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Biopesticides for the control of storage insect pests

by

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1. ABSTRACT

The UK grain industry stores many tonnes of grain each year. Currently, chemical insecticides are used to protect this grain from infestation by stored product insects and mites. Only a limited number of products are available for application and there are concerns about safety, resistance and environmental impact of these conventional pesticides. In principle, the biological control of storage pests could overcome these problems. A previous LINK funded project (LK0914) established the potential for biological control of storage pests in the UK, using an insect-specific fungus *Beauveria bassiana*. The current project (LK0967) has investigated ways to increase the efficacy of the fungus and has also undertaken research to establish that mass production of high quality conidia (asexual spores) is possible, to ensure that formulations have a good shelf-life and has sought feedback from stakeholders with regard to this novel control method.

The work has concentrated on two different fungal isolates, both of which were found from insects in UK grain stores. The main objectives of the current project were:

1. To improve the efficacy of the insect-specific fungi when in contact with insects.
2. To improve the delivery of the insect-specific fungus to the insects.
3. To ensure the consistency and the safety of the product.
4. To ensure that the biopesticide product has wide acceptability and is effective under practical use.

Optimisation of production methods, formulation and delivery systems has resulted in prototype formulations that have good efficacy against a range of storage insect pests under conditions that are likely to be found in UK grain stores. Valuable information on the viability and efficacy of a range of formulation types was obtained, together with studies on the likely effect of the formulations on insect behaviour. Pilot scale trials using three species of stored product beetle have shown that significant levels of control can be achieved. Successful mass production of the conidia is necessary if the biopesticide is to be considered at a commercial level. This project has developed methods to ensure that mass production of the conidia at an industrial scale is possible. During the course of the project, the views of stakeholders and end-users were sought. Specific concerns were addressed and the feedback was used to focus the research aims. The registration and safety issues for the use of a biopesticide were also studied. This project has made a significant contribution to the development of a biopesticide as a structural treatment for grain storage areas in the UK.

2. SUMMARY

Currently, chemical insecticides are used to protect grain in storage from infestation by stored product insects and mites, but only a limited number of products are available for application. However, there are concerns about safety, resistance and environmental impact of these conventional pesticides. In principle, the biological control of storage pests could overcome these problems with conventional chemical agents. A previous Sustainable Arable LINK funded project (LK0914) established the potential for biological control of storage pests in the UK, using an insect-specific fungus *Beauveria bassiana*. The fungus germinates on and penetrates through the insect cuticle, ultimately resulting in the death of the insect. Project LK0914 (whilst providing evidence for the potential effectiveness of *B. bassiana* against stored product insects) also highlighted areas that required further research. These covered both technical areas, for example methods to improve the efficacy of the fungus, and also related to the practical use of the biopesticide product and the potential barriers to uptake of these novel control agents by key stakeholders. These areas were addressed in the current project, which has examined the potential of a biopesticide based on *B. bassiana* as a structural treatment for UK grain stores. The project has focussed on two different isolates of *B. bassiana*, IMI 389521 and IMI 386243, both of which were isolated from insects found in UK grain stores.

The main objectives of the project were:

1. To improve the efficacy of the fungus when in contact with insects.
2. To improve the delivery of the fungus to the insects.
3. To ensure the consistency and the safety of the product.
4. To ensure that the biopesticide product has wide acceptability and is effective under practical use.

2.1. Objective 1. To improve the efficacy of the fungus when in contact with insects

Beauveria bassiana occurs naturally in UK grain stores, and has been shown to have notable potential for the control of various storage insect pests. However, the control was only satisfactory when the tests were undertaken in laboratory bioassays and, especially for the beetle pests, with high humidity for the first 24 hours. To ensure optimal efficacy in practical situations, it was necessary to improve the germination of the fungal conidia and their penetration into the wide range of pests that need to be controlled, under conditions that represent those encountered in UK grain stores.

The first part of this study aimed to improve the pathogenicity and viability of *B. bassiana* isolates by manipulating the conditions under which the conidia are mass-produced. This was achieved by

subjecting the conidia to different degrees of water stress and determining the effect on production levels, viability and pathogenicity. The effect of four different rice treatments on the production level of conidia was assessed with eight different fungal isolates, which had previously been shown to be pathogenic to the saw-toothed grain beetle, *Oryzaephilus surinamensis*. Generally, fewer conidia were produced per gram of conidiated rice when the rice had received no prior treatment and therefore, had the lowest moisture content. The other three treatments resulted in similar levels of conidia produced for each isolate. The viability of the conidia produced for each of the four treatment methods and the ability of the conidia to germinate at lower water activities (corresponding to lower relative humidities) was assessed for two of the isolates. Isolate IMI 389521 proved to have better viability than isolate IMI 386243, showing no significant decrease in germination over time. The production method did not affect the pathogenicity of the conidia at a low humidity; treatment with conidia produced by the four methods resulted in similar levels of insect mortality, which was very low. To ensure that the production methods had not diminished the ability of the isolates to cause insect mortality, conidia of isolate IMI 386243 produced by the four production methods were tested under conditions of high humidity for the first 24 hours. Good levels of mortality were achieved under these conditions; conidia produced under conditions with the greatest water stress had the higher level of pathogenicity. This study demonstrated that the production method can have an effect on the level of mortality caused by the conidia.

It has previously been demonstrated that some stored product insect species have an innate tolerance against infection by *B. bassiana*. Possible reasons for this were examined. It was demonstrated, using scanning electron microscopy, that quantitative and qualitative differences in adherence and germination of conidia could be observed between a susceptible species, *Oryzaephilus surinamensis*, and a tolerant species, *Tribolium confusum*. At each of the post-treatment periods *O. surinamensis* had a greater number of conidia adhering to the cuticle. This species has a greater number of setae, particularly on the ventral abdomen, and the presence of these may have assisted with adherence of the conidia. Germinating conidia were observed more frequently on the cuticle of *O. surinamensis* than for *T. confusum*. The number of conidia found on both species decreased over time, possibly as a result of grooming activities. The findings suggest that conidia that have not germinated and penetrated the cuticle within the first 24-48 hours are unlikely to remain on the cuticle and play a role in the infection process.

Formulation can play a key role in the efficacy of a fungal biopesticide as it may enhance the infectivity of the fungal conidia and allow a product to be stored over a prolonged period. The viability of conidia of isolates IMI 389521 and IMI 386243 in various formulating agents was examined. A range of formulations were considered including both liquid and dust formulations, various bulking agents and liquid emulsions. In addition, the effect of the formulations on insect mortality was also assessed to determine the optimal formulation for use in further studies. The

formulation studies have shown that in an appropriate formulation, at 5°C, the isolates retained excellent germination over a period of 365 days. In general, IMI 389521 performed better than IMI 386243 with higher initial germination and more reproducible results in experiments. At 25°C, the experiments have shown that, in general, good germination may be achieved after 301 days of storage in mineral-based oils and the powder formulations, with viability remaining above 70%. Vegetable oils, in general, gave excellent results at 5°C. Water-based formulations such as 0.05% Tween 80 were not suitable for either isolate, as viability was lost very rapidly at 25°C and less rapidly at 5°C. There was also an apparent isolate difference when exposed to the emulsifier, Codacide. IMI 386243 lost viability rapidly at 25°C, whereas, this effect was not seen with IMI 389521. The oil formulations resulted in greater mortality of *O. surinamensis* in comparison with the water-based Tween 80 formulation. This reflects previous studies that have shown that oil-based formulations may cause higher mortality than water-based formulations. The powder-based formulations also showed potential. In conclusion, based on the viability and efficacy results, oil- and powder-based formulations look to be good candidates for future use as commercial mycoinsecticide formulations.

These studies have resulted in the development of production methods and formulations that result in high levels of insect mortality without the need for a period of high (close to 100%) humidity for a range of storage beetle pest species.

2.2. Objective 2. To improve the delivery of the fungus to the insects.

Optimising the efficacy of the fungus when it is in contact with insects is necessary for success but is not wholly sufficient: it is essential to ensure that the right amount of the agent is brought quickly enough into contact with the target insects, which may be hiding in cracks and crevices, to ensure control of a pest population. Evidence that this requires further research was obtained in the previous project where it was found that insect mortality was high only when insects were either rolled in conidia or were sprayed directly with them. Very low mortality was observed when insects were left on a treated surface. Improving the delivery of the conidia can be achieved in two ways 1) by moving the insects to the conidia and 2) by moving the conidia to the insects.

Moving the insects to the conidia can be achieved in two ways. Firstly, a repellent could be used to treat cracks and crevices to remove insects from these locations in order to make contact with treated surfaces. Secondly, the insects can be attracted to areas where the conidia are present. Both of these methods were investigated.

Following a literature review of possible repellents that could be used in the grain store environment and that satisfied the criteria of ease of application, low mammalian toxicity, and ready availability and registered for similar usage, two compounds, diatomaceous earths and

pyrethrins were chosen for further investigation. The repellency and mobility effects of pyrethrins and a diatomaceous earth were assessed against adult *Oryzaephilus surinamensis* by recording insect behaviour in response to filter paper halves treated with each compound. DEET (N, N - Diethyl-meta-toluamide), a known insect repellent was used as a positive control. There was no significant difference in the time spent on the untreated halves and those treated with the pyrethrins and the diatomaceous earth, indicating no repellent effect. The beetles did however spend significantly less time on the halves treated with DEET. There was also no significant difference in the distance travelled and velocity of beetles on the untreated halves and those treated with DEET and pyrethrins, suggesting that these compounds did not affect mobility. The beetles did, however, travel significantly shorter distances and were slower on the DE treated half compared to the untreated half and when both were compared to the other treatments, suggesting that the DE reduced insect movement even when away from the treated surface. The ability of DE and pyrethrins to remove insects from refuges was examined by creating artificial crevices containing the test compound. At the concentration tested the diatomaceous earth, Silico-sec, reduced the number of insects that were present in the refuge. This was noticeable one hour after the insects were introduced to the arena. Pybuthrin also reduced the number of insects present in the refuge, but not to the same extent as the diatomaceous earth. The work has shown that, on a small scale it is possible to reduce the number of insects present in a refuge at a given time. However, the ability to achieve this at a larger scale remains to be determined and practical issues with regard to treatment of all potential refuges may preclude this as a practical measure to improve uptake by the insects.

An alternative method to improve the contact between the insects and the conidia is to attract insects to areas where the conidia are present. Much work has been undertaken on developing attractants for stored product beetles. It should, therefore, be possible to attract insects to a bait station containing conidia for use in a lure and kill strategy. Treatments used bait stations either with or without dry conidia powder of isolate IMI 389521. In addition, to determine whether the presence of an attractant lure would improve contact with the conidia, a lure developed to attract several species of stored product beetle was placed in some of the 'bait stations' with and without the conidia. There was a significant difference in the mortality of *O. surinamensis* between treatments with and without the conidia in the bait station; mortality was significantly higher in treatments with the conidia. Mortality in the treatment with the lure in the bait station with the conidia was significantly greater than for the conidia without the lure. This study has shown that insects will enter an area where the conidia are present and will pick up a lethal dose of the dry conidia powder. Bait stations containing an appropriate formulation of the fungal isolate, therefore, offer potential of targeted delivery of the conidia to the insects.

The ability to improve contact by moving the conidia to the insects was also investigated by examining the uptake and behavioural responses of *O. surinamensis* to an electrostatically chargeable powder, Entostat™. This is a processed plant wax and has been identified as a potential carrier for active ingredients to be delivered to cracks and crevices in food facilities. Entostat uptake and retention by *O. surinamensis* 24-72 h after exposure to rolled oats mixed with Entostat was quantified. Depending upon initial Entostat concentration in food mixtures, 0.03-0.26 µg powder was extracted from individual beetles 72 h after being transferred from treated to untreated food. SEM images showed that Entostat adhered to all body parts, including joints, between body segments, and at insertions of body hairs. Choice experiments were used to determine whether *O. surinamensis* individuals were repelled by Entostat. In a three-choice experiment with untreated oats, oats mixed with 5% (w/w) Entostat, and oats mixed with 5% (w/w) Entostat and a piece of filter paper containing a beetle attractant, the beetle attractant did not significantly increase the attractiveness of the crack in which it was applied, but the average powder uptake of beetles from cracks treated with the attractant was significantly higher than from the other cracks. The results suggest that considerable amounts of Entostat were taken up even when beetles were offered a choice between treated and untreated cracks. The addition of Entostat, therefore, provided a means by which contact between the insect and the conidia could be improved.

Dispersal of a fungal infection within an insect population is an important consideration to establish maximum effect. After initial infection, conidia may be passed to other uninfected insects by two mechanisms: secondary transmission and secondary cycling. Secondary transmission results from the physical contact with infected live individuals, whereas secondary cycling occurs after contact with dead mycosed insects. The effects of secondary cycling and secondary transmission of a fungal infection on *Oryzaephilus surinamensis* were assessed in the laboratory. Mortality was evaluated at two temperatures (15°C and 20°C) and four relative humidities (70%, 80%, 90% and 100% rh) with different proportions of infected insects. Secondary cycling was more effective at disseminating the fungal infection than secondary transmission, with 20°C and 100% r.h. the most effective conditions for secondary cycling to take place. Secondary transmission had little effect on increasing the mortality of uninfected individuals.

Some storage beetle species have been shown to exhibit refuge-seeking behaviour. This behaviour can limit contact with residual pesticides but may be advantageous for dissemination, both initial and subsequent, of the biopesticide. However, it is possible that insect behaviour may be altered subsequent to infection or at sub-lethal concentrations. In a preliminary study to examine possible effects of infection on insect behaviour, the effect of a lethal and a sub-lethal dose of *B. bassiana* on the refuge-seeking behaviour of *O. surinamensis* was examined. Significantly fewer insects treated with the lethal concentration of *B. bassiana* were present in the

arena at observation points 48 h post-treatment in comparison with the other treatments. The result indicates that insects infected with a lethal concentration of *B. bassiana* were less likely to leave the refuge than insects receiving the other treatments. The number of dead insects found in the refuge was greater than that found in the arena. This could be of benefit in terms of secondary cycling if conditions within the refuge are suitable. It is likely that uninfected insects would enter the refuge containing the dead, conidiating insects, increasing the likelihood of infection.

In order for a biopesticide to be produced and used at a commercial scale it is necessary to mass-produce the fungal isolate to obtain sufficient quantities. The mass production method must result in a product that is of a high quality and that is consistent between different batches. Sylvan-Somycel led the development of the mass production method used in this project. During the process, different aspects of production were examined including the substrate, incubation conditions, extraction process etc. Initial studies used both isolates IMI 386243 and IMI 389521. However, the viability and yields of batches of IMI 386243 were found to be more variable than for isolate IMI 389521. This finding, in conjunction with other data demonstrating greater viability of IMI 389521 under conditions of low water activity, and when formulated in different formulation types, led to the adoption of IMI 389521 as the preferred isolate. The establishment of the mass production method in the latter stages, therefore, focussed on isolate IMI 389521. It is important that the fungus can be produced at an industrial scale and that the product meets established quality control parameters if it is to be used commercially. The scaling up of production from the laboratory was, therefore, an important step if the potential of a biopesticide as a structural grain store treatment is to be realised.

The various studies undertaken to achieve this objective have demonstrated that 'bait stations' offer a potential method for dissemination of the fungal conidia, that the addition of Entostat may improve the uptake of the conidia and that secondary cycling may further spread the infection within an insect population. In addition, a mass production method was established.

2.3. Objective 3. Consistency and safety

An essential part of the project was to show how the safety and quality of the putative biopesticide could be established such that there will be no future technical barriers to its subsequent registration. This included establishing the information that will ultimately be needed for subsequent registration of the biopesticide by industry. In addition, a representative from the Chemicals Regulation Directorate (CRD; formerly the Pesticide Safety Directorate (PSD)) was appointed to the Project Management Group to monitor project progress and provide advice. Discussions with representatives from CRD, particularly with regard to efficacy testing were also held in the latter stages of the project. An overview of the data requirements for a registration package as they currently stand was produced.

The consistency of the mass-produced isolate was confirmed by producing different batches of the chosen isolate and analysing them for compliance with specification standards. The quality of mass-produced conidia by Sylvan-Somycel throughout the project has been very high and the yield has increased with further refinements to the mass production technology since initial laboratory scale mass production experiments were conducted. The particle sizes of IMI 389521 need to be reduced, in general, for good particle suspension in oil formulations and effective spraying. Ideally, droplet sizes for fine oil sprays are 50-100 µm. For dust formulations, particle size is less of an issue for application. The method of application has an important bearing on the ideal size of particle for application. Viability of conidia for IMI 389521 and IMI 386243 from the optimized mass production runs was excellent.

The growing interest in the exploitation of micro-organisms as biological control agents in agriculture has raised concerns about the safety of these organisms. Consequently there is a need for a risk assessment to determine the level of hazard involved and an appreciation that any intervention in an ecosystem will have an impact. Compared with chemical pesticides, mycoinsecticides have features that provide ecologically sound pest control. They are selective to varying degrees, suitable for integrated management techniques, provide an extended period of control by remaining within the environment, and are biodegradable and fundamentally safe. A review within this project of the currently available literature on *B. bassiana* concluded that fungal biocontrol products using *Beauveria* species have a long history of use and have been well tested, but there have been no serious detrimental cases reported. Any potential fungal biocontrol agent will have to undergo testing before it can be released on the market ensuring that all safety issues are covered. However, all the evidence to date indicates that metabolites produced by *Beauveria bassiana* show no risk to humans, animals or the environment.

Finally, as regulatory approval of a biopesticide will require assessment of possible risk due to non-target effects, preliminary studies on the likelihood of non-target effects were made. Some of these, for example carryover on application, are reported for the pilot scale trials under objective 4. Although the biopesticide is intended as a structural treatment, non-target effects would include any effect on the grain in store post-treatment. In this part of the project, the potential effect of the presence of the fungus on the germination of malting barley was examined. The dry conidia powder was mixed with barley at different concentrations and held at different temperatures and varying levels of water availability. Adding a seed-coating of *B. bassiana* increased seed germination under the more temperature and water-stressed conditions; this may have been a combination of conidia providing an absorbent, protective barrier which reduced the rate of imbibition and the germination of the conidia, which may have stimulated seed germination directly by the production of beneficial secondary metabolites or indirectly by out-competing inhibitory

microorganisms on the surface of the seed. However, it was observed that at the more favourable seed germination conditions of 16°C and lower moisture levels, a seed-coating of the fungus had an inhibitory effect on seed germination. The results indicated that a seed-coat treatment of dry *B. bassiana* could affect the timing and/or ability of barley seeds to germinate, and should be investigated further. However, at the levels of *B. bassiana* contamination that could occur on barley in treated premises, no harmful effects were noted.

Although further testing of the formulated product would be needed before the product could be brought to market, the evidence currently available would suggest that such a product would be safe to use for structural treatments. The consistency of the conidia produced using the mass production method has been established and this is also a major step towards the development of a commercially viable product.

2.4. Objective 4. Acceptability and practical use

Use of the biopesticide will depend upon its acceptability to all interested parties and proof that it will function effectively under conditions that are found in typical storage premises. The factors that might affect uptake by industry were examined through consultations with key stakeholder groups. In order to establish the effectiveness of the biopesticide under practical conditions laboratory tests were undertaken on different surface types to ensure that the formulation developed in this project functioned effectively, regardless of surface type. The findings of the laboratory studies of efficacy were validated in pilot- scale trials under conditions which brought together all the difficulties which have to be overcome in real situations, *i.e.* the need to function on combinations of different types of surface under challenging conditions of temperature and humidity, and deal with real infestations of target insects which may try to avoid treatment. Finally, the versatility of the biopesticide for users, and its acceptability to the potential manufacturer and distributor were assessed.

The development of any novel control method should consider not only the technical obstacles and developments, but also needs to consider how such a product would be viewed by stakeholders when used under 'real-world' conditions. The aim of this step was to identify the likely issues, if any, that will inhibit take up of a commercial product based on the biopesticide developed within the project. This was achieved by meeting with representatives of each stakeholder group to outline the project aims and progress, to ascertain any concerns and to identify information that ideally the project would need to provide. The main concerns expressed by the majority of stakeholders fell into the following categories:

1. Efficacy - the range of pests that could be controlled with this type of product, whether limitations are imposed by temperature and humidity conditions, the potential shelf life of the product and the length of the residual effect after treatment.

2. Health and safety considerations for operators - the type of formulation was regarded as a key issue with stakeholders expressing concerns over the use of dust formulations due to potential respiratory risks. This was stated to be one of the main constraints to the use of another novel method for grain store pest control, diatomaceous earths. The potential for the production of secondary metabolites was also a concern.
3. Risk of contamination - the key concern was whether there could be a build-up of inoculum and whether the inoculum could pass further along the processing chain, for example, affecting germination of malting barley or taint in final products
4. Use - cost was seen as a key issue for some stakeholders, whilst others would be willing to accept an increased cost (assuming acceptable efficacy) if this led to a substantial reduction or elimination of the use of organophosphate insecticides.

Many of the concerns raised were similar for all of the groups. The information was used to guide the experimentation within the project and data has been generated that can specifically address some of the issues raised.

In order to establish the effectiveness of the biopesticide under practical conditions, experiments examined the effectiveness of the fungus when applied to different surface types likely to be found in a typical UK grain store. It is important that efficacy of the biopesticide is retained on all surface types and ideally that the viability of the conidia is maintained to provide a degree of persistency. Viability of the fungus was found to be affected by the type of surface, to which it was applied, with a substantial loss of viability observed on the concrete after only one day. The viability on wood and steel also decreased over the 13-day test period, but not to the same degree as the conidia on the concrete. Only one formulation type was tested in the laboratory and it is possible that other formulations may maintain the viability of the conidia to a greater degree. This was explored further in the pilot- scale trials reported below.

The dry conidia powder, either alone or when mixed with Entostat, resulted in high levels of mortality of *O. surinamensis*. The mortality level on wood was slightly less than on steel or concrete. This may have been as a result of the surface of the plywood, which has small grooves in which the conidia can lodge, perhaps making contact with the insects more difficult. Persistency of the conidia on the various surface types was not explored in this laboratory trial, but the effectiveness of the treated surfaces over time was explored in the pilot scale trials.

The laboratory studies had shown that isolate IMI 389521 has good efficacy against storage beetle pests under constant temperature and humidity conditions and that a good level of efficacy was maintained when the isolate was applied to different surface types. The conditions chosen for the laboratory studies were close to optimal for both the fungus and the insects. To act as an effective

structural treatment in a grain store, it is important that efficacy is maintained for a reasonable period under the fluctuating environmental conditions that would typically be encountered in this situation. It was therefore, important to test the fungus in a larger scale experiment under conditions that would typically be encountered in a UK grain store. For the purpose of this experiment, two formulations were chosen for further investigation. The results from the first pilot scale trial indicated that there were differences in the efficacy of the two formulations. However, the data also showed that the different application methods used resulted in a difference in the concentrations of the two formulations applied to the arenas. To establish whether both formulations should remain as candidates for further testing, it was necessary to examine the effect of the two formulations when applied at similar concentrations. The second pilot scale trial examined the effect of the two formulations of IMI 389521 at two target concentrations on the mortality of three species of stored product beetle when applied to plywood arenas. In addition, the viability of the conidia in the two formulations under typical UK grain store conditions was again assessed. A comparison with a currently registered chemical pesticide, pirimiphos methyl, was also made.

The chemical pesticide, Actellic (pirimiphos methyl) when applied at the recommended concentration caused rapid death of all three species of insect. Large numbers of knock down or dead insects were observed within two hours of the introduction of the insects to the treated surface and 100% mortality was recorded for insects recovered from the rings after 14 days. The biopesticide formulations also caused a significantly greater level of mortality than was observed for the control treatments. The viability of the conidia on realistic surfaces, as determined by the % germination, remained high throughout the trial indicating that under the test conditions isolate IMI 389521 retained the potential to infect insects and may, therefore, have residual activity.

A novel insect control agent for use in UK grain stores should ideally integrate with current procedures for best practice. Therefore, the control agent should form part of an integrated pest management (IPM) approach. The cost effectiveness of any novel agent is a key factor in determining likely future commercial exploitation. The development of the mass production methods within this project and determination of the likely maximum quantity for use of the agent on its own have provided data that can be used to examine the likely costs to produce the agent. The cost of the use of the agent on its own should also be considered with the costs of use in combination with another control method. This may also increase the versatility of use of the biological control agent. It is known that the use of a diatomaceous earth (DE) structural treatment has potential against the saw-toothed grain beetle, *O. surinamensis* and it has been reported that a commercial DE synergises the effect of unformulated conidia of *B. bassiana* against some storage pests. The effects of the biopesticide formulation and DE when used alone at a single concentration were compared to the effect of the combination by exposing *O. surinamensis* in

treated Petri dishes to provide information on the versatility of the biopesticide for potential users. The combination of IMI 389521 and Silico-sec resulted in a significant increase in the overall mortality in comparison with either treatment alone at both 15°C and 20°C. At both temperatures, significant synergism was indicated and this was particularly apparent at 15°C.

This part of the project has used the data provided from the three previous objectives and through the pilot scale trials has demonstrated that a biopesticide based on *B. bassiana* has great potential as a structural treatment under typical UK storage conditions. Establishing the key concerns that stakeholders may have in relation to this type of product has enabled data for many of these concerns to be obtained within the course of the project and for this information to be fed back to the stakeholders.

2.5. Conclusions

This study has successfully achieved all four main objectives of the project. Of particular note, is that enhancement of the production and formulation of the conidia has negated a need for a period where the humidity needs to be close to 100% and the conidia do not have to be directly applied to the insects to achieve good efficacy. In addition, mass production methods resulting in consistent, high quality production of conidia with excellent viability and virulence have been developed and significant control of insect populations under practical conditions has been demonstrated.

This research has made a significant step towards the development of a biopesticide, based on *B. bassiana*, as a structural treatment in UK grain stores. In addition to overcoming the main technical obstacles, information has also been collected on the concerns and issues that the development and use of a biopesticide may generate. The research undertaken within this project has, as far as was possible, addressed these concerns and generated important data to demonstrate that this type of product can be used in a practical situation to achieve good levels of control. Information has also been gathered on the registration process for a biopesticides. This will be important in the future development of the product and provides a clear foundation to determine the further studies that would be necessary.

The project has demonstrated that a biopesticide based on *B. bassiana* has potential for control of stored product insects in UK grain stores. Candidate formulations have been identified. However, further work will be needed to fully establish the most appropriate formulation. The mass production process has been optimised, but until the most appropriate formulation and dose rate have been established, it will remain to be seen whether cost-effective production can be realised. The project has made significant progress in the development of a novel structural treatment that would be a benefit to UK farmers.