 14% in the untreated controls. *Beauveria bassiana* will not be further evaluated for this purpose.

Mealy bug (*Pseudococcus viburni*) [HDC project PC 161] - *Beauveria bassiana* was compared to *V. lecanii* against *P. viburni* in laboratory bioassays. Following correction of results to allow for natural mortality, the treatments were found to reduce numbers of mealy bugs by 10% and 6% respectively. The poor results were attributed to the protection provided by the natural waxy layer covering the insects.

Shore flies (*Scatella stagnalis*) - Natural infections of *B. bassiana* have been found on *S. stagnalis* pupae and adults in UK glasshouses, and these observations have been supported by small-scale infectivity studies in the laboratory. In bioassays where *B. bassiana* was applied to growing medium containing second/third instar *S. stagnalis* larvae, 80% of the insects failed to reach maturity. In another bioassay, the pathogen was applied directly to the larvae before they were transferred to rockwool-based algae cultures and the cultures were then kept at 22^{o}C until the adults emerged. In this case, the product had little effect on the larval development. The difference in these effects may be explained by considering the insect's developmental rate and the methods of applying *B. bassiana*. At 20-25^{o}C, the larvae develop rapidly and moult at intervals that are probably shorter than the germination time of the spores. Hence, spores applied directly to the larvae are shed with the insect's cast skin and they rarely become infected. However, when the product is applied to the growing medium, the infection pressure is greater because the insects are constantly exposed to spores. In these circumstances, the majority of insects probably die at the pupal stage, which is of longer duration.

Sciarid flies (*Bradysia* spp.) - An oil-based formulation of *B. bassiana* has been tested against adult sciarid flies in the laboratory but the results were inconclusive because the oil appeared to have a direct effect on the insects. Although the pathogen was identified on dead flies, it was impossible to say whether it had caused death or was simply growing saprophytically on the corpse. Two bioassays within this project evaluated *B. bassiana* against sciarid fly larvae. In the first bioassay, the pathogen was applied to the culture mix before second/third instar sciarid fly larvae were released. The insects were allowed to complete their development and the numbers of adults recorded. There was no significant difference between the numbers of adults emerging in the *B. bassiana* treatments and the untreated controls. The second bioassay was similar except *B. bassiana* was applied directly to the sciarid fly larvae before they were released onto the culture media. Once again, there was no significant difference between the treatments. The larvae may escape infection due to the short development time of the individual instars (as suggested above for shore flies). Further evaluation of *B. bassiana* for the control of sciarid flies is not recommended because effective biological control products (predatory mites and parasitic nematodes) are already commercially available.

Two-spotted spider mite (*Tetranychus urticae*) [HDC project PC 163] - Several strains of *B. bassiana* were included in a screen of 40 isolates of nine species of entomopathogenic fungi. The fungi exhibited a range of pathogenicities to the mites and were ranked in order of virulence. There was no pattern in the ranking that could be attributed to either fungal species or host of origin. The most effective pathogen was a strain of *B. bassiana* that originated from a wasp (*Bephratelloides cubensis*) in Florida, USA. The two strains of *B. bassiana* used in BotaniGard WP and Naturalis-L and were ranked 16 and 18 respectively. Six isolates have been selected for further evaluation.

Methods of applying *B. bassiana*

Beauveria bassiana has been shown to have the potential to control WFT and capsid bugs when applied as either a HV spray or a LV mist in cucumber crops. The latter is relatively inexpensive because it requires minimal labour and is an attractive option because it allows the cost-effective application of a series of treatments. However, further studies are required to determine the optimum frequency and rate of applications against both pests on a larger scale in commercial crops.

Compatibility with biological control agents.

Beauveria bassiana is active against a wide range of invertebrates and it is important to know the impact that it will have on the biological control agents used to control other pests in IPM programmes. So far, results indicate that it is compatible with the thrips predator, *Amblyseius cucumeris*, and the aphid parasitoid, *Aphidius colemani*. Although it was demonstrated that it could infect the spider mite predator, *Phytoseiulus persimilis*, this did not have a significant effect on the populations tested. *Beauveria bassiana* was harmful to the whitefly parasitoid, *Encarsia formosa*, reducing suvival of immature parasitoids by up 62%. However, the pathogen also infects whiteflies directly and can provide effective control of this pest, so the overall effect on this component of the IPM programme may not be detrimental.

Compatibility with fungicides

To integrate *B. bassiana* successfully into IPM programmes, it is essential to determine the compatibility of the pathogen with fungicides commonly used against the diseases of protected crops. A bioassay was developed and used to determine the effect on the germination of *B. bassiana* spores of seven fungicides commonly used on cucumbers and tomatoes. Rovral and Thiovit were relatively safe at their recommended application rates. Scala was safe and Nimrod was relatively safe at one tenth of their recommended rates. Fungaflor, Repulse and Amistar prevented germination at one tenth of their recommended rates. A further bioassay is required to determine the persistence of the effects of the five fungicides that were shown to be harmful to *B. bassiana* at their recommended application rates (to be conducted in project PC 123).

NOTE ON PRODUCT APPROVALS

Naturalis-L, which contains the JW-1 strain of *Beauveria bassiana* in undergoing registration in the UK and it is hoped that approval will be granted by PSD in Autumn 2001. The UK agents and potential distributers are Hortichem Ltd. The second *Beauveria bassiana* product (contains the GHA strain) likely to be registered in the UK is BotaniGard WP and the UK agents and potential distributers are Fargro Ltd. Growers are advised to contact the UK agents for the most up to date information on product approvals.

REQUIREMENTS FOR FUTURE RESEARCH & DEVELOPMENT

Completion of existing studies

- Further studies to improve HV spray techniques for cucumber crops are required to ensure that entomopathogen spores come into contact with the target pest.
- Large-scale evaluation of *B. bassiana*-based products against WFT in commercial cucumber crops will be required when issues relating to HV spray are resolved.
- Space treatments (LV and ULV mists) should be evaluated against capsid bugs in commercial cucumber crops when a *B. bassiana*-based product is registered in the UK and can be used without the destruction of the treated crop.

Opportunities against other crop/pest combinations

- The technology that has been developed for the control of WFT on cucumber crops should be adapted to form the basis of an IPM compatible control strategy for the control of WFT on ornamental crops.
- An IPM compatible package, including *B. bassiana*-based products, should be developed for the control of shore flies in a range of crops.
- Other isolates/strains of *B. bassiana* could be screened for activity against important horticultural pests that have been inadequately controlled by the existing *B. bassiana*-based products.

Enhancing the effect of *B. bassiana*-based products

- The ability of oil adjuvants to enhance the efficacy of *B. bassiana*-based products should be determined, particularly against insects such as mealy bugs that are protected by waxy deposits.
- Some semiochemicals, such as WFT alarm pheromones, increase the activity of insects and have the potential to enhance the effect of entomopathogenic fungi by improving "secondary pick-up" of spores deposited on the leaf surface. This should be further investigated.

Compatibility with other crop protection products

- Further studies are required to provide a complete data package for the compatibility of *B. bassiana* with all life cycle stages of all biological control agents used in IPM programmes in UK protected crops.
- Further bioassays are required to determine the immediate and longer-term effects on *B. bassiana* of the fungicides commonly used in protected crops.

More general subjects

A multi-disciplinary study is required to improve the measurement and definition of microclimates of boundary layers on plant and insect surfaces.

SCIENCE SECTION

1. INTRODUCTION

Beauveria bassiana [Balsamo] Vuillemin has been evaluated for the control of a number of important pests in HDC funded projects. These projects have also included studies to evaluate the compatibility of the pathogen with some important invertebrate biological control agents and with fungicides commonly used against diseases in protected salad crops. A wealth of such information has now been reported in HDC Annual and Final Reports, in abstracts from horticultural conferences and articles in the trade press. A review of work conducted on *Beauveria bassiana* was commissioned by the HDC Protected Crops Panel in order to assist in the development of a co-ordinated R&D programme for integrated pest management in protected crops.

2. BACKGROUND INFORMATION

Beauveria bassiana

Beauveria bassiana is a naturally occurring fungal pathogen of insects and mites that is common in soils throughout the world and indigenous to the UK. It has been known to infect insects since 1835 when it was diagnosed to be the cause of muscardine disease in silkworms. As a species, *B. bassiana* has a very broad host range and is reported to attack representatives of all life cycle stages of all groups of insects and many groups of mites. However, there are many strains of the fungus, with differing host specificity, so it is impossible to generalise on the full range of invertebrate hosts.

When a *B. bassiana* spore comes into contact with a suitable host, a germ tube is produced that penetrates the insect's cuticle allowing the fungus to feed and grow within the insect and eventually kill it. Under suitable conditions, germination usually occurs in 1-2 days, the host is killed in 3-5 days and then the fungus sporulates on the surface of the dead body. The spores are released to infect other hosts.

Commercial exploitation of *Beauveria bassiana*

Two companies have commercialised the pathogen in the USA and both are currently pursuing product registrations in the UK. In all products, spores of the fungus are formulated to mix readily in water and may be applied through standard spray equipment.

Troy Biosciences Inc. (Phoenix, USA) market three products containing the JW-1 strain of *B. bassiana*. This strain has been reported to infect many invertebrate pests including glasshouse whitefly (*Trialeurodes vaporariorum*), tobacco whitefly (*Bemisia tabaci*), silverleaf whitefly (*Bemisia argentifolii*), western flower thrips (*Frankliniella occidentalis*), onion thrips (*Thrips tabaci*), peach-potato aphid (*Myzus persicae*), melon cotton aphid (*Aphis gossypii*) and two-spotted spider mite (*Tetranychus urticae*). The three products, Naturalis-L, Naturalis-O and Naturalis-T, are similar oil-based fomulations, sold for use on edible crops, ornamental crops and turf respectively. Naturalis-L will be registered in the UK and it is this product that has been used in the experiments described in this report. The UK agents and potential distributors are Hortichem Ltd (Amesbury, UK).

Mycotech Corporation (Butte, USA) market two *B. bassiana*-based products containing the GHA strain of the fungus, which has been reported to control several groups of pests including whiteflies, thrips, aphids and grasshoppers. The products, known as BotaniGard WP and BotaniGard ES, are formulated as a wettable powder and oil emulsion respectively. Although we have evaluated both products, this report concentrates on BotaniGard WP because it is most likely to be registered in the UK. The UK representatives are Fargro Ltd (Littlehampton, UK).

Other *B. bassiana*-based products are produced in Eastern Europe and marketed under the trade name Boverin (*eg* Boverin K-BL and Boverin ZH). However, these products are difficult to obtain and were not included in our studies because there has been no attempt to register them in the UK.

Integration of *Beauveria bassiana* in IPM programmes

The development of sustainable systems of pest control, that do not include the application of chemical toxins, will probably require complexes of natural enemies that may be integrated to control single pest species. Many of the studies of biological control by guilds of predators, parasitoids and pathogens have concerned natural assemblages in outdoor agroecosystems and their interactions with pest populations over many seasons. This scenario has been the driver for much of biological control theory. However, it is in sharp contrast to protected crops, where biological control has traditionally been based on multiple introductions of natural enemies over the life of the crop, usually with a single natural enemy species for each pest. Although biological control *per se* is well established in protected crops, the potential benefits of using suites of natural enemies has not yet been explored widely. The authors of this report suggest the use of microbial insecticides, such as *B. bassiana*, as a second line of defence to support preventative pest control measures based on invertebrate natural enemies. This concept has been most fully developed for the control of *F. occidentalis* on cucumber crops (Jacobson *et al.*, 2000) but is advocated for other crop/pest combinations.

If a microbial insecticide is to be used successfully as a second line of defence, then it must be compatible with the primary biological control agents of that pest and all the control agents used against other pests of the same crop. This is of particular concern with *B. bassiana* because it is reported to have such a broad spectrum of activity against invertebrate species.

Factors limiting the success of *Beauveria bassiana*

The strains of *B. bassiana* included in the Naturalis and BotaniGard products were originally selected for activity against whiteflies and are not necessarily the most effective strains against the other major horticultural pests. This is illustrated by the results reported against spider mites.

Although pests may pick up *B. bassiana* spores from the plant surface, the best results are probably achieved when the spray directly hits them. Good spray cover of the parts of the plant inhabited by the pests is therefore essential.

Immature insects moult at regular intervals to allow growth and may escape infection by shedding ungerminated fungal spores on the old skin. In such circumstances, rates of infection may be improved by repeated applications of the microbial insecticide. However, the insect will have an effective defence mechanism if the larvae moult at intervals that are shorter than the germination time of the spores.

Entomopathogenic fungi are generally most successful under conditions of moderate temperature and high relative humidity, and this has led some research workers to attempt to improve their efficacy by artificially elevating humidity in the glasshouse. However, this may not be ideal for crop growth and has sometimes caused secondary problems by encouraging the growth of plant pathogenic fungi. As the microclimate on the surface of the insect is probably close to ideal for spore germination, it is probably more appropriate to concentrate on good spray coverage and ensure that the pathogen comes into contact with as many insects as possible.

Compilation of data

The data produced in HDC funded projects PC 123, PC 129, PC 132, PC 136, PC 139, PC 161, PC 163 and PC 163 has been collated in the following five categories:

- Comparison of *B. bassiana* formulations.
- Efficacy of *B. bassiana* against a range of horticultural pests.
- Relative efficacy of methods of applying *B. bassiana*.
- Compatibility of *B. bassiana* with some of the most important invertebrate biological control agents.
- Compatibility of *B. bassiana* with fungicides commonly used against diseases in protected crops.

3. COMPARISON OF *BEAUVERIA BASSIANA* FORMULATIONS

Numbers of colony forming units

Various combinations of three formulations of *B. bassiana* (Naturalis-L, BotaniGard WP and BotaniGard ES) have been used in over 40 experiments in eight HDC funded projects. Samples have been taken from the spray mixtures used in every treatment to determine the viability of the product.

Preliminary studies developed a bioassay procedure to determine the number of viable spores in suspensions of *B. bassiana*. Initially, the total number of spores were counted using a haemocytometer before culturing on growth media to determine the proportion that were viable. However, this proved to be unreliable with oil-based formulations because it was too difficult to count spores among the oil droplets. The final procedure focused more simply on the number of colony forming units (CFUs) that were present in the formulation. This technique is not absolutely accurate but it provides a good indication of the relative numbers of CFUs in each product. All samples were tested on the day spray mixtures were prepared because storage could reduce viability.

The numbers of spores per ml stated by the producers to be in the undiluted products were approximately:

- Naturalis–L - 2.3×10^7
- BotaniGard WP - 4.4×10^{10}
- BotaniGard ES - 2.1×10^{10}

Overall, 1.66×10^6 CFUs per ml were detected in spray mixtures of Naturalis at the application rate recommended for *F. occidentalis*. However, there was a large variation between the batches (range 0.18×10^6 to 4.6×10^6 per ml). The application rate of BotaniGard WP recommended for *F. occidentalis* and the rate of BotaniGard ES recommended for *T. vaporariorum* yielded 13×10^6 and 2.8×10^6 CFUs per ml respectively. (Jacobson, 2000a)

Direct effect of the oil-based formulation

There was some evidence that the oil in the Naturalis-L formulation had a direct effect on treated insects. This was most noticeable among frail insects, such as adult sciarid flies, which spent a long time grooming after treatment and seemed unable to spread their wings fully. This should be investigated further.

Phytotoxicity

The BotaniGard ES formulation was found to be slightly phytotoxic to tomato leaves. Oedema-like blisters were observed on or close to the veins on the underside of the leaves following three applications of the product at seven day intervals. In other respects, quality and yield of tomatoes were consistent with normal commercial expectations.

4. EFFICACY STUDIES

Experimental approach

A standard procedure, consisting of three distinct phases, has been adopted for all the efficacy studies:

- The **first phase** consists of a laboratory assessment to determine whether the pathogen will infect the insect. Such tests may be completed at any time of the year under conditions that are close to ideal for infection. These studies are relatively quick and inexpensive. If successful, the work progresses to the second phase.
- The **second phase** is done on whole plants but in relatively small experimental glasshouses where the conditions may be strictly regulated and treatments compared to untreated controls. Ideally, each treatment is housed in a separate but identical glasshouse compartment. The crops are artificially infested with the pest species and great care is taken to ensure that the insect populations are even throughout all the glasshouses. It is usually necessary to verify successful results by repeating these experiments at least once before the work progresses to the third phase.
- The **third phase** is done on whole crops in commercial greenhouses and is necessary to validate previous results with scaled-up application methods. These trials are dependent on natural pest infestations, which tend to be unevenly distributed. The results can rarely be compared to untreated controls and have less chance of being statistically valid than the phase two experiments.

This procedure has been used to evaluate *B. bassiana*-based products against the following pest species:

Western flower thrips (*Frankliniella occidentallis*)

Frankliniella occidentalis remains one of the most damaging pests of protected cucumbers in the UK. Crop scale experiments between 1993 and 1995 showed that *F. occidentalis* establishment may be prevented in cucumbers by placing one culture pack containing the predatory mites, *Amblyseius cucumeris*, on each plant immediately after planting (Jacobson *et al*. 2000b). However, the pest will probably become established if the placement of sachets is delayed or crop coverage is incomplete. Even where initial establishment of the pest is suppressed, control may break down during cropping. The most common reasons for this are:

- sudden pest invasion from other crops
- adverse environmental conditions
- harmful effects of chemicals applied against other pests/diseases.

To complete the IPM package against *F. occidentalis* it is vital that effective and compatible remedial control measures are developed to support the preventative action. As there are no selective insecticides that can be integrated with the other control agents in the cucumber IPM programme, remedial action against *F. occidentalis* must be based on biological techniques.

Trials in the USA had demonstrated that the JW-1 strain of *B. bassiana* (Naturalis) was as effective as standard chemical insecticides against a number of glasshouse pests, including thrips (Wright and Kennedy, 1996). Furthermore, results in field crops in climates warmer than the UK suggested that it would be effective at lower relative humidity than *Verticillium lecanii*. Laboratory bioassays and other small-scale studies demonstrated that *B. bassiana* would infect *F. occidentalis* (Jacobson and Fenlon, 1998) and the work progressed to phase two in experimental glasshouses.

In the first such experiment, two high volume sprays of Naturalis-L, applied with a six day interval, reduced numbers of adult and immature *F. occidentalis* by 66% and 75% respectively during the subsequent 14 days. *Amblyseius cucumeris* on leaves were unaffected by the sprays (Jacobson and Chandler, 1999). There was no indication that the applications of *B. bassiana* were harmful to the predators or their prey in opened culture packs that were on the plants when the sprays were applied. The results of these *A. cucumeris* and *B. bassiana* compatibility studies were consistent with results obtained from bioassays in another HDC funded project (see section 6). These results clearly showed that *B. bassiana* had the potential to be used in an IPM programme as a second line of defence to support the primary biological control agent, *A. cucumeris*. However, the humidity was relatively high (mean 87%) in the glasshouses during the experiment, which favoured the development of entomopathogenic fungi, and the experiment was repeated under more challenging conditions of lower relative humidity.

BotaniGard WP, which contains larger numbers of spores per unit volume of product than Naturalis-L, was incorpoated in the second study in experimental glasshouses. Three high volume sprays of BotaniGard WP applied at six day intervals reduced numbers of immature *F. occidentalis* by 83% compared to untreated controls (Jacobson, 2000a). The same number of Naturalis HV sprays reduced numbers of immature *F. occidentalis* by 70%. The treatments were less effective against adult *F. occidentalis* but still reduced numbers by 34% compared to untreated controls. These findings were broadly consistent with the results of the first experiment despite the lower RH (mean 78%) and suggest that infection of *F. occidentalis* by *B. bassiana* is not dependant on high humidity in the aerial environment.

In the experiments reported above, great care was taken to ensure that all parts of the plants (particularly undersides of all leaves) were coated with spray deposits and this was considered to have been of paramount importance. However, spray techniques commonly used in commercial cucumber crops are unlikely to provide such complete cover (Lee and Miller, 2000) and growers must improve their spray practice before they can match the control achieved in these experiments. Evaluation in commercial crops must await approval of the products in the UK.

Capsid bugs (*Lygus rugulipennis* and *Liocoris tripustulatus*)

Two species of capsid bugs cause damage to protected crops in the UK: *Lygus rugulipennis* in cucumber, strawberry and ornamental crops, and *Liocoris tripustulatus* in pepper and aubergine crops. Adults over-winter outdoors and may invade glasshouses as early as April but the main migration occurs during the summer when the second generation appears in the natural habitats. Capsids feed and lay eggs in growing points and young fruit resulting in distorted growth and unmarketable produce. Financial losses are greatest in cucumbers when summer invasions coincide with new plantings. There are currently no IPM compatible control measures available.

Laboratory-based bioassays completed in 1998, demonstrated that *B. bassiana* (Naturalis-L) could infect both species of capsid bugs and the work progressed to phase two in experimental glasshouses (Jacobson, 1998a). Due to limited resouces phase two studies focussed on *L. rugulipennis* on cucumbers.

In the first phase two experiment, a single high volume application of Naturalis-L reduced numbers of adult *L. rugulipennis* by 60% compared to untreated controls (Jacobson, 1999a). This level of control was broadly consistent with that recorded when *B. bassiana* was applied against *Lygus lineolaris* on cotton in the USA and against *Lygus hesperus* in laboratory studies at the University of Idaho (Brown, pers. com.). However, the relative humidity in the glasshouse was relatively high (approximate mean 90%) during this experiment, which favoured the development of the fungal entomopathogen, and the experiment was repeated under more challenging conditions of lower relative humidity.

In the second experiment, a sequence of three high volume sprays of Naturalis-L and BotaniGard WP were applied at daily intervals. The results were similar for both products, reducing numbers of *L. rugulipennis* by approximately 78% compared to the untreated controls (Jacobson, 2000b). The additional control compared to the first experiment was attributed to the three-spray programme. The mean relative humidity during this experimental period was approximately 70% in the crop canopy, which suggests that infection of *L. rugulipennis* by *B. bassiana* is not dependent on high RH in the aerial environment.

Although high volume sprays of *B. bassiana* clearly have potential for the control of *L. rugulipennis* on cucumbers, applications of low volume mists were considered to be an even more attractive option (see section 5) and the latter will be taken forward for evaluation in phase three. However, this must wait until the products become commercially available in the UK.

Lettuce aphids

Protected lettuce is host to at least four different aphid species. Of those that colonise the foliage, the currant lettuce aphid (*Nasonovia ribisnigri*) is specific to lettuce while the peach potato aphid (*Myzus persicae*), the glasshouse potato aphid (*Aulocorthum solani*) and the potato aphid (*Macrosiphum euphorbiae*) occur on a range of different plant species. Growers currently rely on routine and often intensive applications of aphicides to prevent colonisation by these pests. However, food retailers are urging growers to reduce their usage of chemical insecticides and alternative methods of control are urgently sought.

Laboratory bioassays evaluated Naturalis-L and two strains of *Verticillium lecanii* (Vertalec and Mycotal) against *N. ribisnigri* and *M. persicae*. Both strains of *V. lecanii* showed considerable potential and were taken forward to phase two in experimental glasshouses. Although Naturalis-L was less effective in the bioassays, it was also included in the second phase because of its potential to infect insects at lower RH (Tatchell, 1998).

The first phase two experiment evaluated the relative efficacy of Vertalec and Naturalis-L against *N. ribisnigri*, *M. persicae*, *A. solani* and *M. euphorbiae* on young lettuce plants in propagation. The results were disappointing with neither product providing satisfactory control of any of the aphid species (Tatchell, 1999). A second phase two experiment evaluated the efficacy of higher rates of the same products against *N. ribisnigri* on young lettuce plants. Neither product controlled the aphids at up to ten times the recommended application rates under these experimental conditions (Jacobson and Croft, 2000). A third experiment, in which each product was applied three times at three-day intervals, also failed to give satisfactory control (Russell, as yet unpublished data, 2000). The poor control has been at least partly attributed to the growth habit of the lettuce plants, which makes it very difficult to obtain good spray cover on the undersides of the lower leaves.

The work will not progress to phase three experiments in commercial glasshouses.

Macrolophus caliginosus

The predatory mirid bug, *Macrolophus caliginosus*, was originally reared for control of whiteflies but has been found to attack a wide range of pest species. It has been released in UK tomatoes under DoE license since 1995 and has provided a useful contribution to the overall IPM programme. However, the predator has also been found to cause direct damage to tomato plants (Jacobson and Sampson, 1998) and an IPM compatible means of managing the populations in tomato crops has been sought.

In a laboratory bioassay, *Beauvaria bassiana* (Naturalis-L) was compared to *Verticillium lecanii* (Mycotal and Vertalec) against *M. caliginosus* adults and nymphs (Sampson and Jacobson, 1999). Mycotal was the most effective treatment, reducing numbers of *M. caliginosus* by 54%. *Beauvaria bassiana* only reduced numbers of *M. caliginosus* by 26%, which was not much greater than the background mortality of 14% in the untreated controls. Naturalis-L did not progress to a phase two study in experimental glasshouses.

Mealy bug (*Pseudococcus viburni*)

Mealy bugs have been sporadic pests on UK protected tomato crops for many years but there is now evidence that infestations of one species, the obscure mealy bug (*Pseudococcus viburni*), are becoming more common. The pest may be controlled with chemical insecticides but these disrupt biological control of other pests and an IPM compatible control measure is required.

Two *B. bassiana* products (Naturalis-L and BotaniGard WP) were compared to *V. lecanii* (Mycotal) against *P. viburni* in laboratory bioassays. Mortality of mealy bugs in the *B. bassiana* treatments was similar to the untreated controls but there was an additional 8% mortality in the *V. lecanii* treatment (Russell and Sampson, as yet unpublished data, 2000). These poor results were attributed to the natural protective wax produced by the insects. The efficacy of the products may be improved by incorporating oil adjuvants in the spray mixture and this should be tested in further bioassays before deciding whether to progress to phase two studies in experimental glasshouses.

Shore flies (*Scatella stagnalis*)

Scatella stagnalis feed primarily on green algae on the surface of growing media and thrive in many glasshouse crops, eg lettuce, celery, pot plants, bedding plants and herbs. There is no evidence to suggest that any of the life cycle stages directly damage plants but the presence of adult flies and/or their faecal spots on foliage can lead to the rejection of whole batches of produce. Furthermore, the insects have been implicated in the spread of root diseases such as *Pythium spp*. (Jacobson, 1995). An HDC funded project completed in 1997, investigated methods of indirectly reducing *S. stagnalis* numbers by judicious watering and applications of algaecides to control growth of the insect's food material in non-cropped areas of the greenhouse (Jacobson & Croft, 1997). Although these control strategies significantly reduced the numbers of flies in crops, some growers found the remaining population to be unacceptably large and additional control measures were required.

Beauveria bassiana has been found on *S. stagnalis* pupae and adults in UK glasshouses, and these observations have been supported by small-scale infectivity studies in the laboratory (Jacobson, unpublished data, 1997). In other bioassays, Naturalis-L was applied to growing medium containing second and third instar *S. stagnalis* larvae, and 80% of the insects failed to reach maturity (Jacobson *et al*., 1999).

Two further laboratory studies within this project evaluated Naturalis-L against *S. stagnalis* larvae. In the first study, Naturalis-L was applied to rockwool based algae cultures at the rate of 4ml product per litre of water prior to the release of second/third instar *S. stagnalis* larvae. The experiment was aborted because the larvae in the untreated controls became infected with a naturally occurring strain of *B. bassiana* and it was impossible to distinguish this from the applied treatments. In the second study, Naturalis-L was applied directly to the larvae before they were transferred to rockwool-based algae cultures. The cultures were then kept at 22^{o}C until the adults emerged. In this case, the product had no effect on the larval development.

The differences in these effects on the *S. stagnalis* populations may be explained by considering the insect's rate of development and the methods of applying *B. bassiana*. At 20-25^{o}C, the larvae develop rapidly and moult at intervals that are probably shorter than the germination time of the spores. Hence, spores applied directly to the larvae are shed with the insect's cast skin and the insects rarely become infected. However, when the product is applied to the growing medium, the infection pressure is greater because the insects are constantly exposed to spores and this results in fewer insects reaching maturity. Under such circumstances, it is probably the pupae that become infected because this stage is of longer duration than the three larval instars.

It has become clear from experimentation and practical experience that the problems caused by *S. stagnalis* in protected crops are unlikely to be solved by any single control measure. Due to the behaviour and reproductive rate of *S. stagnalis*, effective and sustainable control is more likely to be based on the integration of a suite of control measures, including algaecides, parasitic wasps and entomopathogenic fungi.

It is envisaged that the population growth of *S. stagnalis* could be initially restricted by reducing the availability of the algae food material, parasitic wasps could then be released to suppress the pest's development during crop production and, if necessary, the entomopathogenic fungus could be used as a further line of defence as the crop approaches harvest.

Sciarid flies (*Bradysia* spp.)

Sciarids (also known as fungus gnats) thrive in glasshouses and are commonly found on a wide range of crops. The delicate adult fly lays eggs in growing media of high organic matter. The larvae develop rapidly, feeding primarily on organic matter. However, they can cause both direct and indirect damage to seedlings by grazing on root hairs and transmitting root pathogens. Fresh cuttings are particularly vulnerable to the larvae, which tunnel in the exposed stems.

Naturalis-L has been tested against adult sciarid flies in the laboratory but the results were inconclusive because the oil in the formulation appeared to have a direct effect on the insects. Although the pathogen was identified on dead flies, it was impossible to say whether it had caused death or was growing saprophytically on the corpse (Croft, unpublished data).

Two bioassays within this project evaluated Naturalis-L against sciarid fly larvae. In both cases, sciarid flies were cultured and tested on a mixture of compost and decaying potato. In the first bioassay, Naturalis-L was applied to the culture mix, at the rate of 4ml product per litre of water, before eight second/third instar sciarid fly larvae were released. The insects were allowed to complete their development and the numbers of adults recorded. The results were compared to controls that had been treated with water only and each treatment was replicated five times. There was no significant difference between the numbers of adults emerging in the *B. bassiana* treatments and the untreated controls. The second bioassay was similar except Naturalis-L was applied directly to the sciarid fly larvae before they were released onto the culture media. Once again, there was no significant difference between the treatments.

Although *B. bassiana* is known to infect adult sciarid flies, it appears to have little impact on the development of the larvae. The latter may escape infection due to the development time of the individual instars being shorter than the germination time of the spores (as suggested above for shore flies).

Further evaluation of this product for the control of sciarid flies in IPM programmes is not recommended because effective biological control products (predatory mites and parasitic nematodes) are already commercially available.

Two-spotted spider mite (*Tetranychus urticae*)

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.

Several strains of *B. bassiana* were included in a screen of 40 isolates of nine species of entomopathogenic fungi. Most of these fungi originated from mites or ticks. Others originated from insect hosts but were known from the scientific literature or personal communications to be infective to mites. Isolates of fungi used in six proprietary biopesticides were also included in the study.

A laboratory bioassay was developed to measure the effect of the spores of these entomopathogenic fungi on the survival of *T. urticae*. Fixed age cultures of adult female spider mites were sprayed with a single dose of a suspension of spores and then maintained on a tomato leaf held under controlled conditions of temperature and humidity. The fungi exhibited a range of pathogenicities to the mites and were ranked in order of virulence. There was no pattern in the ranking that could be attributed to either fungal species or host of origin. The most effective pathogen was a strain of *B. bassiana* (isolate 434.99) that originated from a wasp (*Bephratelloides cubensis*) in Florida, USA. The two strains of *B. bassiana* used in BotaniGard WP and Naturalis-L and were ranked 16 and 18 respectively (Chandler, 2000).

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 mite hosts, were chosen from the top ten ranked fungi. Two isolates from proprietary biopesticides were also chosen. These six isolates were:

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 (*B. bassiana* 434.99) was not chosen for further bioassays as it originates from a hymenopteran host and this raises queries about its compatibility with hymenopteran natural enemies in IPM programmes. However, this isolate could be brought back into the study at a later date.

This work is continuing and is expected to move on to phase two studies in experimental glasshouses during 2001.

5. RELATIVE EFFICACY OF METHODS OF APPLYING *B. BASSIANA*.

The efficacy of high volume (HV) sprays of *B. bassiana* have been compared to low volume (LV) mists against *F. occidentalis* (WFT) and *L. rugulipennis* (capsid bug) on experimental cucumber crops. Preliminary studies, using water sensitive papers in various positions throughout the glasshouses, determined the most appropriate techniques and volumes of liquid to apply by both methods. This work ensured that both sides of all leaves were thoroughly coated with spray deposits. Additional tests compared the numbers of viable spores in the *B. bassiana* suspension in the LV spray tank with the number in the spray as it emerged from the spray nozzle. The action of the LV mister did not affect the number or viability of the spores.

The HV sprays were applied to maximum leaf retention using a fully calibrated Oxford Precision Sprayer. The product dilutions were 400 ml Naturalis per 100 litres of water and 125 gm BotaniGard WP per 100 litres water, and the sprays were applied at rates that varied from 2025 to 2919 litres per hectare depending on the amount of foliage present at the time. The LV mists were applied on the same dates as the HV sprays using a fully calibrated Turbair Scamp 240. The mists were applied at rates equivalent to 70 litres per hectare, using more concentrated solutions/suspensions that were calculated to deliver similar quantities of product to the HV sprays.

A sequence of three HV sprays of BotaniGard WP applied at six day intervals reduced numbers of immature *F. occidentalis* by 83% compared to untreated controls. The same number of Naturalis HV sprays, Naturalis LV mists or BotaniGard WP LV mists reduced numbers of immature *F. occidentalis* by about 70%. The treatments were less effective against adult *F. occidentalis* but they all reduced numbers by 34% compared to untreated controls (Jacobson, 2000a).

HV sprays and LV mists of *B. bassiana* have been compared against *L. rugulipennis* in two experiments. In the first, HV spray or LV mist applications of Naturalis-L provided similar results, reducing numbers of adult female *L. rugulipennis* by 60% compared to untreated controls (Jacobson, 1999a). In the second experiment, sequences of three HV spray or LV mists applications of Naturalis-L or BotaniGard WP again provided similar results, this time reducing numbers of capsids by 78% compared to the untreated control (Jacobson, 2000b).

Beauveria bassiana clearly has potential for the control of *F. occidentalis* and *L. rugulipennis* when applied as either an HV spray or a LV mist. The latter is relatively inexpensive because it requires minimal labour and is an attractive option because it allows the cost-effective application of a series of treatments. However, further studies are required to determine the optimum frequency and rate of applications against both pests on a larger scale in commercial crops.

6. COMPATIBILITY OF *B. BASSIANA* WITH SOME IMPORTANT INVERTEBRATE BIOLOGICAL CONTROL AGENTS.

Beauveria bassiana is active against a wide range of invertebrates and it is important to know the impact that it will have on the biological control agents used to control other pests in IPM programmes.

The effects of the entomopathogen on the thrips predator, *Amblyseius cucumeris,* and the whitefly parasitoid, *Encarsia formosa,* were determined in laboratory-based bioassays during 1998/99 (Jacobson, 1999a). There was no evidence to suggest that *B. bassiana* was harmful to *A. cucumeris,* which was consistent with results from another project (Jacobson, 2000a) in which populations of *A. cucumeris* on cucumber plants and in culture packs were unaffected by high volume sprays of the entomopathogenic fungus. However, *B. bassiana* was harmful to *E. formosa.* The results of two experiments demonstrated that *B. bassiana* sprayed on parasitised *Trialeurodes vaporariorum* (glasshouse whitefly) scales, before and after they turned black, reduced survival of the parasitoid by 36% and 62% respectively. *Beauveria bassiana* also infects *T. vaporariorum* (glasshouse whitefly) directly and can provide effective control of this pest, so the overall effect on this component of the IPM programme may not be detrimental.

The effects of *B. bassiana* on the spider mite predator, *Phytoseiulus persimilis*, and the aphid parasitoid, *Aphidius colemani*, were determined in laboratory-based bioassays in 1999/2000 (Jacobson, 2000b). Although it was demonstrated that *B. bassiana* could infect adult *P. persimilis*, this did not have a significant effect on the populations tested. There was no evidence to suggest that *B. bassiana* infected adult *A. colemani* but further studies are required to determine the effect on immature stages of the parasitoid.

Further studies are required to provide a complete data package for the compatibility of *B. bassiana* with all life cycle stages of all biological control agents used in IPM programmes in UK protected crops.

7. COMPATIBILITY OF *B. BASSIANA* WITH FUNGICIDES COMMONLY USED AGAINST DISEASES IN PROTECTED CROPS.

To integrate *B. bassiana* successfully into protected crop IPM programmes, it is essential to determine the compatibility of the fungal pathogen with the fungicides commonly used against the diseases of these crops.

A bioassay was developed and used to determine the effect of seven fungicides, that are commonly used on cucumber and tomato crops, on the germination of *B. bassiana* spores (Jacobson, 2000a). Rovral and Thiovit were relatively safe at their recommended application rates. Scala was safe and Nimrod was relatively safe at one tenth of their recommended rates. Fungaflor, Repulse and Amistar prevented germination at one tenth of their recommended rates. A further bioassay is required to determine the persistence of the effects of the five fungicides that were shown to be harmful to *B. bassiana* at their recommended application rates.

7. REQUIREMENTS FOR FUTURE RESEARCH & DEVELOPMENT

Completion of existing studies

- Further studies to improve HV spray techniques for commercial cucumber crops are required to ensure that entomopathogen spores come into contact with the target pest (PC 136).
- Large-scale evaluation of *B. bassiana*-based products against WFT in commercial cucumber crops will be required when the difficulties relating to HV spray techniques are resolved.
- Space treatments (LV and ULV mists) should be evaluated against capsid bugs in commercial cucumber crops when a *B. bassiana*-based product is registered in the UK and can be used without the destruction of the treated crop (PC 123).

Opportunities against other crop/pest combinations

- The technology that has been developed for the control of WFT on cucumber crops should be adapted to form the basis of an IPM compatible control strategy for the control of WFT on ornamental crops.
- An IPM compatible package, including *B. bassiana*-based products, should be developed for the control of shore flies in propagation houses and lettuce, celery, pot plant, bedding plant and herb crops.
- Other isolates/strains of *B. bassiana* could be screened for activity against important horticultural pests that have been inadequately controlled by the existing *B. bassiana*-based products.

Enhancing the effect of *B. bassiana*-based products

- The ability of oil adjuvants to enhance the efficacy of *B. bassiana*-based products should be determined, particularly against insects such as mealy bugs that are protected by waxy deposits.
- Some semiochemicals, such as WFT alarm pheromones, increase the activity of insects and have the potential to enhance the effect of entomopathogenic fungi by improving "secondary pick-up" of spores deposited on the leaf surface. This should be further investigated.

Compatibility with other crop protection products

- Further studies are required to provide a complete data package for the compatibility of *B. bassiana* with all life cycle stages of all biological control agents used in IPM programmes in UK protected crops.
- Further bioassays are required to determine the immediate and longer-term effects on *B. bassiana* of the fungicides commonly used in protected crops.

More general subjects

- A multi-disciplinary study is required to improve the measurement and definition of microclimates of boundary layers on plant and insect surfaces.

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