Progress in the development of a biopesticide for the structural treatment of grain stores

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Abstract

Chemical insecticides are used to protect stored grain from infestation by stored product insects and mites. In the UK only a limited number of products are available for application and there are concerns about safety, pest resistance and environmental impact of these conventional pesticides. Biological control offers an alternative to the use of chemical insecticides. The potential for biological control of storage pests in the UK using an insect-specific fungus, *Beauveria bassiana*, to treat the structure of the stores, has previously been established. However, this study also highlighted areas where improvements were needed; specifically to improve the uptake of the fungal conidia by the pests and to improve their germination and penetration into the pests. In addition it was necessary to ensure that potential formulations had a good shelf-life and to develop a mass production method to consistently produce high quality fungal conidia. A four year project has recently been completed examining these areas in detail. The work has concentrated on two different fungal isolates of *B. bassiana*, both of which were found from insects in UK grain stores. Optimisation of production methods, formulation and delivery systems has resulted in prototype formulations that exhibit good viability over periods up to one year and that have good efficacy against a range of storage insect pests under conditions that are likely to be found in UK grain stores. Pilot scale trials using three species of stored product beetle have shown that significant levels of control can be achieved. An overview of the key findings is presented. The study has made a significant contribution to the development of a biopesticide as a structural treatment for grain storage areas in the UK.

Keywords: Biological control; *Beauveria bassiana*; *Oryzaephilus surinamensis*; Structural treatment; Biopesticide

1. Introduction

Cereals are an important component in the human diet and are similarly important in livestock feedstuffs. Cereals, whilst in storage, are at risk of infestation by insects, resulting in quality deterioration and losses. Pesticides are commonly used for control of stored product insects; in 2002 over 9 tonnes of active ingredients were used as fabric treatments to protect the harvest in Great Britain (Dawson et al., 2004a, b). Concerns about the safety of some insecticides, in particular organophosphates, have resulted in proposed changes to EU legislation, which would result in removal of some currently approved pesticide products (Pesticides Safety Directorate, 2008). Concerns have also arisen with regard to insect resistance to commonly used products. Resistance has been reported for all insecticide classes for one of more key pest species (Whalon et al., 2008), including stored product insects. With an increasing emphasis on food security, alternative approaches for control of stored product insects are needed.

Biological control of storage pests is one such novel approach. While bacteria, fungi, protozoa and viruses all have potential as natural microbial control agents (biopesticides), it is the insect-specific (entomopathogenic) fungi such as *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin for which most is currently known and that are the best candidates. Research to investigate the potential of entomopathogenic fungi for control of stored product insects has increased over the past decade, with studies examining the potential of both *M. anisopliae and B. bassiana* on a range of species. The majority of these studies have examined the efficacy when the fungus is applied to grain (Lord, 2001; 2005; 2007a, b; Akbar et al., 2004; Vassilakos et al., 2006; Michalaki et al., 2006; Kavallieratos et al., 2006; Cherry et al., 2007; Hansen and Steenberg, 2007; Athanassiou et al., 2008).

The potential of *B. bassiana* as a structural treatment for UK stores was investigated by Cox et al. (2004) and, although 100% mortality of one or more species of stored product insect and mites was achieved in laboratory studies, it was concluded that it would be essential to improve the germination of the conidia on the insect pest and the uptake of the fungal conidia by the pests. In particular it had been found that high levels of insect mortality could only be achieved under conditions of high humidity and when insects were treated directly with the conidia (Cox et al., 2004). The current project therefore focussed on improving efficacy by overcoming these conditions. The studies were undertaken to achieve three objectives (1) to improve the efficacy of the entomopathogenic fungus when in contact with insects (2) to improve the delivery of the entomopathogenic fungus to the insects and (3) to demonstrate that prototype biopesticides formulations are effective under practical conditions. Initially several different isolates of *B. bassiana* that had been isolated from insects found in UK grain stores (Cox et al., 2004) were used. From these, two isolates, IMI 386243 and IMI 389521, were chosen for further studies, and a single isolate was used to demonstrate the potential of prototype formulations under practical UK conditions.

2. Materials and methods

2.1. Optimization of the conidia

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 were mass produced. 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* L. (Coleoptera: Silvanidae) (Cox et al., 2004). Conidia were produced using a two stage method, whereby inoculum were grown in a liquid broth initially and then inoculated onto sterile rice (Jenkins et al., 1998). Levels of water added to the substrate were varied, as it was hypothesised that conidia grown in conditions of water stress would be more viable at lower humidities. Isolates were tested for their ability to germinate at lower water availability levels on polyethylene glycol (PEG) adjusted agar. The method was a simulation to test the viability of conidia at lower levels of humidity. The effect of the different production methods on the pathogenicity of the conidia was assessed in bioassays using *O. surinamensis*.

2.2. Formulation studies

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 of time. 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 against *O. surinamensis* in laboratory bioassays.

2.3. Improving contact between insects and conidia

Optimising the efficacy of the conidia when in contact with insects is an important factor, but, to achieve adequate control, it is also essential that a sufficient number of conidia are brought quickly enough into contact with the target insects, which may be hiding in cracks and crevices. 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; either a repellent could be used to treat cracks and crevices to remove insects from these locations in order to make contact with treated surfaces, or the insects can be attracted to areas where the conidia are present. The ability of a diatomaceous earth (DE) and pyrethrins to act as repellents and remove insects from refuges was examined by creating artificial crevices containing the test compound. To determine whether insects could be attracted to areas treated with the conidia, ‘bait stations’ either with or without dry conidia powder and the presence or absence of a lure, developed to attract several species of stored product beetle, were used.

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^{TM}. 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.

2.4. Pilot scale trial

In order to establish the effectiveness of the biopesticide under practical conditions a larger scale experiment was undertaken to ensure that efficacy was maintained for a reasonable period of time under the fluctuating environmental conditions that would typically be encountered in a UK grain store. The pilot scale trial examined the effect of two prototype formulations at two target concentrations on the mortality of three species of stored product beetle when applied to plywood arenas housed within a grain store. A comparison with a currently registered chemical pesticide, pirimiphos methyl, was also made.

3. Results

3.1. Optimization of the conidia

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 (Table 1). This study demonstrated that the production method can have an affect on the level of mortality caused by the conidia.

Table 1 Mean % mortality (± S.E.) of *Oryzaephilus surinamenisis* 14 d after treatment with a solution containing $1x10^7$ conidia/mL^{-1} *Beauveria bassiana*. Conidia were produced using a method in which different quantities of water were added. The amount of water added is indicated by the figures 1-4, with 1 being the lowest and 4 the highest.

Water added	1	2	3	4
% mortality	74 ± 5	73 ± 4	44 ± 6	50 ± 3

3.2. Formulation studies

The formulation studies demonstrated that in an appropriate formulation, at 5°C, the isolates retained excellent germination over a period of 365 d. In general IMI 389521 performed better than IMI 386243 with higher initial germination and more reproducible results in experiments. At 25°C the experiments showed that, in general, good germination was retained after 301 d of storage in an appropriate formulation, with viability remaining above 70%. Water based formulations were not suitable for either isolate as viability was lost very rapidly at 25°C and less rapidly at 5°C.

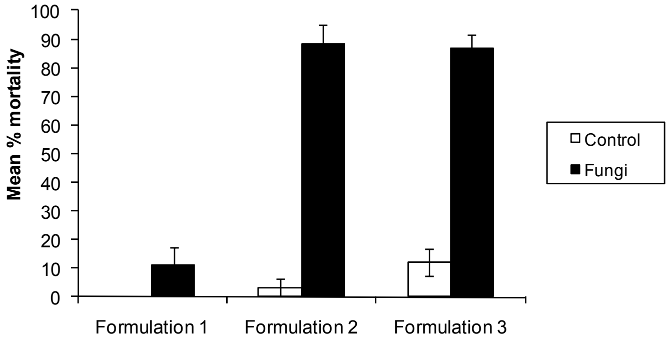

Figure 1 Mean (± S.E.) % mortality of *Oryzaephilus surinamensis* 14 d after treatment with *Beauveria bassiana* formulated in different carriers at a concentration of $1x10^9$ conidia/mL^{-1}.

The oil formulations resulted in greater mortality of *O. surinamensis* in comparison with the water-based formulation (Figure 1). 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.

3.3. Improving contact between insects and conidia

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 1 h after the insects were introduced to the arena (Table 2). Pybuthrin also reduced the number of insects present in the refuge, but not to the same extent as the diatomaceous earth (Table 2). 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.

Table 2 The mean percentage of *Oryzaephilus surinamensis* observed in arenas containing refuges with different treatments at various time points after introduction (n = 5).

Elapsed	Control			Silico-sec			Pybuthrin		
Time (h:m)	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.
00:15	89.4	98.0	76.0	79.0	92.0	72.0	87.4	94.0	84.0
00:30	85.6	96.0	80.0	86.4	90.0	82.0	84.6	90.0	76.0
00:45	82.2	94.0	72.0	84.0	92.0	78.0	84.2	88.0	78.0
01:00	80.2	94.0	74.0	81.0	88.0	74.0	81.0	86.0	70.0
01:30	59.8	80.0	28.0	74.4	82.0	60.0	59.8	68.0	38.0
02:00	42.4	56.0	16.0	61.2	78.0	44.0	49.4	68.0	34.0
02:30	39.8	56.0	18.0	61.6	70.0	56.0	46.2	62.0	32.0
03:00	36.2	50.0	14.0	60.6	66.0	52.0	49.0	66.0	34.0
04:00	33.6	46.0	18.0	53.8	68.0	40.0	45.2	58.0	34.0
05:00	35.2	54.0	20.0	54.0	64.0	46.0	43.8	62.0	28.0
24:00	21.2	32.0	14.0	54.4	64.0	36.0	36.6	52.0	20.0

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 (Figure 2). 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.

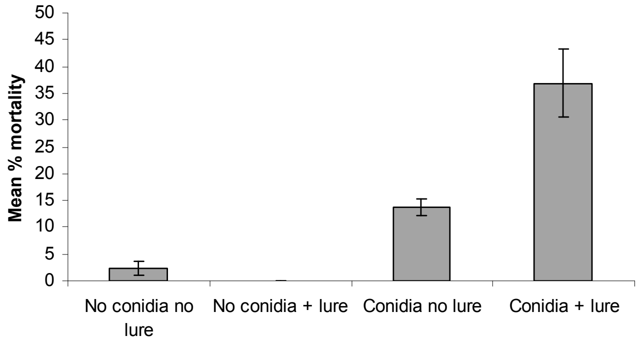

Figure 2 Mean (± S.E.) % mortality of *Oryzaephilus surinamensis* 14 d after exposure to different treatments in 'bait stations' (n = 10).

Entostat uptake and retention by *O. surinamensis* 24-72 h after exposure to rolled oats mixed with Entostat was quantified. SEM images showed that Entostat adhered to all body parts, including joints, between body segments, and at insertions of body hairs (Nansen et al., 2007). Choice experiments were used to determine whether *O. surinamensis* individuals were repelled by Entostat. The results suggest that considerable amounts of Entostat were taken up even when beetles were offered a choice between treated and untreated cracks (Nansen et al., 2007). The addition of Entostat therefore provided a means by which contact between the insect and the conidia could be improved.

3.4. Pilot scale trial

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 2 h of the introduction of the insects to the treated surface and 100% mortality was recorded for insects recovered from the rings after 14 d. The biopesticide formulations also caused a significantly greater level of mortality (45-95% dependant on species, concentration and formulation) 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.

4. Discussion

During the course of the project significant progress was made; 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 that 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 determined 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. Candidate formulations have been identified but 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 farmers and storekeepers.

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