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FINAL REPORT - SRDC PROJECT BSS246

EXPANDED REGISTRATIONS FOR *METARHIZIUM* STRAINS AGAINST CANEGRUBS

by

P R Samson*, R J Milner and G K Bullard*****

SD02020

****BSES, PMB57, Mackay Mail Centre 4741 (ph. 07 49545100)***
*****CSIRO Entomology, GPO Box 1700, Canberra 2600***
Bio-Care Technology Pty Ltd, RMB 1084 Pacific Highway, Somersby NSW 2250 (ph. 02 43402246)

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CONTENTS

Page No.

SUMMARY

Eight fungicides and three liquid insecticides are registered in Queensland for application to sugarcane at planting, and these may come into contact with *Metarhizium* during application from cane planters. Seven of the chemicals were tested for deleterious effects on two *Metarhizium* isolates, FI-147 and FI-1045 (BioCane™), in laboratory and field experiments.

In growth studies on medium, the fungicides Cane Strike® and Sportak® were about 10 times more toxic than Shirtan® and Tilt®, while toxicity of the latter fungicides was about 100 times that of the three insecticides Lorsban®, Talstar® and Regent® (based on active ingredient). When the amount of active ingredient in each product and field application rates are considered, the expected order of harmfulness in commercial use would be Regent < Talstar < Lorsban < Cane Strike < Tilt < Shirtan < Sportak.

In a field experiment where *Metarhizium* granules were sprayed with each chemical (except Regent) at very high rates and then covered with soil, only Shirtan showed any toxic effect on spore viability, with a reduction from 82% to 69%. No harmful effect of any chemical was detected in counts of colony-forming units in soil samples or in bioassays of treated soil using negatoria and greyback canegrubs. No reduction was found in viability of FI-1045 on nine farms, where BioCane granules were applied though commercial planters with fungicide, compared with granules buried in untreated soil. Thus, we believe that BioCane is compatible with these chemicals in practice, and a label change for BioCane to include application at planting has been drafted with Bio-Care Technology Pty Ltd.

Metarhizium isolate FI-1045 reduced numbers of southern one-year canegrub in four efficacy trials using band application in plant cane, and its effect on numbers and yield could not be separated statistically from that of suSCon® (Blue or Plus). In a fifth trial, FI-1045 was less effective than suSCon Plus but spore concentrations in soil were lower than expected shortly after application. In a sixth trial, neither FI-1045 nor suSCon Blue reduced grub numbers in a third ratoon but this was more than 3 years after application. An application rate of FI-1045 of 5 g/m was quite effective, although 10 g/m seemed to give results more comparable to suSCon. There are not enough results to evaluate the efficacy of isolate mixtures. All results of field trials using band application in plant crops have been positive provided expected spore concentrations in soil were achieved. There is still good potential for BioCane against this pest.

Trials established in a previous project, BSS134, showed great potential for use of isolate FI-147 against negatoria canegrub. Good results were achieved at rates below 5 g/m using coulters in ratoon crops. However, we have been unable to collect any additional data for ratoon application. For band application in plant crops, we have results from only one trial, with grub numbers reduced by both FI-147 and suSCon Blue in the first ratoon. Neither product improved cane yields, probably because only a light infestation was present. This trial produced useful non-target data showing no effect of *Metarhizium* on numbers of earthworms. More data are needed to justify registration of isolate FI-147 for control of negatoria canegrub and to give confidence in recommending its use. Unfortunately, the current low incidence of negatoria canegrub, particularly in southern Queensland, makes the collection of additional data problematic.

Results have been obtained in only two trials against French's grub, despite the establishment of 17 trials targeting this species. Spore concentrations were below expectation in one trial in plant cane, possibly because of inadequate soil cover due to the drills being largely filled in by the time of application, and suSCon Blue gave a better yield than *Metarhizium* in the second ratoon. In the second trial where products were applied using coulters into a ratoon, FI-147 alone or mixed with FI-1045 improved yields of the succeeding ratoon, although no significant effect of treatment on grub numbers had been detected previously. Bioassays showed that this species is very susceptible to isolate FI-147, similar to negatoria canegrub, and is a good candidate for biological control with *Metarhizium*. However, like negatoria, its low frequency and unpredictability of occurrence has precluded collection of sufficient efficacy data to be convincing.

Childers canegrub was not a primary target of BSS246, and no new trials were established. However, one trial from BSS134 was continued into the current project. Although grub numbers were not reduced one year after treatment, soil samples collected more than two years after treatment still produced high rates of infection of Childers grubs confined within the treated soil. Trial results against Childers canegrub have been disappointing in both projects. However, suSCon Blue was also unsuccessful in all trials where used, even in the one trial where it was applied according to label recommendations, and so there is no evidence that *Metarhizium* is any less effective than suSCon Blue (which is known to work in most situations). There may be environmental factors that caused the poor performance of *Metarhizium* (and suSCon Blue) in some of our trials.

No field trials were established against noxia canegrub, but we carried out bioassays to evaluate the potential for *Metarhizium* to be used against this localised but troublesome pest. Noxia grubs responded poorly to a range of isolates, and none would be worth testing in the field.

Two tests of persistence of *Metarhizium* spores in soil were conducted in PVC rings, either completing research started in project BSS134 or testing new hypotheses. In the longer running experiment, the annual survival rate of FI-1045 in northern Queensland was 40-56% at three sites while annual survival of FI-147 in southern Queensland was 49-52%. There was little difference between survival of several formulations, including spores on rice granules, and there was little evidence that differences in trash blanketing between sites had much effect on spore persistence. In a second experiment, the rate of decline of spore levels seemed to be unaffected by the starting concentration in soil, but longer-term data are needed.

Bioassays of BioCane cultures against greyback canegrub were conducted in each of 2000-2002 to confirm virulence. This is an important component of the maintenance program for BioCane, to ensure continuing efficacy of the commercial product.

Efficacy trials that are still capable of producing useful results will be monitored for at least another year using BSES resources, and the possibility of seeking registration for BioCane against southern one-year canegrub will be considered after harvest results are collected for two infested trials later in 2002.

1.0 BACKGROUND

Canegrubs chew the roots from sugarcane plants, causing poor yield and death. Nineteen species of canegrub have been found in canefields. Good control of canegrubs has been provided by insecticides for most of the past 50 years. The organochlorine insecticides BHC and heptachlor were used until the 1980s, when they were phased out. Controlled-release (CR) formulations of chlorpyrifos, eg suSCon® Blue and suSCon® Plus, are now used on more than 90% of the area treated for canegrubs each year. The only insecticidal alternatives to CR chlorpyrifos are two 'knockdown' products, Confidor® Guard and Rugby® 100 g. These are used to kill grubs that are feeding at or soon after the time of application, and are not suitable for all canegrub species or all districts. There are no products other than CR chlorpyrifos that give residual protection to cane.

Although insecticides are currently giving good control of most canegrubs, the industry should not be complacent. Failure of insecticidal control is the rule rather than the exception in most industries. Reasons include pest resistance to the chemical, enhanced microbial breakdown in soil, and environmental problems. Failure can quickly lead to a crisis if alternative management systems are not available. The sugar industry has already experienced its own crisis, with suSCon Blue failing in the Burdekin district and a consequent explosion in greyback grub numbers. No fall-back management system was in place, and cane losses in that region increased enormously. The research needed to develop management options for canegrubs, with their long life cycles, can take many years. The industry should be proactive in ensuring that a range of management options is available for the major canegrub species, to avoid future crises.

The *Metarhizium* fungus has characteristics that make it very suitable as a management tool for canegrubs. The spores can be mass-produced at an economic cost, they are hardy, and they can survive for years in soil. Bio-Care Technology Pty Ltd is already producing one *Metarhizium* product for greyback canegrub, BioCane™, and the company is interested in other products. This project aimed to use established research and commercial infrastructure to develop *Metarhizium*-based biopesticides for several other major canegrubs, southern one-year, negatoria and French's, building on work started in a previous SRDC-funded project, BSS134.

The three targeted canegrubs are all serious pests of sugarcane. French's canegrub is widespread in central and northern Queensland, and caused greater losses than greyback before the advent of suSCon Blue. Its counterpart in southern Queensland, negatoria canegrub, is the most widespread canegrub species in that region. Southern one-year canegrub occurs frequently on sandy soils in southern districts.

Promising results in BSS134 with BioCane isolate FI-1045 against southern one-year canegrub were followed through in the current project. BioCane is now registered against greyback, and another target pest could readily be included in its efficacy claim. This would also broaden the economic basis for BioCane production.

A major focus of the new project was isolate FI-147. This isolate is highly pathogenic to negatoria and French's canegrubs. BSS134 evaluated ratoon treatment for negatoria canegrub, with excellent results, but more data are needed for registration, particularly for longer time periods after application. Application in plant crops was assessed, because

this may be more effective than ratoon application and may be a preferred option for growers. French's canegrub was the subject of trials established late in BSS134, and was an important target in the new project.

Some canefields in certain districts may be subject to infestation by more than one canegrub species. In northern and central Queensland, greyback and French's canegrub can occur together, and in southern Queensland, negatoria and southern one-year canegrubs sometimes occur together on sandy loams. No single isolate of *Metarhizium* will control all of these species, but a combination of FI-147 and FI-1045 may control all four. The economics of a combined product was investigated for these situations.

Expanded use of *Metarhizium* in plant crops required an investigation of the possible effect of planting fungicides and insecticides on spore viability. There is a risk that these chemicals will adversely affect *Metarhizium*, but their possible impact on field efficacy under realistic conditions of use has not been studied.

2.0 OBJECTIVES

This project addressed the issue of canegrub management, and its heavy or sole dependence on a single chemical insecticide (controlled-release chlorpyrifos, ie suSCon Blue and suSCon Plus). The industry would be in a disastrous situation if these products failed, and alternative management systems must be evaluated and made available for major canegrub species.

Objectives of the project were to:

- extend the registration of the existing commercial isolate FI-1045 (BioCane) to include southern one-year canegrub;
- complete efficacy testing and initiate registration of a product based on isolate FI-147 for control of French's and negatoria canegrubs;
- investigate the economics of using a combined product containing both isolates in situations where mixed infestations of canegrubs occur;.
- define the conditions for successful application of *Metarhizium*, including compatibility with planting fungicides.

Achievement of these objectives has been as follows:

- Additional data have been collected that demonstrate the efficacy of FI-1045 against southern one-year canegrub (Section 7.0), but more data would be desirable for registration, particularly for second ratoon crops. There are seven trials established in 2000 or 2001 that may produce the data required, and these will continue to be monitored using BSES resources. The possibility of seeking registration for BioCane against southern one-year canegrub will be reconsidered after harvest results are collected for two infested trials later in 2002.
- Insufficient results have been collected to support the registration of FI-147 (Sections 9.0 and 10.0), despite the establishment of a large number of trials, due to the infrequent and unpredictable occurrence of negatoria and French's canegrubs. However, the data that are available indicate that the isolate should be effective. The collection of additional data remains problematic, but established trials that are potentially useful will be monitored for at least one more year.

- There are also insufficient results to judge the effectiveness of a combination of isolates.
- The viability of *Metarhizium* spores and their infectivity to canegrubs were unaffected by a range of planting insecticides and fungicides at commercial application rates (Section 6.0). BioCane can therefore be safely applied at planting, and a label change to allow this use has been drafted with Bio-Care Technology Pty Ltd.

3.0 RECOMMENDATIONS FOR FURTHER R,D&E

1. Continue to sample efficacy trials that are still in place and that are capable of producing useful results ie seven against southern one-year canegrub, one against Nambour canegrub, three against negatoria canegrub in southern Queensland, and seven against negatoria or French's canegrubs in central Queensland.

2. Conduct further research on the behaviour of Childers canegrub, to identify factors that limit the effectiveness of *Metarhizium* and other products.

3. Investigate the mechanisms of the infection process and host response, using greyback, negatoria, southern one-year and Childers canegrubs as examples of canegrub types that are susceptible to different groups of *Metarhizium* isolates (see Holdom and Li 1996, and our results), with a view to improving virulence.

4. Study the genome of *Metarhizium* isolates with a view to improving strains to give faster kill at the same or lower rates of application, developing multi-purpose strains, and perhaps identifying genes that could be used in genetically modified sugarcane.

4.0 PUBLICATIONS

Milner, R.J. and Samson, P.R. (2000) The development of *Metarhizium*-based biopesticides for use against sugarcane whitegrubs in Australia. *XXI International Congress of Entomology, Iguassu Falls, Brazil, 20-26 August.*

Samson, P., Robertson, L., Bakker, P., Cocco, R., Horsfield, A., Logan, D., Kettle, C., Harris, W., Allsopp, P., McGill, N., Milner, R. and Bullard, G. (2001) Development of *Metarhizium*-based biopesticides for use against sugarcane whitegrubs in Australia. *Proc. Int. Soc. Sugar Cane Technol*. 24: 354-360.

Milner, R.J., Samson, P.R. and Bullard, G.K. (2002) FI-1045: a profile of a commercially useful isolate of *Metarhizium anisopliae* var. *anisopliae*. *BioControl Sci. Technol*. 12: 43-58.

5.0 GENERAL METHODOLOGY

The effect on *Metarhizium* of insecticides and fungicides used at planting was assessed in laboratory and field experiments. This information was used to develop recommendations for *Metarhizium* use.

Efficacy of *Metarhizium* isolates FI-1045 or FI-147 against target canegrubs was measured as both grub numbers and crop yield. Spore levels were monitored in trials, to ensure that the product had been applied correctly and was surviving in soil, and as additional data for registration.

The canegrubs and crop ages targeted with each isolate were:
- southern one-year canegrub with FI-1045 applied in plant crops;
- negatoria canegrub with FI-147 applied in plant and young ratoon crops;
- French's canegrub with FI-147 applied in plant and young ratoon crops.

These were identified within BSS134 as targets with a good chance of success. *Metarhizium* was applied in high-risk crops before significant pest infestations established, because research in BSS134 showed that *Metarhizium* acts too slowly to prevent damage by grubs present at the time of application (Samson *et al.* 1997).

Most new trials included the selected *Metarhizium* isolate at three application rates, 2.5, 5 and 10 g/m of row, the standard insecticide if one was available, and untreated controls. In addition, a 1:1 mixture of the two isolates FI-147 and FI-1045 was tested at application rates of 5 and 10 g of combined product/m.

Product was checked for spore content at the time of production and again just before application. Spore levels in soil were measured shortly after application, after 6 months, and annually thereafter. A plastic marker was included in one plot from each treatment to aid in locating the treated band for spore sampling. Numbers of grubs were assessed annually and crop yields measured in infested trials. Grubs from trials in central Queensland were identified as either French's or negatoria canegrubs by a genetic test (restriction fragment length polymorphism, RFLP).

Some additional research was carried out to assess the potential for *Metarhizium* against several other canegrubs (Nambour, Childers, noxia), and to support current BioCane use.

6.0 EFFECT OF CHEMICALS ON *METARHIZIUM* PERSISTENCE

Metarhizium is currently not recommended for application to sugarcane at the time of planting, and instead it is applied during fill-in of the planting furrows. In part, this is to avoid contact between *Metarhizium* spores and chemicals applied during the planting operation that may harm spore viability. Application of BioCane after planting is a standard and desirable procedure in the Burdekin region where setts are covered by a considerable depth of soil when the final row profile is achieved. However, this requirement will limit *Metarhizium* acceptability in other regions where it may be difficult to obtain adequate soil cover over granules applied at fill-in.

Pesticides currently registered for application during planting are eight fungicides against pineapple disease, methoxy ethyl mercuric chloride (Shirtan®), flusilazole (Cane Strike), prochloraz (Sportak®, Protak®), propiconazole (Cane Sett Treatment, Bumper®, Tilt®), triadimenol (Bayfidan®, Tridim®), triadimefon (Triadimefon®, Triad®), carbendazim (Carbendazim®, Bavistin®, Spin Flo®) and benomyl (Benlate®); three liquid insecticides

against wireworms, chlorpyrifos (Lorsban®, etc), bifenthrin (Talstar®) and fipronil (Regent®), as well as controlled-release canegrub treatments containing chlorpyrifos (eg suSCon Blue). The fungicides are likely to be of greatest concern to *Metarhizium* viability. Virtually all cane is treated with fungicide at planting.

This section of the report describes experiments to define the risk of at-planting chemicals harming *Metarhizium* efficacy, with a view to extending the use recommendations to include application at-planting.

6.1 Chemicals and growth on media

Seven chemicals were tested for their ability to inhibit the growth of two isolates of *Metarhizium*, FI-1045 and FI-147, *in vitro*. Two preliminary experiments showed that doses equivalent to those in the spray tank were universally inhibitory. Consequently this experiment was undertaken using a wide range of doses and using the same doses for all chemicals tested. The doses were chosen based on published results (eg Pung *et al*. 1993): 0.2, 2, 20 and 200 mg ai kg^{-1}. Chemicals were evaluated together except for Regent, which was tested later. The medium used was Sabouraud's Dextrose Agar (SDA). A double concentration of the agar medium was prepared and autoclaved and, before cooling, was mixed in a 1:1 ratio with a double concentration of the chemical. The mixture was then poured into 9 cm Petri dishes and allowed to dry. The plates were then inoculated in the centre with a single 1 µL droplet of a 10^8 spores/mL suspension of FI-1045 or FI-147. Three plates were inoculated per treatment with three plates of controls on SDA plates. All plates were incubated at 25°C and the colony diameters measured after 5-6 days and again after 10 or 13 days; only the first results are presented here. The percentage inhibition was determined by expressing the size of the colonies on the chemical plates as a percentage of the size of the control colonies.

Table 1
Percentage inhibition of radial growth of *Metarhizium* isolates after 5-6 d at 25°C, with different pesticides incorporated in the medium.

Product	Isolate	Dose (mg ai kg^{-1})			
		0.2	**2**	**20**	**200**
Lorsban	FI-147	0	0	29	41
	FI-1045	0	0	10	48
Talstar	FI-147	0	0	8	37
	FI-1045	0	0	0	41
Regent	FI-147	0	0	0	59
	FI-1045	0	0	22	53
Shirtan	FI-147	0	59	100	100
	FI-1045	0	52	100	100
Cane Strike	FI-147	72	100	100	100
	FI-1045	62	84	100	100
Sportak	FI-147	74	100	100	100
	FI-1045	58	79	100	100
Tilt	FI-147	8	60	100	100
	FI-1045	0	16	47	79

Toxicity of Cane Strike and Sportak was about 10 times that of Shirtan and Tilt (Table 1). Toxicity of the latter fungicides was about 100 times that of the three insecticides Lorsban, Talstar and Regent. In field application, expected amounts of active ingredient applied per 100 m are: Lorsban, 11.25 g; Talstar, 0.56 g; Regent, 0.22; Shirtan, 0.90 g; Cane Strike, 0.012 g; Sportak, 0.54 g; Tilt, 0.30 g (from Table 2, assuming fungicide use is 400 L/ha). Therefore, the expected order of toxicity at field rates (with toxicity relative to that of Regent in brackets) is Regent (1) < Talstar (2.5) < Lorsban (51) < Cane Strike (53) < Tilt (137) < Shirtan (407) < Sportak (2,444).

6.2 Field trial of *Metarhizium* with planting chemicals

The chemicals tested in a field trial were the four most popular at-planting fungicides, Shirtan, Cane Strike, Sportak and Tilt (the same active ingredient as Cane Sett Treatment), and two wireworm treatments, Lorsban and Talstar. Two controls were included, ie *Metarhizium* alone, at the start and end of application. The trial design was eight treatments of two isolates with four replicates, ie 64 plots, in a randomised complete block design, with each plot measuring one row x 10 m.

Experimental details are given in Table 2 (note Regent was not included). Fungicide registered rates are in mL/100 L in the tank, whereas insecticide rates (Lorsban, Talstar) are in mL/ha or mL/100 m of row. In the trial, the spray rate was 2.2 L/100 m. Assuming 6,667 row m/ha, this is equivalent to 147 L/ha; this is similar to or slightly higher than the recommended rate for insecticides (eg Talstar notes suggest 60-100 L/ha), but much lower than the possible use of diluted fungicide per hectare of up to 1,000 L. We focused on the rate of product per unit area rather than the concentration in the tank. For the fungicides, we assumed that 1,000 L would be used per hectare in commercial practice, which would be an extremely high use rate; we then calculated the amount of product in that 1,000 L and used that in our trials. As our spray rate was only 147 L/ha, the fungicides were much more concentrated in our tank than they would be in practice. For the insecticides, we aimed to get five times the registered rate per hectare.

Table 2
EC01-7 (Compatibility of *Metarhizium* isolates with planting chemicals) - experimental details

Product	ai g/L	Registered product rate		Target rate	Tank conc.
		mL/100 L	mL/100 m row	mL/100 m row[a]	mL/100 L
Lorsban	500		22.5	112.5	5114
Talstar	100		5.6	28.1	1277
Regent	200		1.1	na	na
Shirtan	120	125		18.7	850
Cane Strike	1.6	125		18.7	850
Sportak	450	20		3	136
Tilt	250	20		3	136

[a] Measured use of spray was 15% over target.

Open furrows were made in cultivated ground with a commercial planter, and the base of each furrow was covered with a coloured plastic marker. *Metarhizium* was applied at about 10 g/m (11.6 g/m FI-147 and 9.6 g/m FI-1045), oversprayed with each treatment in

a band about 16 cm wide and then covered immediately with soil in a single operation, with the *Metarhizium* applicator, sprayer and covering tines all mounted on a single implement. Extra soil was added in a second operation. Initial cover was 10-12 cm of loose soil, and more soil was then added to give about 18 cm of cover. The soil surface temperature was about 17°C at the start of application at 8.30 am and about 32°C at completion at noon. Treatments were applied on 31 May 2001.

Metarhizium-coated granules (rice or bentonite) were collected on 13 June 2001, with five granules collected from each of two holes in each plot and transferred to 1 mL of 0.02% Teric® (surfactant) water. Soil samples were collected on 12 July 2001, by taking soil from the treated zone (indicated by plastic marker) for a length of 1.2 m; samples were sieved, mixed and subsampled for counting of colony-forming units. Remaining soil was stored at 10°C. These retained samples were subsequently bioassayed, those containing FI-147 with negatoria canegrubs starting 10 December 2001 and those containing FI-1045 with greyback canegrubs starting 28 February 2002. Soil was weighed into plastic cups, 140 g/cup, with 10 cups per plot, and one grub was added to each cup. Cups were stored at 25°C. Grubs were fed carrot and checked at 11, 28 and 42 d for overt infection or death without symptoms. The angular transformation was applied to the proportions responding and means of the transformed values were compared by the least significant difference test after analysis of variance.

For spore viability (Figure 1), there was a significant difference between treatments (P = 0.003), with the mean for Shirtan of 69% significantly lower than for every other treatment (mean = 82%). There was no significant difference between isolates (P = 0.051) and no significant interaction between isolate and treatment (P = 0.090).

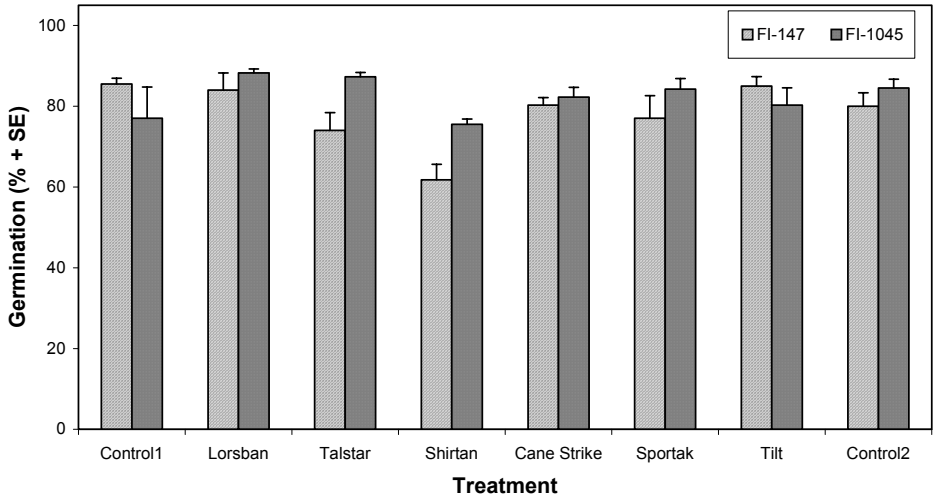


Figure 1. Spore viability on granules sprayed with different insecticides or fungicides (see Table 2 for application rates).

For spore concentrations (Figure 2), there was no significant difference between isolates (P = 0.17) or chemical treatments (P = 0.66), and there was no significant interaction between isolate and treatment (P = 0.74). These data were more variable than the viability data (compare with Figure 1), due to the greater sampling error.

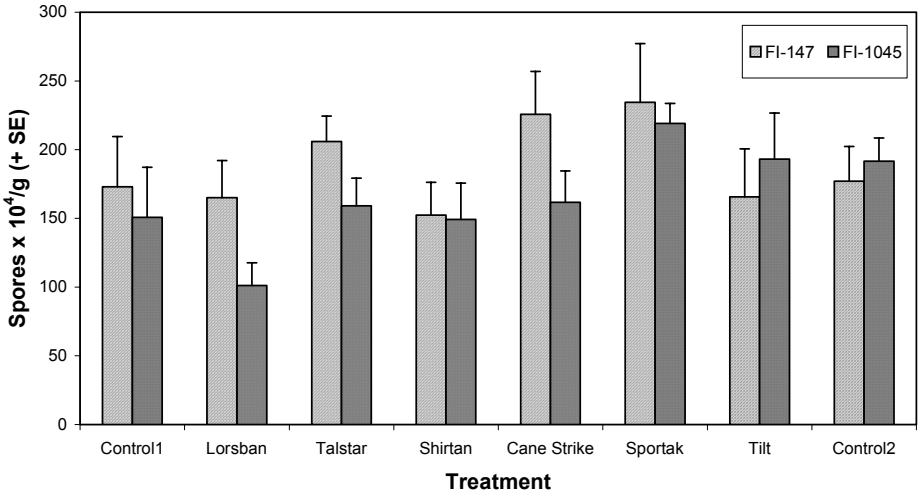


Figure 2. Spore concentrations in soil treated with FI-147 or FI-1045 granules and oversprayed with different insecticides or fungicides (see Table 2 for application rates)

Mortality of negatoria canegrubs confined in soil differed significantly between treatments at 11 and 28 d ($P < 0.001$) but not at 42 d ($P = 0.11$) (Table 3). The early difference was mainly due to different responses in the two insecticide treatments compared with the fungicide treatments and the control; Lorsban and Talstar produced high mortality of grubs at both 11 d and 28 d. If the insecticide treatments were omitted from the analysis, a significant difference between the remaining treatments was detected only for total mortality after 11 d ($P = 0.006$), with mortality in the Cane Strike treatment being higher than in all others. Overt infection differed significantly between treatments on all occasions ($P < 0.001$). Again this was due mainly to different responses with insecticides; Talstar produced high overt infection of grubs as early as 11 d, while in the Lorsban treatment most grubs died with no pathogenic symptoms (Table 3). In the Talstar treatment, most of the grubs classed as 'infected' at 11 d (19 of 27) were hard but without visible sporulation, which became evident later. If the two insecticide treatments were omitted, then overt infection did not differ significantly between the remaining treatments on any occasion ($P = 0.13$, 0.56 and 0.60 at 11, 28 and 42 d, respectively).

Mortality of greyback canegrubs differed significantly between treatments on all occasions ($P < 0.001$) (Table 3). The difference was again mainly due to high mortality in the two insecticide treatments compared with the fungicide treatments and the control. If the insecticide treatments were omitted from the analyses, there was no significant difference between the remaining treatments ($P = 0.81$, 0.80 and 0.53 at 11, 28 and 42 d, respectively). Overt infection differed significantly between treatments on all occasions ($P < 0.001$). This was also due mainly to different responses with insecticides; Talstar produced high overt infection of grubs as early as 11 d, while in the Lorsban treatment all grubs died with no pathogenic symptoms (Table 3). If the two insecticide treatments were omitted, then overt infection did not differ significantly between the remaining treatments on any occasion ($P = 0.063$, 0.56 and 0.051 at 11, 28 and 42 d, respectively). In this bioassay, many greyback grubs became hard initially, indicating *Metarhizium* infection,

but for unknown reasons did not subsequently produce external spores – these individuals were still classed as exhibiting overt infection in analyses.

Table 3
Response of negatoria and greyback canegrubs in soil treated with FI-147 or FI-1045 granules and oversprayed with different insecticides or fungicides.

Days	Grub response (% ± SE)						
	Control 1	Lorsban	Talstar	Shirtan	Cane Strike	Sportak	Tilt
Negatoria/FI-147							
Total mortality							
11	0 ± 0 d	100 ± 0 a	83 ± 9 b	5 ± 3 d	20 ± 4 c	0 ± 0 d	5 ± 3 d
28	80 ± 6 bc	100 ± 0 a	100 ± 0 a	65 ± 9 c	85 ± 3 b	75 ± 3 bc	80 ± 7 bc
42	97 ± 3 a	100 ± 0 a	100 ± 0 a	90 ± 4 a	100 ± 0 a	98 ± 3 a	95 ± 5 a
Overt infection							
11	0 ± 0 d	18 ± 3 b	68 ± 9 a	5 ± 3 cd	13 ± 5 bc	0 ± 0 d	5 ± 3 cd
28	80 ± 6 a	18 ± 3 b	83 ± 9 a	65 ± 9 a	78 ± 3 a	75 ± 3 a	80 ± 7 a
42	93 ± 3 a	18 ± 3 b	83 ± 9 a	90 ± 4 a	93 ± 3 a	98 ± 3 a	95 ± 5 a
Greyback/FI-1045							
Total mortality							
11	8 ± 8 c	100 ± 0 a	43 ± 6 b	5 ± 3 c	3 ± 3 c	3 ± 3 c	0 ± 0 c
28	43 ± 13 b	100 ± 0 a	100 ± 0 a	55 ± 14 b	53 ± 10 b	63 ± 13 b	48 ± 3 b
42	65 ± 10 b	100 ± 0 a	100 ± 0 a	73 ± 3 b	73 ± 8 b	78 ± 6 b	83 ± 3 b
Overt infection							
11	0 ± 0 c	0 ± 0 c	33 ± 3 a	5 ± 3 b	0 ± 0 c	0 ± 0 c	0 ± 0 c
28	33 ± 6 b	0 ± 0 c	83 ± 11 a	48 ± 10 b	45 ± 12 b	55 ± 10 b	48 ± 3 b
42	55 ± 6 c	0 ± 0 d	83 ± 11 a	63 ± 5 bc	63 ± 8 bc	68 ± 5 bc	83 ± 3 ab

Mortality of negatoria in untreated soil was zero at 11 d and 3 ± 3% at 28 and 42 d, respectively, with zero infection at all times. Corresponding mortality figures for greyback were zero at 28 d and 3 ± 3% at 42 d, with no infection. Means in rows followed by the same letter were not significantly different ($P = 0.05$).

6.3 Survey of planters

BioCane FI-1045 was applied between planting boards on a range of planters during commercial planting operations, at Mackay in 2001 or Bundaberg in 2002 (Table 5). Granules were applied to two 10 m lengths of row in each of two adjacent rows. Controls with no fungicide were represented by BioCane granules buried beyond the ends of rows to the same depth as the granules in the row. Details were obtained from the farmer/contractor on fungicides and any other chemicals applied on the planter, and rates of these as the dilution in the tank and total tank use per hectare. Granules were retrieved 24-42 d later; five granules were collected from each of two holes in each treated row and combined, or from the untreated holes, and placed in 1 mL Teric water; we also placed 10 granules from a non-applied sample (ie retained in container and refrigerated) into Teric water.

There was no significant reduction in viability of spores in any of nine trials (Table 5). The confidence intervals of the difference between granules exposed or not exposed to

fungicide indicated no serious impairment in any trial. Spore viabilities in soil were high, and at least as high as the viability of spores on non-applied granules. In several trials in 2002, the viability of non-applied granules was very poor, indicating deterioration of the granules during refrigerated storage. Granules retrieved from fields in 2002 were whitish and seemed to have few adhering spores, suggesting that most spores had sloughed off into the soil since application.

6.4 Relevance of viability measurements on retrieved granules

It is possible that apparent viability of spores on granules retrieved from soil may not be a reliable indicator of spore mortality, because dead spores may be lost from granules before retrieval or may be much more difficult to see in the laboratory germination test than healthy spores. The observation that granules recovered from some commercial planting operations had lost most of their spores (see above) increased this concern, because it is possible that non-viable spores may be lost more quickly than healthy ones.

To test the relevance of the germination test, samples of BioCane granules were heated at in an oven 45-47°C for 80 or 150 minutes, to kill a proportion of spores, and the treated granules then buried in a plant and a ratoon cane field at Mackay on 20 May 2002. Three samples each of 10 granules from each heat treatment were tested for germination before burial. Granules were retrieved on 11 and 24 June, three samples per treatment with ten granules per sample, and germination measured. Unreplicated samples of treated granules that had been stored refrigerated were tested at the same times.

Table 4
Apparent viability of spores on granules retrieved from a plant or a ratoon canefield after burial following pre-heating for either 80 or 150 minutes.

Period of heating (minutes)	Treatment	Germination (% ± SE) at times after burial		
		0 d	22 d	35 d
80 min	Refrigerated	10 ± 3	17	10
	Plant cane		34 ± 3	25 ± 5
	Ratoon cane		37 ± 10	38 ± 5
150 min	Refrigerated	10 ± 3	12	7
	Plant cane		29 ± 5	25 ± 1
	Ratoon cane		47 ± 3	26 ± 3

Heating reduced the viability of spores to about 10% (Table 4). Germination of spores on retrieved granules was better than on granules that had not been buried but assessed immediately after heat treatment or stored refrigerated (Table 4). Thus, measurements of germination of spores on retrieved granules overestimated viability, but effects of treatment were still apparent.

6.5 Conclusion

Although *in vitro* tests show the potential for harm from some chemicals, particularly the fungicides Sportak and Shirtan, this was not evidenced in the field. A label change for BioCane to be applied at planting has been agreed with Bio-Care Technology Pty Ltd, and a new label drafted.

Table 5
Viability of spores on BioCane granules retrieved from soil after application through different types of commercial planters.

Type of planter	Trial	Date	Cover (cm)	Temp (°C)[a]	mL/100 L	L/ha	Viability (%)			*P* [b]	95% CI of difference
							Granules	Untreated	Treated		
Shirtan 125 mL/100 L											
Stick spray	A	29/08/2001	10	22	170	250	83	96	93	0.26	-9, 3
	D	5/09/2001	10	22	110	560	88	89	89	0.98	-16, 16
Billet spray	G	19/03/2002	6	31	125	< 900	4	86	84	0.83	-19, 16
Billet dip	I	19/03/2002	10	30	100	1000	na	80	82	0.59	-7, 12
	J [c]	20/03/2002	12	27	140	720	na	85	87	0.37	-4, 9
Sportak 20 mL/100 L											
Stick spray	B	30/08/2001	10	22	20	370	85	88	94	0.018	1, 10
Cane Sett Treatment or Tilt 20 mL/100 L											
Stick spray	M	21/03/2002	5	32	20	250	14	85	84	0.84	-13, 11
Billet spray	E	5/09/2001	8	25	20	370	91	84	95	0.1	-3, 26
Billet dip	F	11/09/2001	6	na	30	300	88	89	86	0.16	-8, 2

[a] At granule level 10 min after planting.
[b] Treated against untreated by *t*-test.
[c] Combined dip and spray.

7.0 TRIALS AGAINST SOUTHERN ONE-YEAR CANEGRUB

7.1 1997 plant cane trials (ES97-6, -7, -10)

Three trials were established against southern one-year canegrub in plant cane in late 1997 as part of BSS134. They continued to be monitored under BSS246.

All trials used FI-1045 only, at four application rates, 5, 7.5, 10 and 25 g product/m, as well as suSCon Blue at 3.2 g/m (21 kg/ha). Products were applied in the half-open drill and covered with soil. In ES97-10, target rates of FI-1045 of 10 and 25 g/m were also applied using paired coulters, to compare efficacy of the two application techniques. Each treatment was applied to plots measuring five rows by 12 or 13 m, and was replicated five times in randomised complete-block designs.

Two trials were sufficiently infested in the third ratoon early in 2001 to justify sampling.

Table 6
Numbers of southern one-year canegrubs in ES97-6 in March 2001 and harvest yield of the third ratoon on 10 July 2001 (mean ± SE), after treatment of plant cane in September 1997.

Variable	FI-1045 g/m				suSCon	Control
	5	7.5	10	25	21 kg/ha	
Grubs/stool	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	0.8 ± 0.3	1.2 ± 0.3	1.2 ± 0.4
Cane (t/ha)	77 ± 4	80 ± 3	72 ± 3	77 ± 4	72 ± 3	72 ± 3

Numbers of grubs were low in ES97-6 and there was no significant difference between treatments (Table 6, $P = 0.61$). There was also no significant difference in grub numbers between the treated plots taken together and untreated plots ($P = 0.34$). At harvest, there was no significant difference among the full set of treatments for cane yield (Table 6, $P = 0.18$), nor was there any significant difference in mean yield between treated plots and untreated controls ($P = 0.23$) or between *Metarhizium*- and suSCon-treated plots ($P = 0.13$).

Table 7
Numbers of southern one-year canegrubs in ES97-10 in March 2001 and harvest results in third ratoons on 17 September 2001 (mean ± SE), after treatment of plant cane in the drill in October 1997 or by coulters in December 1997.

Variable	FI-1045 in drill, g/m				FI-1045 by coulters, g/m		suSCon	Control
	5	7.5	10	25	10	26	21 kg/ha	
Grubs/stool	0.7±0.2	0.8±0.3	0.4±0.2	1.0±0.3	0.9±0.2	0.7±0.2	0.5±0.2	2.0±0.5
Cane (t/ha)	58 ± 6	61 ± 8	66 ± 5	61 ± 7	52 ± 7	66 ± 7	66 ± 8	50 ± 6

Numbers of grubs were also low in ES97-6 and there was no significant difference between treatments (Table 7, $P = 0.06$). However, the mean number of grubs in all treated plots was significantly lower than the number in the controls ($P = 0.002$). This is surprising for the coulter treatments, because this application method had not reduced

grub numbers by March in a previous trial (ES96-15, BSS134). At harvest, there was no significant difference among the full set of treatments for cane yield (Table 7, $P = 0.14$). However, mean yield in treated plots was significantly greater than in control plots ($P = 0.037$), following the pattern of grub numbers above. There was no significant difference between mean yields of *Metarhizium*- and suSCon-treated plots ($P = 0.30$). Mean yields of plots treated with *Metarhizium* in the drill and by coulters were significantly different at 10 g/m ($P = 0.044$) but not at 25-26 g/m ($P = 0.52$).

Good spore concentrations were present in the half-open drill treatments in each trial over three year's monitoring (Tables 8-10). The levels in plots treated with coulters in ES97-10 are apparently much too low (Table 10), but there have been problems finding the marker in these treatments.

Table 8
Concentration of spores (x 10^4)/g of wet soil at different times after treatment in ES97-6

Trt.	FI-1045	3 week		6 month		1 year		2 year		3 year	
	g/m	a	b	a	b	a	b	a	b	a	b
1	5	19	25	5.0	6.3	10	11	16	28	40	17
2	7.5	14	19	20	11	8.6	25	42	26	11	22
3	10	23	16	16	27	33	30	33	41	14	13
4	25	40	32	38	27	130	59	76	107	36	39
6	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		

(a and b are duplicate determinations from a single soil sample)

Table 9
Concentration of spores (x 10^4)/g of wet soil at different times after treatment in ES97-7

Trt	FI-1045	3 week		6 month		1 year		2 year		3 year	
	g/m	a	b	a	b	a	b	a	b	a	b
1	5	68	78	20	16	9.7	9.0	16	17	5.3	6.6
2	7.5	13	12	17	17	11	10	15	20	2.6	4.6
3	10	60	70	13	33	5.7	8.3	11	15	4.0	9.3
4	25	154	179	84	84	65	31	55	51	19	25
6	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 10
Concentration of spores (x 10^4)/g of wet soil at different times after treatment in ES97-10

Trt.	FI-1045	3 week		6 month		1 year		2 year		3 year	
	g/m	a	b	a	b	a	b	a	b	a	b
1	5	25	29	12	19	34	28	20	16	27	18
2	7.5	40	41	25	10	27	16	50	56	33	25
3	10	59	38	39	99	36	19	25	31	19	29
4	25	84	32	92	133	23	28	103	63	53	39
5	10.5 [a]	48	49	16	13	24	24	18	18	6.7	8.7
6	26.2 [a]	178	169	39	47	0.3	1.3	17	27	8.7	15.5
8	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] coulter.

7.2 1999 plant cane trials (ES99-4 and ES99-6)

Two trials were established against southern one-year canegrub in August 1999 in plant cane.

Each trial included FI-1045 as the primary isolate. ES99-4 also included two treatments containing FI-1045 and FI-147 in a 1:1 mix (w/w) to investigate the economics of a two-isolate preparation for mixed infestations. We used batches M156-158 of FI-1045 and batches M166 and M167 of FI-147, all with approximately 3.0 x 10^9 spores/g. Each treatment was applied to plots measuring four rows by 15 m or five rows by 12 m, and was replicated five or six times in randomised complete-block designs.

Setts were uncovered shortly after planting and product was applied by hand into the furrow. The suSCon Blue was applied by hand at 3.2 g/m of row. Granules were covered with soil immediately.

Numbers of southern one-year canegrubs in ES99-6 were reduced by FI-1045 at 5 and 10 g/m and by suSCon Blue in early 2001 in comparison with untreated plots (Table 11). There was no significant difference in grub numbers between rates of FI-1045 or suSCon Blue. However, some damage was evident in cane outside the trial that had been treated with FI-1045 at 10 g/m. Soil samples were taken from this outside area on 3 May 2001 and spore concentrations were satisfactory (101 x 10^4/g); this area was sampled again in October 2001 (two years after application) and counts were high (58 x 10^4/g).

Picticollis grubs were also present in EC99-6, and were apparently not affected by treatment; there was no significant difference between individual treatments (Table 11) or between the treated plots taken together and untreated controls (P = 0.10). However, picticollis numbers were probably too low to provide a satisfactory test.

Table 11
Numbers of southern one-year and picticollis canegrubs in ES99-6 in February and April 2001 and cane yield in the first ratoon on 25 July 2001 (mean ± SE), after treatment of plant cane in August 1999.

Grubs/stool or yield	FI-1045		suSCon	Control	*P*
	5 g/m	10 g/m	21 kg/ha		
Southern 1-year					
27/2/2001	0.4 ± 0.1 b	0.5 ± 0.2 b	0.4 ± 0.1 b	2.0 ± 0.6 a	0.034
12/4/2001	0.6 ± 0.1 b	0.3 ± 0.1 b	0.6 ± 0.2 b	2.1 ± 0.5 a	0.003
Picticollis					
27/2/2001	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.40
12/4/2001	1.1 ± 0.3	0.3 ± 0.1	0.3 ± 0.2	0.9 ± 0.3	0.10
Cane (t/ha)	48 ± 7	52 ± 5	53 ± 6	40 ± 9	0.38

Means in rows followed by the same letter were not significantly different (P = 0.05).

At harvest in 2001, there was no significant difference among the full set of treatments for cane yield (Table 11), nor was there any significant difference in mean yield between

treated plots and untreated controls (P = 0.11) or between *Metarhizium*- and suSCon-treated plots (P = 0.70).

Spore concentrations were satisfactory up to two years after treatment (Tables 12-13).

Table 12
Concentration of FI-1045 spores (x 10^4)/g of wet soil at different times after treatment in ES99-4

Trt.	FI-1045 g/m	3 week		6 month		1 year		2 year	
		a	b	a	b	a	b	a	b
1	2.5	11	10	32	43	6.6	8.6	9.3	9.7
2	5	3.3	3.0	21	23	6.6	5.6	6.7	8.3
3	10	21	19	45	75	14	20	32	27
4	2.5*	10 (6.0)	9.6 (5.7)	10 (8.3)	9.0 (9.0)	14 (7.3)	12 (7.3)	7.3 (4.0)	5.0 (2.7)
5	5*	20 (20)	20 (20)	23 (26)	23 (21)	23 (10)	21 (6.6)	16 (3.0)	18 (3.3)
7	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3

*Plus an equal amount of FI-147.
(for treatments 4 and 5, concentrations in brackets are FI-147)

Table 13
Concentration of spores (x 10^4)/g of wet soil at different times after treatment in ES99-6

Trt.	FI-1045 g/m	3 week		6 month		1 year		2 year	
		a	b	a	b	a	b	a	b
1	5	11	13	25	22	15	21	30	31
2	10	45	56	69	63	35	34	45	44
4	0	0.0	1.0	0.0	0.0	0.0	1.0	0.0	0.0

7.3 2000 plant cane trials (ES00-3, -8)

Two trials were established against southern one-year canegrub in plant cane in 2000, in March and August.

FI-1045 was the primary isolate, plus two treatments containing FI-1045 and FI-147 in a 1:1 mix (w/w). In ES00-3 we used batch M170 of FI-1045 and batch M173 of FI-147, with 3.0-3.1 x 10^9 spores/g in original pouches and with 500 g bentonite subsequently added to each 2.5 kg pouch. In ES00-8 we used batch M185 of FI-1045 and batch M186 of FI-147, with 3.7 and 3.2 x 10^9 spores/g, respectively, before addition of bentonite. Each treatment was applied to plots measuring four rows by 18 m in ES00-3, and five rows by 13 m in ES00-8, and was replicated five times in randomised complete-block designs.

Setts were uncovered shortly after planting and product was applied by hand into the furrow. The suSCon Blue was applied by hand at 3.15 g/m of row. Granules were covered with soil immediately.

One trial was infested in the first ratoon in 2002. Although there was no significant effect of treatments on grub numbers in the overall analysis of variance (Table 14, P = 0.20), the mean number in all treated plots was significantly lower than the number in controls (P = 0.029).

Table 14
Numbers of southern-one year canegrubs (mean ± SE) in ES00-8 in the first ratoon in April 2002, after treatment of plant cane in August 2000.

	FI-1045 g/m			FI-1045+FI-147 g/m		suSCon	Control
	2.5	5	10	2.5+2.5	5+5	21 kg/ha	
Grubs/stool	1.8±0.4	1.5±0.4	1.1±0.2	1.4±0.3	2.7±0.7	0.8±0.2	3.2±0.8

Spore counts were satisfactory (Tables 15, 16).

Table 15
Concentration of FI-1045 spores (x 10^4)/g of wet soil at different times after treatment in ES00-3

Trt.	FI-1045 g/m	3 week		6 month		1 year		2 year	
		a	b	a	b	a	b	a	b
1	2.5	80	87	15	14	12	10	6.0	8.0
2	5	34	36	26	28	13	11	12	15
3	10	194	172	58	58	33	30	73	57
4	2.5*	57 (50)	60 (54)	14 tot	27 tot	56 tot	43 tot	25 (13)	22 (10)
5	5*	82 (97)	123 (82)	54 tot	66 tot	39 tot	34 tot	15 (11)	23 (18)
7	0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0

*Plus an equal amount of FI-147.
(for treatments 4 and 5, concentrations in brackets are FI-147, or a total (tot) level of both isolates is given)

Table 16
Concentration of FI-1045 spores (x 10^4)/g of wet soil at different times after treatment in ES00-8

Trt.	FI-1045 g/m	3 week		6 month		1 year	
		a	b	a	b	a	b
1	2.5	27	29	na	na	13	14
2	5	13	18	na	na	47	55
3	10	47	64	na	na	100	81
4	2.5*	60 tot	na	na	na	13 tot	32 tot
5	5*	39 tot	na	na	na	52 tot	60 tot
7	0	0.0	0.0	na	na	0.0	0.0

*Plus an equal amount of FI-147.
(for treatments 4 and 5, a total (tot) level of both isolates is given)

7.4 2001 plant cane trials (ES01-4, -5, -6, -7)

Four trials were established against southern one-year canegrub in plant cane in August-September 2001.

FI-1045 was the only isolate used, at two rates of 5 and 10 g/m. We used batches M203 in ES01-4, M204 in ES01-5 and EC01-6, and both M204 and M206 in ES01-7 in separate replicates. Spore levels were 2.5-2.6 x 10^9/g in original material with 500 g bentonite subsequently added to each 2.5 kg pouch. Each treatment was applied to plots measuring 3-5 rows by 14-25 m (70-75 m total), and was replicated six to eight times in randomised complete-block designs.

Setts were uncovered shortly after planting and product was applied by hand into the furrow in a band 20-25 cm wide. The suSCon Plus was applied by hand at 6 g/m of row. Granules were covered with soil immediately.

There was a significant effect of treatment on numbers of southern one-year canegrubs in the plant crop in ES01-5 (P = 0.030). Grub numbers in the suSCon Plus plots were significantly lower than in untreated plots (Table 17). Only the higher rate of FI-1045 was sampled; grub numbers did not differ significantly from those in the controls.

Table 17
Numbers of southern-one year canegrubs (mean ± SE) in ES01-5 in March 2002, after treatment of plant cane in August 2001.

	FI-1045 10 g/m	suSCon Plus	Control
Grubs/stool	0.8 ± 0.2 ab	0.3 ± 0.1 b	1.2 ± 0.2 a

Means followed by the same letter were not significantly different (P = 0.05).

Numbers of southern one-year canegrubs in the plant crop in ES01-7 were significantly affected by treatment (P < 0.001). There were fewer grubs in plots treated with either rate of FI-1045 or with suSCon Plus than in untreated plots; there was no difference in grub numbers between *Metarhizium* and suSCon treatments (Table 18).

Table 18
Numbers of southern-one year canegrubs (mean ± SE) in ES01-7 in March 2002, after treatment of plant cane in September 2001

	FI-1045 5 g/m	FI-1045 10 g/m	suSCon Plus	Control
Grubs/stool	0.9 ± 0.2 b	0.5 ± 0.1 b	0.5 ± 0.1 b	1.7 ± 0.2 a

Means followed by the same letter were not significantly different (P = 0.05).

Spore counts were mostly satisfactory, including the two different batches in ES01-7, although counts in ES01-5 were lower than in the other trials.

Table 19
Concentration of spores (x 10^4)/g of wet soil at different times after treatment in ES01-4

Trt.	FI-1045 g/m	3 week a	b
1	5	20	25
2	10	31	27
4	0	0.0	0.0

Table 20

Concentration of spores (x 10^4)/g of wet soil at different times after treatment in ES01-5

Trt.	FI-1045 g/m	3 week a	b
1	5	9.3	9.6
2	10	7.3	21.0
4	0	0.0	0.0

Table 21
Concentration of spores (x 10^4)/g of wet soil at different times after treatment in ES01-6

Trt.	FI-1045 g/m	3 week a	b
1	5	67	50
2	10	63	77
4	0	0.0	0.0

Table 22
Concentration of spores (x 10^4)/g of wet soil at different times after treatment in ES01-7

Trt.	FI-1045 g/m	3 week a	b
1	5 M204	43	80
	5 M206	88	113
2	10 M204	70	96
	10 M206	141	146
4	0	0.0	0.0

8.0 TRIAL AGAINST NAMBOUR CANEGRUB

8.1 2000 plant cane trial (ES00-11)

One trial was established in plant cane in September 2000 against Nambour canegrub, a species closely related to southern one-year canegrub.

FI-1045 was the only isolate used, batch M185 containing 3.7 x 10^9 spores/g before addition of bentonite. Each treatment was applied to plots measuring five rows by 13 m, and was replicated five times in a randomised complete-block design.

Cane was planted with little or no soil cover. Product was applied by hand into the furrow in a band 22-25 cm wide, and suSCon Blue was similarly applied at 3.15 g/m of row. Granules were covered with soil immediately.

Spore counts were satisfactory (Table 23).

Table 23

Concentration of spores (x 10^4)/g of wet soil at different times after treatment in ES00-11

Trt.	FI-1045 g/m	3 week		6 month	
		a	b	a	b
1	2.5	14	30	7.7	10.3
2	5	27	32	42	33
3	10	69	68	69	66
5	0	0.0	0.0	0.3	0.0

9.0 TRIALS IN SOUTHERN QUEENSLAND AGAINST NEGATORIA CANEGRUB

9.1 1996 ratoon trial (ES96-30)

One trial was established against negatoria canegrub using coulters in ratoons in 1996 under project BSS134 and was still available for sampling in BSS246.

No grubs were found in February 2001.

High spore concentrations were still present three years after application (Table 24).

Table 24
Concentration of spores (x 10^4)/g of wet soil at different times after treatment in ES96-30

Trt.	FI-147 g/m	3 week		6 month		1 year		2 year		3 year	
		a	b	a	b	a	b	a	b	a	b
1	2.5	10	7.3	20	34	10	12	3.6	4.0	8.7	8.0
2	3.9	38	34	21	14	20	18	2.6	12	7.3	6.3
3	5.3	53	49	30	58	34	17	14	12	18	20
4	8.1	66	51	6.7	6.0	48	40	1.3	3.6	15	12
5	29.4	92	166	85	118	34	84	58	94	33	71
7	0	0.6	0.0	0.0	0.0	0.0	0.3	0.6	0.3	0.0	0.0

9.2 1998 plant cane trial (ES98-11)

One trial was successfully established against negatoria canegrub in November 1998 in plant cane.

The trial used batch M142 of FI-147, with a spore content of 3.6 x 10^9 spores/g. Each treatment was applied to plots measuring four rows by 20 m long, and was replicated six times in a randomised complete-block design.

Soil was removed to sett level just after planting and treatments were applied, including suSCon Blue at 3.2 g/m (21 kg/ha).

Spore levels were satisfactory up to two years after treatment (Table 25).

Three other trials were established against negatoria canegrub in 1998 (see Appendix 1), but subsequently were found to have very low spore levels in soil and were abandoned. These included two trials with treatments applied in the half-open drill and one with treatments applied in a ratoon using coulters.

Table 25
Concentration of spores (x 10^4)/g of wet soil at different times after treatment in ES98-11

Trt.	FI-147	3 week		6 month		1 year		2 year	
	g/m	a	b	a	b	a	b	a	b
1	2.5	31	26	8.0	10	53	11	14	12
2	5	33	15	66	51	47	50	11	20
3	10	79	57	39	49	102	73	25	26
5	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

9.3 1999 plant cane trials (ES99-12, -18)

Two trials were established against negatoria canegrub in October 1999 in plant cane.

Each trial included FI-147 as the primary isolate, with two treatments containing FI-147 and FI-1045 in a 1:1 mix (w/w). Trials used batches M166 and M167 of FI-147 and batches M156-158 of FI-1045, all with approximately 3 x 10^9 spores/g. Each treatment was applied to plots measuring four rows by 15 or 16 m long, and was replicated five times in randomised complete-block designs.

Product was applied by hand into the furrow, immediately after planting over uncovered setts in ES99-12 and in the half-open drill in ES99-18, and then covered with soil. The suSCon Blue was applied at 3.2 g/m of row.

Spore concentrations were satisfactory in both trials up to one year after application, but appeared to fall away in ES99-18 at two years; the reason for this is unknown (Tables 26 and 27).

Table 26
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in ES99-12

Trt.	FI-147	3 week		6 month		1 year		2 year	
	g/m	a	b	a	b	a	b	a	b
1	2.5	27	18	9.6	15	34	23	4.7	12.3
2	5	22	37	14	12	na	na	16	32
3	10	15	21	106	82	37	12	25	26
4	2.5*	29 tot	55 tot	21 (15)	25 (30)	12 (6.6)	13 (6.6)	13 (2.0)	18 (3.7)
5	5*	30 tot	37 tot	22 (26)	30 (34)	16 (9.0)	18 (8.3)	14 (15)	14 (15)
7	0	na	na	0.0	0.0	0.0	0.0	0.0	0.0

*Plus an equal amount of FI-1045.

(for treatments 4 and 5, concentrations in brackets are FI-1045, or a total (tot) level of both isolates is given)

Table 27
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in ES99-18

Trt.	FI-147	3 week		6 month		1 year		2 year	
	g/m	a	b	a	b	a	b	a	b
1	2.5	3.7	6.0	6.3	11	15	9.7	2.0	4.7
2	5	12	15	12	8.3	14	18	3.3	3.0
3	10	30	12	23	13	24	23	1.7	2.3
4	2.5*	27 (17)	7.7 (16)	12 (17)	8.0 (22)	8.7 (7.7)	8.7 (7.0)	1.0 (4.0)	0.0 (2.7)
5	5*	14 (19)	16 (14)	11 (20)	16 (18)	8.3 (7.0)	10 (6.7)	4.7 (4.7)	3.3 (5.7)
7	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Plus an equal amount of FI-1045.
(for treatments 4 and 5, concentrations in brackets are FI-1045)

9.4 2000 plant cane trials (ES00-4, -7, -9)

Three trials were established in plant cane in 2000, one in March and two in August.

In ES00-4 we used batch M173 of FI-147 and M170 of FI-1045, with 3.0-3.1 x 10^9 spores/g in original pouches and with 500 g bentonite subsequently added to each 2.5 kg pouch. In ES00-7 and ES00-9 we used batch M185 of FI-1045 and batch M186 of FI-147, with 3.7 and 3.2 x 10^9 spores/g, respectively, before addition of bentonite. Each treatment was applied to plots measuring five rows by 14 m in ES00-4, five rows by 13 m in ES00-7, and four rows by 16 m in ES00-9, and was replicated five times in randomised complete-block designs.

Product was applied by hand into the furrow, immediately after planting over uncovered setts, and then covered with soil. The suSCon Blue was applied at 3.2 g/m of row.

Spore counts in soil were satisfactory (Tables 28-30).

Table 28
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in ES00-4

Trt.	FI-147	3 week		6 month		1 year		2 year	
	g/m	a	b	a	b	a	b	a	b
1	2.5	44	56	13	36	22	25	15	25
2	5	79	75	63	35	29	26	28	26
3	10	118	157	95	76	32	31	20	19
4	2.5*	32 (26)	35 (32)	83 tot	73 tot	37 tot	32 tot	9.7 (13.0)	6.0 (8.0)
5	5*	68 (66)	83 (84)	76 tot	76 tot	75 tot	71 tot	24 (18)	20 (10)
7	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Plus an equal amount of FI-1045.
(for treatments 4 and 5, concentrations in brackets are FI-1045, or a total (tot) level of both isolates is given)

Table 29
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in ES00-7

Trt.	FI-147 g/m	3 week a	3 week b	6 month a	6 month b	1 year a	1 year b
1	2.5	16	76	7.7	5.3	12.7	6.0
2	5	20	48	7.0	7.7	24	31
3	10	196	68	26	22	53	50
4	2.5*	46 tot	48 tot	16 tot	12 tot	12 (6.3)	11 (8.3)
5	5*	32 tot	36 tot	23 tot	20 tot	13 (54)	25 (22)
7	0	0.0	0.0	0.0	0.0	0.0	0.0

*Plus an equal amount of FI-1045.
(for treatments 4 and 5, concentrations in brackets are FI-1045, or a total (tot) level of both isolates is given)

Table 30
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in ES00-9

Trt.	FI-147 g/m	3 week a	3 week b	6 month a	6 month b	1 year a	1 year b
1	2.5	31	53	14.7	7.7	7.0	4.3
2	5	39	40	13	61	17	16
3	10	49	48	46	52	76	111
4	2.5*	20 tot	30 tot	14 tot	15 tot	17 (21)	16 (23)
5	5*	64 tot	na	55 tot	57 tot	9.3 (38)	6.3 (19)
7	0	0.3	2.0	0.0	0.0	0.0	0.3

*Plus an equal amount of FI-1045.
(for treatments 4 and 5, concentrations in brackets are FI-1045, or a total (tot) level of both isolates is given)

10.0 TRIALS IN CENTRAL QUEENSLAND AGAINST FRENCH'S/NEGATORIA CANEGRUBS

10.1 French's/negatoria comparative bioassay

A bioassay was carried out at Mackay with negatoria and French's canegrubs being exposed simultaneously to FI-147 in peat. Negatoria grubs were obtained from Stephen Orr, Homebush, and French's grubs from Eddie Micallef (Alligator Creek) and Sam Scibberas (control plots in EC98-4 and EC99-4). A single dose was used, 2 x 10^5/g, with no controls. Batch M196 of FI-147 was used, 3.3 x 10^9 spores/g, with dilutions of spores prepared by washing spores off the rice granules.

Table 31
Response of French's and negatoria canegrubs in peat treated with FI-147 at 2 x 10^5 spores/g

Canegrub	n	% infection/mortality at following weeks					
		3	4	6	8	10	12
French's (Micallef)	13	31/61	61/92	69/100			
French's (Scibberas)	10	40/40	60/60	100/100			
Negatoria (Orr)	22	18/27	27/36	50/59	64/73	68/82	72/86

The results indicate that French's canegrubs are at least as susceptible as negatoria to FI-147 (Table 31). This agrees with results of bioassays carried out by CSIRO on the two species at different times.

10.2 1998 plant cane and ratoon trials (EC98-2, -3, -4, -5, -9)

Five trials were established in central Queensland (primarily targeting French's canegrub) in 1998 under BSS134, four in plant cane and one (EC98-5) in ratoons. Trials were established in August-November.

All trials used FI-147; EC98-2, -3 and -5 used batch M138 (2.9 x 10^9 spores/g) while EC98-4 and -9 used batch M149 (3.2 x 10^9 spores/g). The suSCon Blue was applied at 3.2 g/m in each trial. Each treatment was applied to plots measuring four rows by 20 m or five rows by 15 m, and was replicated five times in randomised complete-block designs.

Treatments in plant cane were applied by hand in a band about 20 cm wide and then covered with soil. In EC98-2 and EC98-3, setts were uncovered shortly after planting, treatments were applied and covered immediately with soil using shovels. In EC98-4 and EC98-9, treatments were applied just before filling-in of the planting drill, and were covered using a tractor-drawn implement. In the latter case, a delay of more than one hour would have elapsed between the times of application and covering in some plots. Treatments in ratoon cane in EC98-5 were applied behind paired coulters spaced 21 cm apart and 15-20 cm deep.

Some grubs were found in EC98-4 in December 2000. Numbers of grubs did not differ significantly between treatments (Table 32). However, the mean number in all treated plots was significantly lower than in untreated plots when groups of means were examined using linear contrasts ($P = 0.017$). Note that spore concentrations in soil were less than expected (see below and Table 35). A total of 18 grubs was tested by RFLP; all were *L. frenchi*.

Table 32
Numbers of French's canegrubs (mean ± SE) in EC98-4 in December 2000, and harvest yields of second ratoons on 10 October 2001, after treatment of a plant crop in November 1998

	FI-147 g/m			suSCon	Untreated
	2.5	5	10	21 kg/ha	
Grubs/stool	0.3 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.7 ± 0.2
Cane (t/ha)	47.9 ± 5.5	50.3 ± 7.3	53.9 ± 4.7	65.6 ± 7.7	45.1 ± 4.8
Sugar (t/ha)	5.9 ± 0.6	6.0 ± 1.0	5.8 ± 0.5	8.0 ± 1.0	5.3 ± 0.8
CCS	12.4 ± 0.4	11.8 ± 0.4	10.9 ± 0.5	12.2 ± 0.5	11.8 ± 0.8

Means were not significantly different ($P = 0.16$)

At harvest in 2001, there was no significant difference among the full set of treatments for yield or ccs (Table 32). Also, mean cane and sugar yields in all treated plots were not significantly different from the mean in untreated plots ($P = 0.12$ and 0.24, respectively), unlike the analysis of grub numbers above. However, cane and sugar yields in suSCon-treated plots were significantly greater than the mean in *Metarhizium*-treated plots ($P = 0.024$ and 0.043, respectively).

Spore concentrations were low in EC98-4 at 1-3 years (Table 35), probably because of the small depth of soil cover. Concentrations were also low in EC98-3 (Table 34), and appeared to fall away in EC98-2 at 3 years (Table 33), for unknown reasons.

Table 33
Concentration of spores (x 10^4)/g of wet soil at different times after treatment in EC98-2. Final soil cover was about 15 cm

Trt.	FI-147	3 week		6 month		1 year		2 year		3 year	
	g/m	a	b	a	b	a	b	a	b	a	b
1	2.5	90	74	29	27	8	16	14	12	0.0	1.0
2	5	62	83	37	35	28	41	31	33	7.3	2.3
3	10	82	100	57	57	72	64	102	89	2.3	0.0
5	0	0.6	0.0	0.3	0.3	0.3	0.7	0.0	0.3	0.0	0.0

Table 34
Concentration of spores (x 10^4)/g of wet soil at different times after treatment in EC98-3. Final soil cover was >20 cm

Trt.	FI-147	3 week		6 month		1 year	
	g/m	a	b	a	b	a	b
1	2.5	20	16	0.3	0.6	3.3	1.7
2	5	38	51	29	48	9.3	12
3	10	74	64	3.0	8.6	7.3	14
5	0	0.3	0.0	0.6	0.3	0.0	0.0

Table 35
Concentration of spores (x 10^4)/g of wet soil at different times after treatment in EC98-4. Final soil cover was about 5 cm.

Trt.	FI-147	3 week		6 month		1 year		2 year		3 year	
	g/m	a	b	a	b	a	b	a	b	a	b
1	2.5	0.0	0.3	6.3	7.3	12	13	1.6	4.3	1.0	0.7
2	5	10	169	9.0	5.0	7.6	5.0	3.0	5.3	2.7	3.0
3	10	50	38	3.3	11	13	15	30	33	6.7	4.7
5	0	1.0	3.6	1.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0

Table 36
Concentration of spores (x 10^4)/g of wet soil at different times after treatment in EC98-5 (ratoon application). Depth of application was about 15 cm.

Trt.	FI-147	3 week		6 month		1 year		2 year	
	g/m	a	b	a	b	a	b	a	b
1	2.7	18	12	19	21	7.0	9.6	12	10
2	5.2	21	24	21	19	9.6	10	26	22
3	9.2	66	112	85	74	66	59	44	44
5	0	0.0	0.0	0.0	0.0	2.0	0.6	0.0	0.0

Table 37
Concentration of spores (x 10^4)/g of wet soil at different times after treatment in EC98-9. Final soil cover was about 15 cm.

Trt.	FI-147	3 week		6 month		1 year		2 year		3 year	
	g/m	a	b	a	b	a	b	a	b	a	b
1	2.5	3.3	8.3	7.7	9.7	14	8.0	4.7	4.7	4.7	7.7
2	5	18	20	38	25	24	23	15	23	4.7	3.7
3	10	17	16	158	42	95	50	36	41	29	24
5	0	0.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.7

10.3 1999 plant cane and ratoon trials (EC99-1, -2, -3, -4, -5, -6, -7)

Seven trials were established in central Queensland in August-December 1999, four in plant cane and three (EC99-2, -3, -4) in ratoons.

Each trial included FI-147 as the primary isolate, with two treatments containing FI-147 and FI-1045 in a 1:1 mix (w/w) to investigate the economics of a two-isolate preparation for mixed infestations with greyback canegrub. FI-147 was batches M166 and M167, and FI-1045 was batches M156-158, all with approximately 3 x 10^9 spores/g in original pouches. Bentonite (500 g) was subsequently added to the approximately 2.5 kg product in each pouch. Each treatment was applied to plots measuring four rows by 20 m, and was replicated five times in randomised complete-block designs.

All treatments were applied using either a worm-drive Cane Country box (*Metarhizium*) or a fluted-roller Microband box (suSCon Blue). A series of three cogs with successive doubling of teeth was attached to the ground wheel of the Cane Country box to allow changing of rates between adjacent plots. The suSCon Blue was applied at a target rate of

3.2 g/m; calculated rates varied from 2.9-3.3 g/m in individual trials. Plant cane treatments were applied before filling-in of the drill, and soil cover was immediately added by reversed discs mounted on the implement. Ratoon treatments were applied behind paired coulters 23-30 cm apart and up to 15 cm deep.

A light infestation of French's grubs was present in EC99-4 3 months after treatment (coulters in ratoon). All three instars were present in late January 2000 (Table 38), presumably representing progeny of beetle flights in 1999 ('small' grubs, ie first and second instars) and 1998 ('large' grubs, ie third instars). There was no significant difference in numbers of either small or large grubs between treatments (Table 38). Grubs that were retrieved undamaged from the trial were held at 25°C in untreated soil for five weeks. Some of these grubs subsequently died with overt *Metarhizium* infection (Table 38), predominantly large (third instar) grubs in plots treated with FI-147. This sampling is not a good assessment of the effect of treatments, because grub numbers were low and insufficient time had elapsed since application of *Metarhizium*, but it does indicate some effect.

In the same trial late in 2000 (19 December), there was again no significant difference in grub numbers between treatments (Table 38), and the mean number of grubs in treated plots did not differ significantly from the number in untreated controls ($P = 0.08$). However, the overall grub density was low, with a gradient within replicates, and no grubs were found in many plots. There was little subsequent development of *Metarhizium* infection in grubs held for another three weeks in the laboratory (Table 38). A total of 23 grubs was tested by RFLP; all were *L. frenchi*.

The trial was sampled again in 25 January 2001, only in plots where we had found grubs the previous month. Grub numbers were lower than in December 2000 (note that tabulated means only include the sampled plots), with no significant difference between treatments (Table 38).

Table 38
Numbers of French's grubs/stool (mean ± SE) in EC99-4 (ratoon application in October 1999) in January 2000, 13 weeks after treatment, or in December 2000 or January 2001.

Grubs/ stool and subsequent infection	FI-147 g/m			FI-147 + FI-1045 g/m		suSCon Blue	Control
	2.7	5.3	10.7	2.9+2.9	5.9+5.9		
Jan. 2000							
Small [a]	0.6±0.3	0.1±0.1	0.1±0.1	0.3±0.2	0.0±0.0	0.4±0.2	0.2±0.1
Infect/total [b]	0/8	0/3	1/3	0/6	0/1	0/9	0/1
Large [a]	0.1±0.1	0.3±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.2±0.1	0.4±0.1
Infect/total [b]	1/5	2/7	3/3	0/2	0/1	0/5	0/3
Dec. 2000							
Live	0.8±0.2	0.7±0.2	0.3±0.1	0.1±0.1	0.4±0.2	0.2±0.1	0.9±0.2
Cadavers	0.04	0.00	0.08	0.00	0.08	0.00	0.00
Infect/total [c]	0/3	1/8	½	na	0/7	na	0/10
Jan. 2001 [d]							
Live	0.4±0.3	0.3±0.2	0.1±0.1	0.1±0.1	0.1±0.1	0.1±0.1	0.8±0.3
Cadavers	0.05	0.13	0.00	0.00	0.00	0.00	0.00

[a] Small and large grubs represent ages of < 1 year and > 1 year, respectively.
[b] After 6 weeks incubation in untreated soil at 25°C.
[c] After 3 weeks incubation in untreated soil at 25°C.
[d] Only plots with grubs present in December 2000 were sampled, no. replicates = 2-4/treatment.
Numbers of small and large grubs in January 2000 and live grubs in December 2000 and January 2001 did not differ significantly between treatments (P = 0.10, 0.66, and 0.19 and 0.11, respectively).

At harvest in 2001, there was no significant difference among the full set of treatments for cane or sugar yield or ccs (Table 39). However, mean cane yield in all treated plots was significantly greater than the mean in untreated plots (P = 0.021) while the difference between the corresponding mean sugar yields just failed to reach statistical significance (P = 0.051). The average increase in cane yield following treatment was 16.5 t. There was no significant difference between means of *Metarhizium*- and suSCon-treated plots for either cane or sugar yield (P = 0.93 and 0.88, respectively). There was also no significant difference between mean yields of plots treated with the single FI-147 isolate (two higher rates only) and the isolate mixture (P = 0.38 and 0.90 for cane and sugar, respectively).

Table 39
Harvest results in second ratoons (mean ± SE) for EC99-4 on 10 October 2001, after treatment of a first ratoon crop in October 1999.

	FI-147 g/m			FI-147+FI-1045 g/m		suSCon	Control
	2.7	5.3	10.7	2.9+2.9	5.9+5.9	21 kg/ha	
Cane (t/ha)	52.7±10.5	52.1±9.0	52.7±3.6	54.1±10.9	61.7±7.7	54.1±5.9	38.1±6.6
Sugar (t/ha)	6.6±1.4	6.3±1.7	5.5±0.6	5.6±1.1	6.5±1.2	6.3±0.8	4.4±0.5
ccs	12.5±0.9	11.7±1.2	10.6±0.9	10.8±0.9	10.3±1.3	11.6±1.0	12.0±0.8

Means for cane and sugar yield and ccs were not significantly different (P = 0.30, 0.47 and 0.73, respectively).

In EC99-6, negatoria grubs were present at a low density in summer 2000/2001. In December, numbers of live grubs did not differ significantly between treatments (Table 40). Many grubs from treated plots that were held in the laboratory for four weeks died from overt *Metarhizium* infection, but the soil in which the grubs were held temporarily (up to one week) before transfer to untreated soil came from the sample plots and so may have been contaminated. In February, all larval instars were present (Table 40); numbers of third instars were significantly lower in every treated plot than in untreated controls. Some of the survivors died of *Metarhizium* when held in the laboratory in untreated soil. Few cadavers were found on either sampling occasion. Numbers of earthworms in February 2001 were unaffected by treatment. A total of 15 grubs collected in December 2000 was tested by RFLP; all were *L. negatoria*.

Table 40
Numbers of negatoria grubs and earthworms/stool (mean ± SE) in EC99-6 on 12 December 2000 and 1 February 2001, after treatment of plant cane in November 1999.

Grubs/ stool and subsequent infection	FI-147 g/m			FI-147 + FI-1045 g/m		suSCon Blue	Control	*P*
	2.8	5.6	11.2	3.1+3.1	6.3+6.3			
Dec. 2000								
Live	0.4±0.1	0.6±0.2	0.2±0.1	0.4±0.2	0.4±0.1	0.1±0.1	0.7±0.2	0.16
Cadavers	0.00	0.00	0.00	0.00	0.04	0.00	0.00	
Infect/total [a]	1/1	6/7	na	1/4	1/5	na	1/8	
Feb. 2001								
Live I	0.1±0.1	0.2±0.1	0.0±0.0	0.1±0.1	0.1±0.1	0.0±0.0	0.2±0.1	0.61
Live II	0.0±0.0	0.2±0.1	0.0±0.0	0.0±0.0	0.2±0.1	0.1±0.1	0.3±0.1	0.23
Live III	0.3±0.1b	0.4±0.1b	0.2±0.1b	0.1±0.1b	0.3±0.1b	0.2±0.1b	1.0±0.2a	0.00
Cadavers III	0.00	0.00	0.04	0.00	0.00	0.00	0.00	
Infect/tot III [b]	0/5	4/6	0/5	0/3	1/6	0/3	0/21	
Earthworms	4.8±0.6	5.6±0.5	5.0±0.6	4.3±0.6	6.0±0.8	4.7±0.6	3.2±0.5	0.11

[a] After 4 weeks incubation in untreated soil at 25°C; however for the first week grubs were kept in small tubes with soil from the sampled plots.
[b] After 8 weeks incubation in untreated soil at 25°C.
Means followed by the same letter were not significantly different ($P = 0.05$).

There were some poor growth patches in EC99-6 early in 2001 that may have corresponded with grub infestation, and the suSCon-treated plots appeared better than plots treated with *Metarhizium*; however, the field also had problems with low calcium levels and high counts of *Pachymetra* and lesion nematodes.

At harvest in 2001, there was no significant difference among the full set of treatments for cane or sugar yield or ccs ($P = 0.41$, 0.55 and 0.55, respectively) (Table 41), unlike the analysis of February grub numbers above. Cane and sugar yields averaged over all treated plots were not significantly different from the means in untreated plots ($P = 0.13$ and 0.25, respectively), nor were there significant differences between means of *Metarhizium*- and suSCon-treated plots ($P = 0.26$ and 0.30, respectively).

Table 41
Harvest results in first ratoons (mean ± SE) for EC99-6 on 18 September 2001, after treatment of a plant crop in November 1999.

	FI-147 g/m			FI-147+FI-1045 g/m		suSCon	Control
	2.8	5.6	11.2	3.1+3.1	6.3+6.3	21 kg/ha	
Cane (t/ha)	87.5±5.6	89.6±3.5	97.4±7.5	94.1±7.7	97.6±8.0	100.4±5.4	84.7±9.1
Sugar (t/ha)	13.9±1.0	14.4±0.8	15.7±1.1	15.3±1.2	15.4±1.3	16.0±0.9	13.9±1.6
CCS	15.9±0.4	16.0±0.3	16.1±0.1	16.2±0.2	15.7±0.1	15.9±0.2	16.4±0.2

Spore concentrations up to one year after application were satisfactory in ES99-1 to EC99-6 (Tables 42-47).

Spore concentrations were very low in EC99-7 soon after application, and no FI-1045 type colonies were found; subsequent spore determinations have been inconsistent (Table 48). This was the last trial established in 1999, with treatments applied in December using product manufactured as early as July. Samples from batches 166 and 167 of FI-147 were sent to Bio-Care Technology for testing on 15 December, and recorded satisfactory germinations of >90% and 80-85%, respectively. FI-147 and FI-1045 tested on 18 November recorded germinations of 85-90% and >90%, respectively.

Table 42
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in EC99-1

Trt.	FI-147	3 week		6 month		1 year		2 year	
	g/m	a	b	a	b	a	b	a	b
1	2.5	27	12	7.3	8.0	12	8.7	13	12
2	5.0	29	38	26	21	11	11	3.7	5.0
3	10.0	82	105	57	54	22	16	5.0	9.7
4	2.7*	12 (21)	21 (39)	13 (8.3)	19 (21)	5.7 (7.0)	12 (9.7)	3.0 (3.7)	4.0 (2.3)
5	5.5*	12 (20)	18 (27)	41 (53)	48 (49)	16 (9.3)	18 (12)	12 (18)	16 (13)
7	0	2	0	0.6	0.3	0.0	0.7	0.0	0.3

*Plus an equal amount of FI-1045.
(for treatments 4 and 5, concentrations in brackets are FI-1045)

Table 43
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in EC99-2 (ratoon application)

Trt.	FI-147	3 week		6 month		1 year		2 year	
	g/m	a	b	a	b	a	b	a	b
1	2.6	129	108	41	36	25	12	35	27
2	5.1	99	149	26	44	23	22	13	14
3	10.3	325	393	102	121	12	44	39	46
4	2.7*	60 (96)	278 tot	51 (47)	57 (30)	13 (11)	8.7 (9.7)	27 (19)	20 (16)
5	5.4*	227 tot	219 tot	55 (22)	65 (44)	20 (18)	22 (19)	61 (47)	61 (36)
7	0	0	1	0.0	0.0	0.0	0.0	0.3	0.0

*Plus an equal amount of FI-1045.
(for treatments 4 and 5, concentrations in brackets are FI-1045, or a total (tot) level of both isolates is given)

Table 44
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in EC99-3 (ratoon application)

Trt.	FI-147 g/m	3 week a	3 week b	6 month a	6 month b	1 year a	1 year b	2 year a	2 year b
1	2.7	36	13	na	na	63	57	13	23
2	5.4	99	90			40	40	8.3	12
3	10.8	283	135			52	45	25	19
4	2.7*	36 (28)	28 (20)			37 tot	41 tot	9.7 (8.3)	7.3 (3.0)
5	5.5*	210 tot	197 tot			90 tot	21 tot	7.7 (0.3)	8.3 (0.3)
7	0	0.0	0.0			0.0	0.0	0.0	0.0

*Plus an equal amount of FI-1045
(for treatments 4 and 5, concentrations in brackets are FI-1045, or a total (tot) level of both isolates is given)

Table 45
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in EC99-4 (ratoon application)

Trt.	FI-147 g/m	3 week a	3 week b	6 month a	6 month b	1 year a	1 year b	2 year a	2 year b
1	2.7	71	57	39	53	26	10	31	29
2	5.3	516	377	207	279	28	31	37	59
3	10.7	407	351	135	157	104	41	18	18
4	2.9*	104 (23)	110 (24)	47 (23)	37 (31)	19 tot	22 tot	4.3 (7.7)	3.3 (4.3)
5	5.9*	115 (21)	92 (21)	53 (43)	44 (31)	41 tot	33 tot	8.0 (10.3)	11 (16)
7	0	0.7	0.3	0.0	0.0	0.0	0.0	0.0	0.0

*Plus an equal amount of FI-1045
(for treatments 4 and 5, concentrations in brackets are FI-1045, or a total (tot) level of both isolates is given)

Table 46
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in EC99-5

Trt.	FI-147 g/m	3 week a	3 week b	6 month a	6 month b	1 year a	1 year b	2 year a	2 year b
1	3.2	15	82	14	36	9.3	12	2.7	7.7
2	6.4	22	22	52	44	8.7	8.3	5.0	4.7
3	12.8	98	133	58	46	17	19	13.3	6.0
4	2.7*	48 tot	60 tot	16 (19)	11 (9.0)	16 (23)	14 (12)	5.0 (2.3)	4.3 (3.7)
5	5.4*	40 tot	39 tot	31 (16)	21 (18)	11 (7.7)	11 (13)	3.7 (5.3)	8.3 (9.7)
7	0	0.0	0.3	0.0	0.0	0.0	2.2	0.0	0.0

*Plus an equal amount of FI-1045
(for treatments 4 and 5, concentrations in brackets are FI-1045, or a total (tot) level of both isolates is given)

Table 47
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in EC99-6

Trt.	FI-147	3 week		6 month		1 year		2 year	
	g/m	a	b	a	b	a	b	a	b
1	2.8	16	15	16	30	11	10	8.3	12.3
2	5.7	32	29	19	21	27	24	31	20
3	11.3	53	54	31	73	17	36	25	15
4	3.1*	17 (30)	14 (27)	18 (18)	12 (9.0)	19 (14)	25 (20)	2.7 (4.7)	5.0 (8.0)
5	6.3*	13 (31)	18 (24)	39 (20)	27 (16)	32 (19)	39 (22)	9.3 (8.0)	6.7 (9.0)
7	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Plus an equal amount of FI-1045.
(for treatments 4 and 5, concentrations in brackets are FI-1045, or a total (tot) level of both isolates)

Table 48
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in EC99-7

Trt.	FI-147	3 week		6 month		1 year		2 year	
	g/m	a	b	a	b	a	b	a	b
1	2.4	1.3	1.3	9.3	12	12	8.0	0.7	0.7
2	4.8	2.6	4.0	13	18	1.6	1.3	1.3	2.3
3	9.6	8.3	6.0	33	30	4.3	7.3	1.7	2.0
4	2.6*	6.7 (0.0)	6.0 (0.0)	13 (13)	14 (9.3)	25 tot	18 tot	4.7 (2.0)	7.0 (0.7)
5	5.3*	8.3 (0.0)	3.0 (0.0)	20 (20)	28 (24)	17 tot	28 tot	6.0 (9.7)	4.7 (6.3)
7	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Plus an equal amount of FI-1045
(for treatments 4 and 5, concentrations in brackets are FI-1045, or a total (tot) level of both isolates is given)

10.4 2000 plant cane and ratoon trials (EC00-1, -3, -4, -6)

Four trials were established in 2000, two in plant cane (EC00-4 and EC00-6) and two in ratoons (EC00-1 and EC00-3).

We used batches M186 of FI-147 (3.2 x 10^9 spores/g) and M190 of FI-1045 (3.9 x 10^9 spores/g) in EC00-1 and EC00-3, and M194 of FI-147 (3.0 x 10^9 spores/g) and M192 of FI-1045 (3.4 x 10^9 spores/g) in EC00-4 and EC00-6 (all spore contents were before addition of bentonite at 500 g/pouch). Each treatment was applied to plots measuring four rows by 20 m except in EC00-4 (three rows by 10 m), and was replicated five times in randomised complete-block designs.

In EC00-4, product was applied by hand just before fill-in and then covered using chip hoes. In other trials, treatments were applied using either a worm-drive Cane Country box (*Metarhizium*) or a fluted-roller Microband box (suSCon Blue). The suSCon Blue was applied at a target rate of 3.2 g/m; calculated rates varied from 3.0-3.4 g/m in individual trials. Plant cane treatments were applied before filling-in of the drill, and soil cover was immediately added by reversed discs mounted on the implement. Ratoon treatments were applied behind paired coulters 22 cm apart and about 15 cm deep.

Spore levels were satisfactory in all trials (Tables 49-52).

Table 49
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in EC00-1 (ratoon application)

Trt.	FI-147	3 week		6 month		1 year	
	g/m	a	b	a	b	a	b
1	2	11	15	32	6.7	6.7	17.7
2	4	35	28	13	18	49	29
3	8	121	114	49	26	43	30
4	3*	7.3 (1.7)	7.0 (1.7)	4 (8)	12 (11)	9.0 (11)	10 (20)
5	6*	24 (19)	31 (16)	7 (11)	24 (25)	27 (27)	19 (27)
7	0	0.0	0.0	0.0	0.0	0.0	0.0

*Plus an equal amount of FI-1045.
(for treatments 4 and 5, concentrations in brackets are FI-1045)

Table 50
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in EC00-3 (ratoon application)

Trt.	FI-147	3 week		6 month		1 year	
	g/m	a	b	a	b	a	b
1	2.7	41	77	2.7	3.3	1.0	2.7
2	5.4	75	137	15	13	6.3	4.7
3	10.8	81	83	18	28	12	11
4	2.9*	139 tot	122 tot	28 (28)	15 (20)	4.3 (9.3)	5.7 (8.3)
5	5.8*	151 tot	171 tot	16 (20)	19 (16)	21 (44)	12 (22)
7	0	0.0	0.0	0.0	0.0	0.0	0.0

*Plus an equal amount of FI-1045.
(for treatments 4 and 5, concentrations in brackets are FI-1045, or a total (tot) level of both isolates is given)

Table 51
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in EC00-4

Trt.	FI-147	3 week		6 month		1 year	
	g/m	a	b	a	b	a	b
1	2.5	6.3	7.0	11.7	6.7	9.3	8.3
2	5	17	27	20	18	9.0	10.3
3	10	39	30	46	33	20	28
4	2.5*	7.3 (5.7)	7.0 (8.0)	22 (17)	11 (15)	6.0 (7.3)	3.7 (19)
5	5*	15 (15)	37 (29)	27 (24)	14 (9)	14 (15)	13 (17)
7	0	0.0	0.0	0.0	0.0	0.0	0.0

*Plus an equal amount of FI-1045
(for treatments 4 and 5, concentrations in brackets are FI-1045)

Table 52
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in EC00-6

Trt.	FI-147 g/m	3 week		6 month		1 year	
		a	b	a	b	a	b
1	2.7	6.3	4.3	5.3	3.0	1.3	1.7
2	5.5	47	30	17	29	15	14
3	11.0	42	89	32	60	15	14
4	2.6*	33 tot	30 tot	2.7 (3.7)	8.7 (6.7)	7.3 (2.7)	4.3 (0.3)
5	5.2*	18 tot	22 tot	38 (42)	33 (19)	13 (11)	7.7 (8.0)
7	0	0.0	0.0	0.0	0.0	0.0	0.0

*Plus an equal amount of FI-1045
(for treatments 4 and 5, concentrations in brackets are FI-1045, or a total (tot) level of both isolates is given)

10.5 2001 ratoon trial (EC01-11)

One trial was established in ratoon cane in 2001.

FI-147 and FI-1045 were batches M205 (2.7 x 10^9 spores/g) and M211 (2.4 x 10^9 spores/g), respectively (all spore contents are before addition of bentonite at 500 g/pouch). Four rates of FI-147 alone were applied plus a 2:1 mixture of FI-147:FI-1045; this variation from 1:1 was adopted because of doubt about the spore content of the FI-147 product at the time of application. Each treatment was applied to plots measuring four rows by 20 m, and was replicated five times in a randomised complete-block design.

Treatments were applied using either a worm-drive Cane Country box (*Metarhizium*) or a fluted-roller Microband box (suSCon Blue). The suSCon Blue was applied at a target rate of 3.2 g/m; the calculated rate was 3.3 g/m. Treatments were applied at a depth of about 15 cm behind a single coulter only, because it was not possible to obtain sufficient depth with two coulters.

Very high spore concentrations were present to six months (Table 53); note that concentrations would be expected to be double those of double coulter treatments.

Table 53
Concentration of FI-147 spores (x 10^4)/g of wet soil at different times after treatment in EC01-11

Trt.	FI-147 g/m	3 week		6 month	
		a	b	a	b
1	2.7	280	267	92	69
2	5.4	238	174	119	153
3	10.8	230	383	110	122
4	21.6	640	657	262	266
5	5.5*	181 (54)	168 (49)	52 (16)	62 (20)
7	0	5.0	4.7	0.0	0.0

*Plus half as much of FI-1045
(for treatment 5, concentrations in brackets are FI-1045)

11.0 TRIAL AGAINST CHILDERS CANEGRUB

11.1 1998 plant cane trial (ES98-10)

One trial was established against Childers canegrub in 1998 in BSS134. Plant cane was targeted, because results of previous applications in ratoon cane had been poor. We used FI-137, which had not previously been evaluated in field trials; batch M147 (3.0×10^9 spores/g).

The trial was established in October 1998. Treatments were two high rates of FI-137, 10.3 and 25.8 g/m, and suSCon Blue at 3.2 g/m (21 kg/ha). Treatments were sprinkled across the bottom of the half-open drill, in a 25 cm band, and then covered with 50-60 mm of soil. More soil cover was added during hilling-up. Each treatment was applied to plots measuring four rows by 16 m long, and was replicated six times in a randomised complete-block design.

A light infestation of grubs was present in the young first ratoon crop (Final Report BSS134). Neither FI-137 nor suSCon Blue significantly reduced numbers of live grubs (Table 54, $P = 0.41$).

Table 54
Live Childers canegrubs on 2 December 1999 in young first ratoon cane treated with *Metarhizium* or suSCon Blue soon after planting in 1998

	Rate of FI-137 (g/m)		suSCon Blue	Untreated
	10	26		
Grubs/stool	2.7 ± 0.4 a	2.4 ± 0.4 a	1.9 ± 0.3 a	3.3 ± 0.5 a

Means followed by the same letter were not significantly different ($P = 0.05$).

Soil was collected from the treated zone after two years and bioassayed with Childers grubs, in two sets of tests. Soil was very pathogenic to grubs, with 100% mortality in 7-9 weeks at 10 g/m and 4-6 weeks at 26 g/m (Table 55). Spore concentrations at this time were about 10 and 23 x 10^4/g, respectively (Table 56).

Table 55
Response of Childers canegrubs to treated soil collected from ES98-10 two years after treatment

Time (wk)	% mortality, Test 1			% mortality, Test 2		
	10.3 g/m	26 g/m	Control	10 g/m	26 g/m	Control
1	0	0	0	0	0	8
2	17	8	0	42	17	8
3	50	67	17	67	75	8
4	75	100	17	75	83	8
5	75	100	17	83	92	25
6	83	100	17	92	100	25
7	100	100	17	92	100	33
8				92	100	42
9				100	100	50

All insects sporulated except one in the controls in Test 1 and one at 26 g/m and two in the Controls in Test 2.

Spore concentrations were high up to two years after application (Table 56).

Table 56
Concentration of spores (x 10^4)/g of wet soil at different times after treatment in ES98-10

Trt.	FI-137	3 week		6 month		1 year		2 year	
	g/m	a	b	a	b	a	b	a	b
1	10.3	39	30	10	12	21	18	11	9.3
2	25.8	58	61	25	26	61	65	25	20
4	0	0.7	0.3	0.0	0.6	1.6	0.3	1.0	0.0

12.0 BIOASSAYS AGAINST NOXIA CANEGRUB

Table 57
Response of noxia canegrub to *Metarhizium* isolates in a dipping test. Results are % mortality or sporulation (in brackets) after 12 weeks at 25°C

Isolate	10^7/mL	10^5/mL
FI-147	100 (61)	44 (22)
FI-192	44 (17)	39 (17)
FI-522	28 (11)	39 (5)
FI-1045	50 (17)	44 (17)
FI-1186	44 (11)	28 (5)
FI-1248	39 (5)	39 (10)
FI-1400	22 (5)	28 (11)
FI-1415	28 (11)	28 (11)

Control response (%) was 30 (10)

A dipping test was carried out in Canberra against noxia canegrub using a range of isolates at two concentrations. FI-147 was the best of the isolates tested (Table 57), but response was only moderate. FI-1415, which was isolated from a noxia cadaver, was not very pathogenic.

One bioassay was carried out at Mackay in July 2000 with third instar grubs from Howard Sparke's farm near Bundaberg. FI-1045 (batch M182, 3.8 x 10^9 spores/g, >90% germination) and FI-147 (batch M173, 2.0 x 10^9 spores/g, 70% germination) were diluted and mixed with peat to give spore concentrations of 10^5 or 10^6/g (dry weight). Nineteen grubs were tested at each concentration.

Response to both isolates was very poor (Table 58), and contrasts with measured response (overt infection) of negatoria canegrub to FI-147 of 65% and 95% at 10^5 and 10^6 spores/g, respectively (Samson and Milner 1999).

Table 58
Response of noxia canegrubs to two *Metarhizium* isolates in peat after 12 weeks at 25°C

Treatment	% mortality	% overt infection
Control	53	21
FI-147 @ 10^5/g	53	32
FI-147 @ 10^6/g	84	42
FI-1045 @ 10^6/g	79	42

13.0 EFFICACY SUMMARY (BSS134 AND BSS246)

13.1 Southern one-year canegrub

A trial against southern one-year canegrubs using paired coulters to cut *Metarhizium* into a ratoon crop (ES96-15) showed that this treatment, though killing grubs, acted too late to prevent cane damage. A subsequent trial comparing coulter and band application (ES97-10) indicated that the latter gave a greater benefit in yield. All other trials against southern one-year canegrub therefore used band application in plant cane (Table 60).

In four trials using band application, *Metarhizium* isolate FI-1045 reduced southern one-year grub numbers and its effect on grub numbers or yield could not be separated statistically from that of suSCon (Blue or Plus) (Table 60). In a fifth trial, ES01-5, FI-1045 was less effective than suSCon Plus but spore concentrations in soil were lower than expected shortly after application (Table 20). In trial ES97-6, neither FI-1045 nor suSCon Blue reduced grub numbers in a third ratoon but this was a long time after application.

The average of grub counts over these trials, excluding ES01-5, suggests that a rate of FI-1045 of 10 g/m was slightly more effective than 5 g/m and gave comparable results to suSCon Blue or Plus (Table 59). However, 5 g/m was still effective. There are not enough results to evaluate the efficacy of isolate mixtures. Also, there are no efficacy data for second ratoons (Table 59).

Simultaneous bioassays of FI-1045 against southern one-year canegrub and greyback canegrub showed southern one-year is the more susceptible species. All the field trial results using band application in plant crops have been positive where expected spore concentrations in soil were achieved. There is good potential for BioCane against this pest.

Table 59
Results (mean ± SE) of efficacy trials against southern one-year canegrub, with 5 or 10 g/m of FI-1045 or FI-1045:FI-147 (1:1 mixture) or 21 or 40 kg/ha of suSCon Blue or Plus applied in a band in plant crops

Crop	Trial	5 g	10 g	5 g mix	10 g mix	suSCon	Control
Grubs							
P	ES01-5		0.8 ± 0.2			0.3 ± 0.1	1.2 ± 0.2
	ES01-7	0.9 ± 0.2	0.5 ± 0.1			0.5 ± 0.1	1.7 ± 0.2
1R	ES99-6	0.6 ± 0.1	0.3 ± 0.1			0.6 ± 0.2	2.1 ± 0.5
	ES00-8	1.5 ± 0.4	1.1 ± 0.2	1.4 ± 0.3	2.7 ± 0.7	0.8 ± 0.2	3.2 ± 0.8
3R	ES97-6	0.6 ± 0.2	0.7 ± 0.2			1.2 ± 0.3	1.2 ± 0.4
	ES97-10	0.7 ± 0.2	0.4 ± 0.2			0.5 ± 0.2	2.0 ± 0.5
Mean [a]		0.9	0.6			0.7	2.0
Cane (t/ha)							
1R	ES99-6	48 ± 7	52 ± 5			53 ± 6	40 ± 9
3R	ES97-6	77 ± 4	72 ± 3			72 ± 3	72 ± 3
	ES97-10	58 ± 6	66 ± 5			66 ± 8	50 ± 6
Mean		61	63			64	54

[a] Excluding ES01-5.

13.2 Negatoria canegrub

Two trials established in the previous project BSS134, in different fields on the same farm, showed great potential for use of FI-147 against negatoria canegrub. Good results were achieved at rates below 5 g/m using coulters in ratoon crops (Table 60). However, we have been unable to collect any additional data for ratoon application. For band application in plant crops, we have results from only one trial, with grub numbers reduced by both FI-147 and suSCon Blue in the first ratoon. Neither product improved cane yields, probably because only a light infestation was present (Table 60). This trial produced useful non-target data showing no effect of *Metarhizium* on numbers of earthworms. More data are needed to justify registration of isolate FI-147 for control of negatoria canegrub and to give confidence in recommending its use. Unfortunately, the current low incidence of negatoria canegrub, particularly in southern Queensland, makes the collection of additional data problematic.

13.3 French's canegrub

Results have been obtained in only two trials against French's grub (Table 60), despite the establishment of 17 trials targeting this species (Table 61). Infestations were variable in both trials. Spore concentrations were low in one trial in plant cane, possibly because of inadequate soil cover due to the drills being mostly filled in by the time of application, and suSCon Blue gave a better yield than *Metarhizium*. In the second trial where products were applied using coulters into a ratoon, FI-147 alone or mixed with FI-1045 improved yields of the succeeding ratoon, although no significant effect of treatment on grub numbers had been detected earlier in the year.

Bioassays show that this species is susceptible to isolate FI-147, similar to negatoria canegrub, and is a good candidate for biological control with *Metarhizium*. However, like

negatoria, its low frequency and unpredictability of occurrence have precluded collection of sufficient efficacy data to be convincing.

13.4 Childers canegrub

Childers canegrub was not a primary target of BSS246, and no new trials were established. However, one trial from BSS134 was continued into the current project.

Results against Childers canegrub have been disappointing (Table 60). Preventative application into ratoons using coulters did not reduce grub numbers, and plant cane application with a side-band dressing was also unsuccessful. In a trial in which application was thought to be ideal, ES98-10, grub numbers the following year were not reduced by treatment. Hence, there is no field evidence that preventative application of *Metarhizium* (various isolates) can control Childers canegrub. However, suSCon Blue was also unsuccessful in all trials where used, including ES98-10 where it was applied according to label recommendations. Thus there is also no evidence that *Metarhizium* is any less effective than suSCon Blue, which is known to work in most situations. Soil samples taken from ES98-10 more than two years after *Metarhizium* treatment still gave high rates of infection of Childers grubs confined within the treated soil. There may be environmental factors that caused the poor performance of *Metarhizium* (and suSCon) in some of our trials.

13.5 Noxia canegrub

No field trials were established against noxia canegrub, but bioassays were conducted by CSIRO and BSES to evaluate the potential for *Metarhizium* to be used against this localised but troublesome pest. Noxia grubs responded poorly to a range of isolates, and none would be worth testing in the field.

Table 60
Summary of efficacy results from preventative application of *Metarhizium* in BSS134 or BSS246

Trial	Details	Result
Southern one-year canegrub		
ES96-15	Coulter into ratoon Oct 96	FI-1045 (8 & 34 g/m) and FI-1186 reduced grub numbers by May but not April 1997. FI-147 not effective. Nine grubs/stool in controls. No suSCon in trial. No effect of treatment on yield in October 1997.
ES97-6	Band in plant cane Sep 97	No reduction in grub numbers by FI-1045 (5-25 g/m) or suSCon Blue in 3R in March 2001. 1.2 grubs/stool in controls. No effect of treatment on 3R yield in 2001.
ES97-10	Band (Oct 97) or coulter (Dec 97) in plant cane	FI-1045 at 5-25 g/m by band or coulter and suSCon all reduced grub numbers in 3R in March 2001. Two grubs/stool in control. Mean yield of treated plots greater than that of untreated in 3R in 2001. Band treatment gave greater yield than coulter at 10 g/m.

Trial	Details	Result
ES99-6	Band in plant cane Aug 99	FI-1045 (5 & 10 g/m) and suSCon Blue reduced grub numbers in 1R in February and April 2001. Two grubs/stool in controls. Possible poor control with FI-1045 at 10 g/m in adjacent rows. No effect of treatment on 1R yield in 2001.
ES00-8	Band in plant cane Aug 00	FI-1045 and suSCon Blue reduced grub numbers in 1R in April 2002. 3.2 grubs/stool in controls.
ES01-5	Band in plant cane Aug 01	Grub numbers in plant crop in March 2002 reduced by suSCon Plus but not by FI-1045 at 10 g/m. 1.2 grubs/stool in controls. Note: spore counts below expectation.
ES01-7	Band in plant cane Sep 01	FI-1045 at 5 and 10 g/m and suSCon Plus reduced grub numbers in plant crop in March 2002. 1.7 grubs/stool in controls.
Negatoria canegrub		
ES96-16	Coulter into ratoon Oct 96	Grub numbers in September and November 1997 reduced by FI-147 at rates as low as 2.3 g/m; 7 g/m equivalent to suSCon Blue. Five grubs/stool in control.
ES96-31	Coulter into ratoon Dec 96	Grub numbers in December 1997 reduced by FI-147 at rates down to 2.6 g/m; only top rate of 29 g/m equivalent to suSCon Blue. Five grubs/stool in controls. Yield increased by FI-147 at 4 g/m; 6 g/m equiv. to suSCon.
EC99-6	Band in plant cane Nov 99, Mackay	Grub numbers equally reduced by FI-147 at or above 2.8 g/m (alone or mixed with FI-1045) and by suSCon Blue in 1R in February 2001. 1.0 grubs/stool in Controls. suSCon plots looked better than some *Metarhizium* plots but may be chance effect - variable site. No effect of treatment on 1R yield in September 2001.
French's canegrub		
EC98-4	Band in plant cane Nov 98	Fewer grubs in treated plots (FI-147 2.5-10 g/m and suSCon Blue) in young 2R in December 2000 (contrast in ANOVA). 0.7 grubs/stool in control. Very variable in grub numbers and cane growth. Yield greater in suSCon plots than in FI-147 plots in 2R in October 2001. Note: spore counts low, poor soil cover.
EC99-4	Coulters in ratoon Oct 99	Some large grubs infected in January 2000 but light infestation. No effect of treatments (FI-147 at 2.7-11 g/m plus mix with FI-1045, suSCon Blue) on grub numbers in December 2000 or January 2001. 0.9 grubs/stool in controls. Very variable infestation. Yield in October 2001 increased by all treatments.
Childers canegrub		
ES95-13	Coulters in ratoon Oct 95	No reduction in grub numbers by FI-114 (2.4-27 g/m) or FI-147 (2.0-26 g/m) in January 1997. 1.8 grubs/stool in controls.
ES95-20	Coulters in ratoon	No reduction in grub numbers by FI-114 (3.4-19 g/m) or FI-

Trial	Details	Result
	Nov 95	147 (2.9-24 g/m) in January 1997. 1.5 grubs/stool in controls.
ES95-22	Side-band in plant cane Dec 95	No reduction in grub numbers by FI-114 (2.6-26 g/m), FI-147 (2.7-27 g/m) or suSCon Blue in 1R in December 1996 (1.1 grubs/stool in controls) or January 1998 (1.5 grubs/stool in Controls).
ES96-26	Coulters in ratoon Nov 96	No reduction in grub numbers by FI-114 or FI-1186 at rates to 24 and 29 g/m respect., or by suSCon Blue, in November 1997. Seven grubs/stool in controls. Little or no yield improvement.
ES96-32	Coulters in ratoons Nov 96	No reduction in grub numbers by FI-1186 at rates to 35 g/m, or by suSCon Blue, in December 1997. 4.8 grubs/stool in controls.
ES98-10	Band in plant cane Oct 98	No reduction in grub numbers by FI-137 (10 & 26 g/m) or by suSCon Blue in 1R in December 1999. 3.3 grubs/stool in Controls. Soil samples pathogenic in January 2001.

13.6 Current status of existing trials

Many trials established within this project are still in place in fields and are capable of producing useful results. This includes trials established in 2000 or 2001, or older trials that have been infested during previous sampling. There are eight against southern one-year or Nambour canegrub, three against negatoria canegrub in southern Queensland, and seven against negatoria or French's canegrub in central Queensland (Table 61). These should be checked for infestations in 2002/3, and yields should be measured for two trials infested with southern one-year canegrubs in 2002, ES00-8 and ES01-7. Measurement of spore concentrations would also be desirable for infested trials.

Table 61
Numbers of canegrubs found in untreated plots each year.

Year treated	Crop age treated	Trial code	Grubs/stool 1999/00	Grubs/stool 2000/1	Grubs/stool 2001/2	Sample 2002/3
Southern 1-yr or Nambour canegrub						
1997	Plant	ES97-6	0.3	**1.2***	na	
		ES97-7	0.0	0.0	na	
		ES97-10	0.5	**2.0***	na	
1999	Plant	ES99-4		0.0	0.0	
		ES99-6		**2.0***	0.5	Yes
2000	Plant	ES00-3			0.0	Yes
		ES00-8			**3.2***	Yes
		ES00-11			0.0	Yes
2001	Plant	ES01-4			0.0	Yes
		ES01-5			**1.2***	Yes
		ES01-6			0.2	Yes
		ES01-7			**1.7***	Yes
Negatoria canegrub in southern Queensland						
1998	Plant	ES98-11	0.0	0.0	0.0	
1999	Plant	ES99-12		0.0	0.2	
		ES99-18		0.0	0.1	
2000	Plant	ES00-4			0.0	Yes
		ES00-7			0.0	Yes
		ES00-9			0.0	Yes
French's/negatoria canegrubs in central Queensland (F, French's; N, negatoria in sample)						
1998	Plant	EC98-2	0.1	0.0	0.0	
		EC98-3	0.04	na	na	
		EC98-4	0.6 F	**0.7* F**	0.0	
		EC98-9	0.1 N	0.0	0.2 F	
	Ratoon	EC98-5	0.0	0.1	na	
1999	Plant	EC99-1		0.3 F/N	0.4 N	Yes
		EC99-5		0.0	0.0	
		EC99-6		**0.7* N**	0.1 N	Yes
		EC99-7		0.1 N	0.0	
	Ratoon	EC99-2	0.0	0.0	0.4 N	Yes
		EC99-3	0.0	na	0.0	
		EC99-4	**0.4* F**	**0.9* F**	0.6 F	
2000	Plant	EC00-4			0.2 F	Yes
		EC00-6			0.4 F	Yes
	Ratoon	EC00-1			0.0	
		EC00-3			0.6 F	Yes
2001	Ratoon	EC01-11				Yes

* Treatments sampled as well as controls; otherwise controls only.

14.0 PERSISTENCE TESTS IN PVC RINGS

14.1 Effect of locations and formulations

The first PVC ring experiment, started in BSS134, was completed. The persistence of spores of two isolates, FI-1045 (BioCane) and FI-147, was studied in three canefields each in northern and southern Queensland, respectively. Four forms of *Metarhizium* were included, a rice-based granule as in the BioCane product, a wettable powder made from spores extracted from rice and mixed 1:1 with attapulgite, a suspension of spores from rice, and a suspension of spores grown on agar. Spores were mixed in soil as dry granules or as an aqueous suspension, at a nominal rate of 1 x 10^7/g. Treated soil was transferred to PVC rings of 50-55 mm internal diameter and 35 mm height, 100 g/ring. Rings were buried in three rows in each field to a depth of 175 mm at their base, and sitting on a thin layer of white sand. Three replicate rings of each formulation, one from each row, were retrieved from each field every six months and spore concentrations measured.

Spore concentrations over 3.5 years are shown in Figures 3-4. Assuming a linear relationship between spore concentrations, log-transformed, and time, there was little difference between the annual survival rate of several formulations, including rice granules (Table 62). The annual survival rate of FI-1045 in northern Queensland, averaged over the different formulations, was 40-56% at three sites. Annual survival of FI-147 in southern Queensland was 49-52%.

It was thought that a green cane trash blanket (GCTB) may influence *Metarhizium* persistence, eg by ameliorating soil temperature. Sites at Innisfail, Heidke and Webber had a GCTB each year, Moller had a GCTB in 1998 but was burnt in 1997, while trash at Tully and the Burdekin was burnt each year. These differences seem to have had no effect on spore persistence.

Table 62
Predicted annual survival rates (%/year) for FI-1045 and FI-147 in different formulations and at different sites, assuming linear relationships between concentration, log-transformed, and time at each location

Site	BioCane™	WP ex-rice	Spores ex-rice	Spores ex-agar	Mean
Innisfail (FI-1045)	47	41	41	40	42
Tully (FI-1045)	57	55	54	69	56
Burdekin (FI-1045)	44	42	37	33	40
Heidke (FI-147)	54	48	50	70	52
Moller (FI-147)	51	53	54	44	52
Webber (FI-147)	56	47	44	60	49
Mean	54	50	49	58	

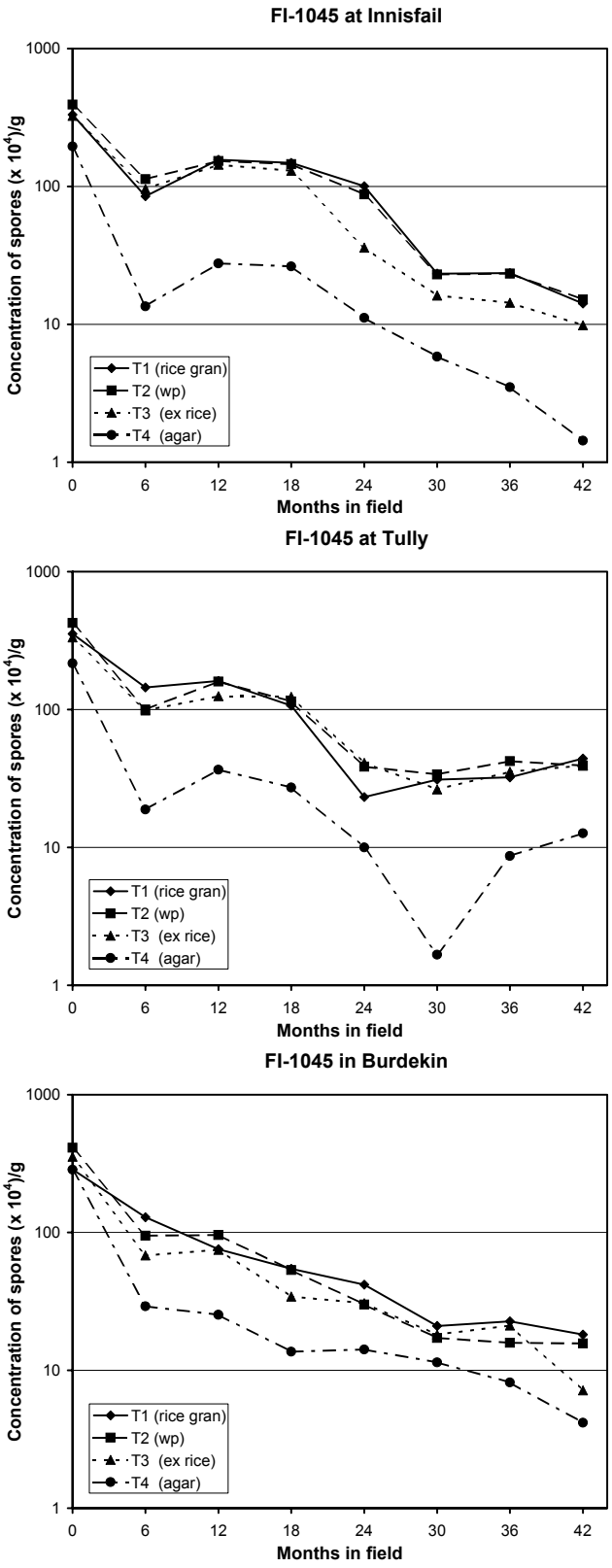


Figure 3. Persistence of FI-1045 in PVC rings in three districts in northern Queensland

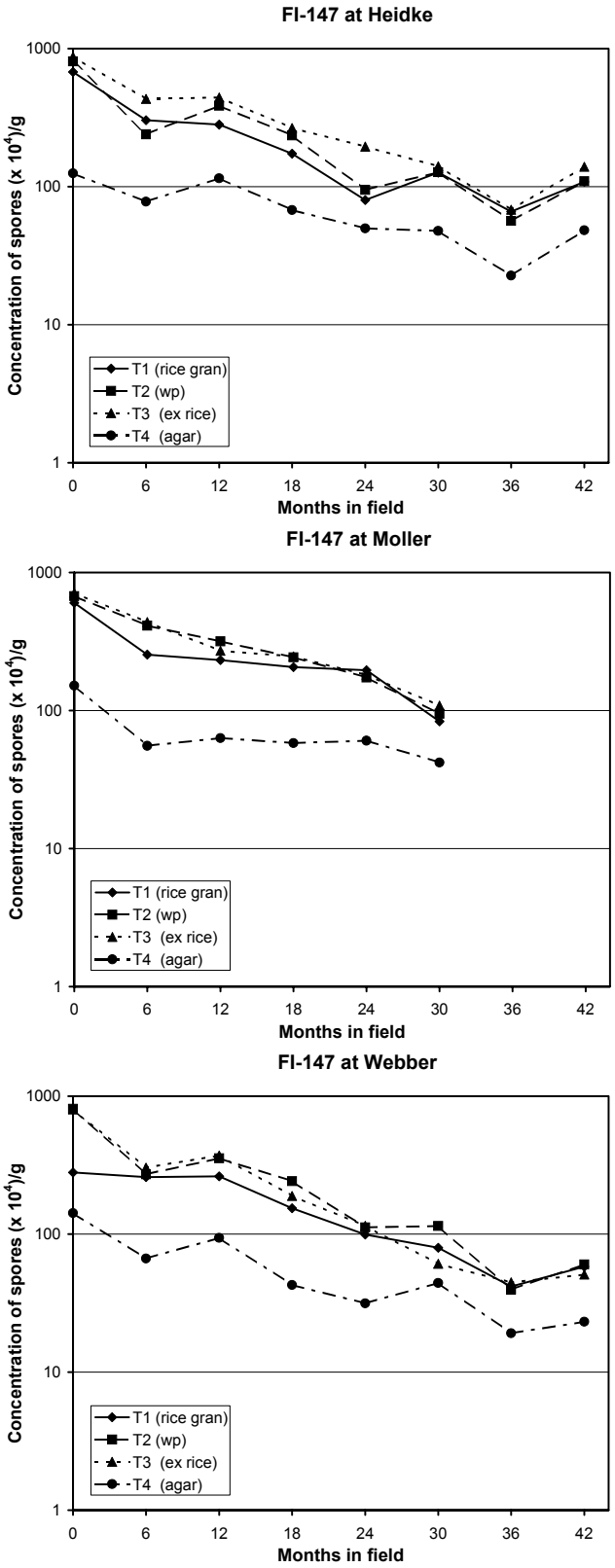


Figure 4. Persistence of FI-147 in PVC rings at three farms near Bundaberg in southern Queensland

14.2 Effect of spore concentration

This experiment aimed to determine whether the rate of decline of spore populations in soil is affected by spore concentration.

The trial site was a canefield at Mackay, planted in 2000 (Les Trueman, Te Kowai, Q135). Rings (55 mm internal diameter, 35 mm height) were filled with 100 g of the native soil, treated as below, and buried at 175 mm depth on 2 April 2001.

Eight treatments were included:

- FI-1045 at 10^7/g all at a central point in 2 mL suspension;
- FI-1045 at 10^7, 10^6, 10^5 and 10^4/g spread through soil;
- FI-1045 as four BioCane granules;
- FI-147 at 10^7/g spread through soil;
- untreated control.

Sufficient rings were prepared to allow retrieval on nine occasions – 0, 1, 3, 6 months and 1, 2, 3, 4, 5 years – with three replicates on each occasion.

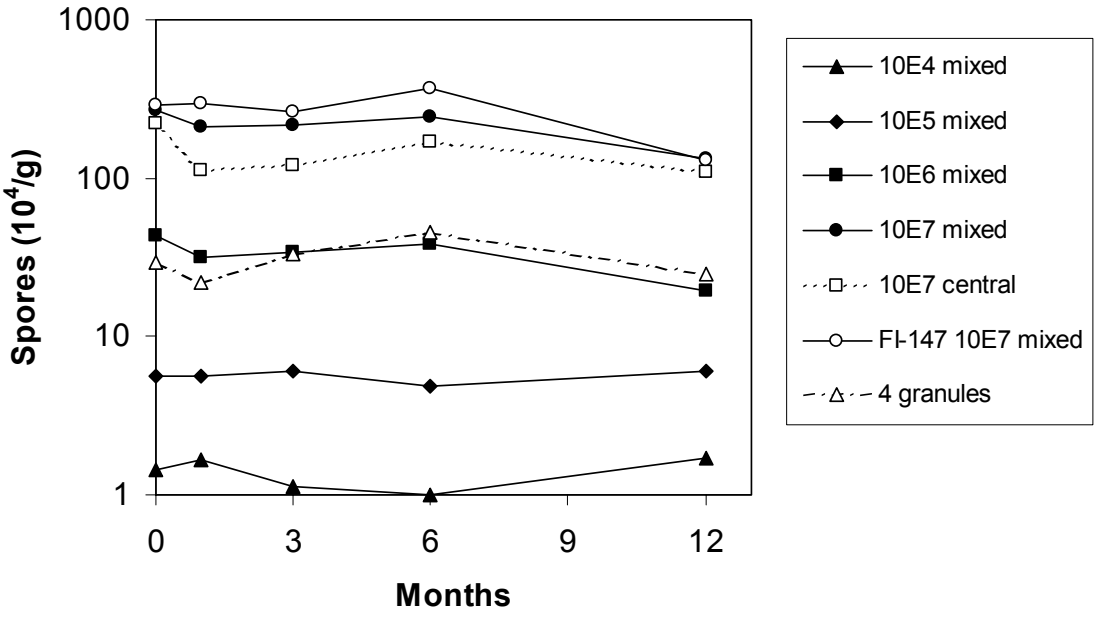


Figure 5. Spore concentrations in PVC rings containing FI-1045 or FI-147 (one treatment only) at different initial concentrations or distributions

The efficiency of extraction seemed to be higher at lower doses, ie measured concentrations at time zero were closer to target concentrations (Figure 5), which could be explained by colonies being on top of each other at the high doses. Counts for FI-147 and FI-1045 were very similar at the same dose of 10^7 spores/g. Rates of decline of spore concentration may be lower at 10^4 and 10^5 spores/g and for BioCane granules than for some other treatments, but results are needed for longer time periods after burial.

BioCane granules were estimated to weigh about 13.5 mg each (n=60). Therefore four granules should weigh about 54 mg, equalling 1.8 x 10^8 spores, so the count per gram of soil in the rings should be about 180 x 10^4. The actual count at the start (0 months) was much below this – the reason is unknown.

15.0 BIOCANE BIOASSAYS

It is vital that a genetically stable and pathogenic culture of FI-1045 be maintained indefinitely for annual production of BioCane. A protocol was established to maintain the integrity of FI-1045. Each year, the culture of FI-1045 from the previous year was passaged through greyback canegrubs. Three 'daughter' cultures were produced, each corresponding to an infected grub cadaver. The daughter cultures were verified in two ways, using the parent culture and historic records as a comparison:

1. by RAPD (random amplified polymorphic DNA) testing and by growth characteristics (CSIRO);
2. by bioassay (BSES).

Based on these results, a decision was made on which culture(s) would be used for commercial production of BioCane™ in that year.

15.1 2000

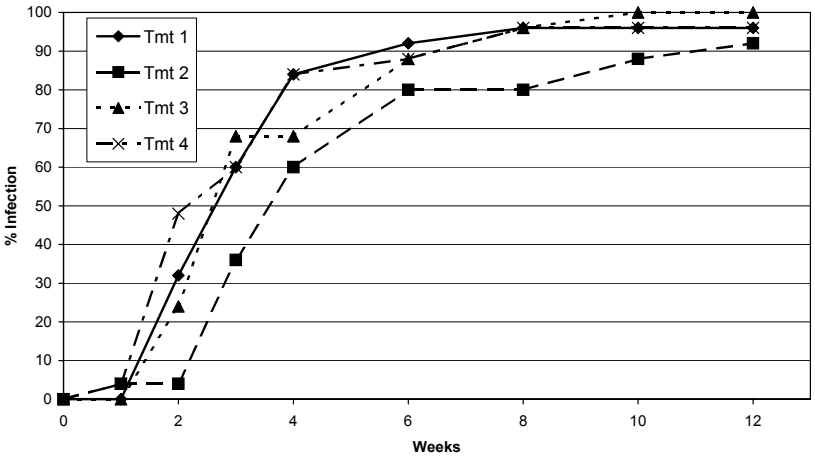
Four cultures were compared in 2000:

1. BC623 (fresh spore granules);
2. BC623 (spore granules from an old culture batch M157);
3. BC648 (spore granules produced with standard procedures);
4. BC648 (spore granules produced with new production procedures).

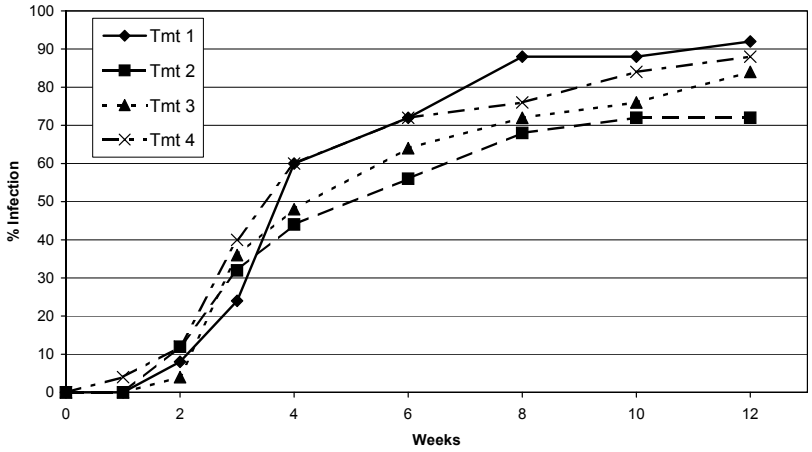
Each culture was tested at five spore concentrations, 10^7, 10^6, 10^5, 10^4, and 10^3/g peat, with 25 greyback grubs at each concentration, plus 50 grubs as untreated controls. Grubs were confined individually in garden peat in 150 mL plastic cups with snap-on lids, and fed slices of carrot. They were checked every week for the first four weeks and every fortnight thereafter, for a period of 12 weeks. Any dead grubs were left in their cups to check for sporulation at the next observation. Date of death and/or sporulation was recorded for each grub.

The low concentrations of 10^3 and 10^4 spores/g provided no useful information (Figure 6). There was little difference in response between the four cultures at the three higher concentrations, though response to the older granules (Treatment 3) may have been slightly slower than to the fresh granules.

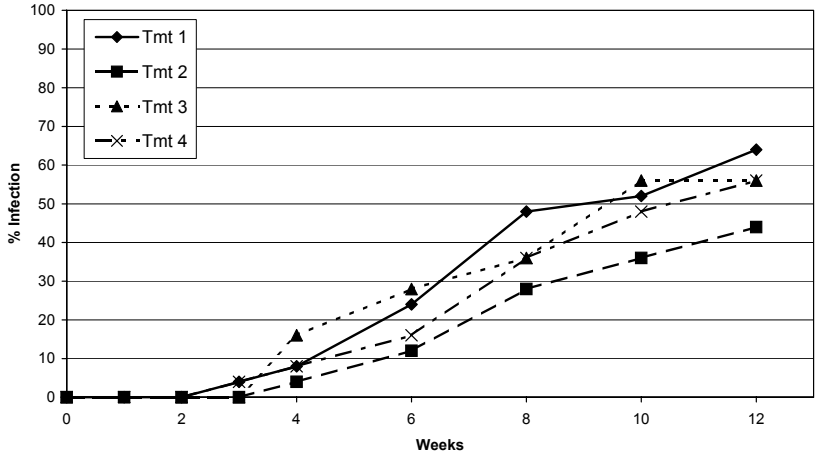
Bio-Cane Bioassay 2000
10^7



Bio-Cane Bioassay 2000
10^6



Bio-Cane Bioassay 2000
10^5



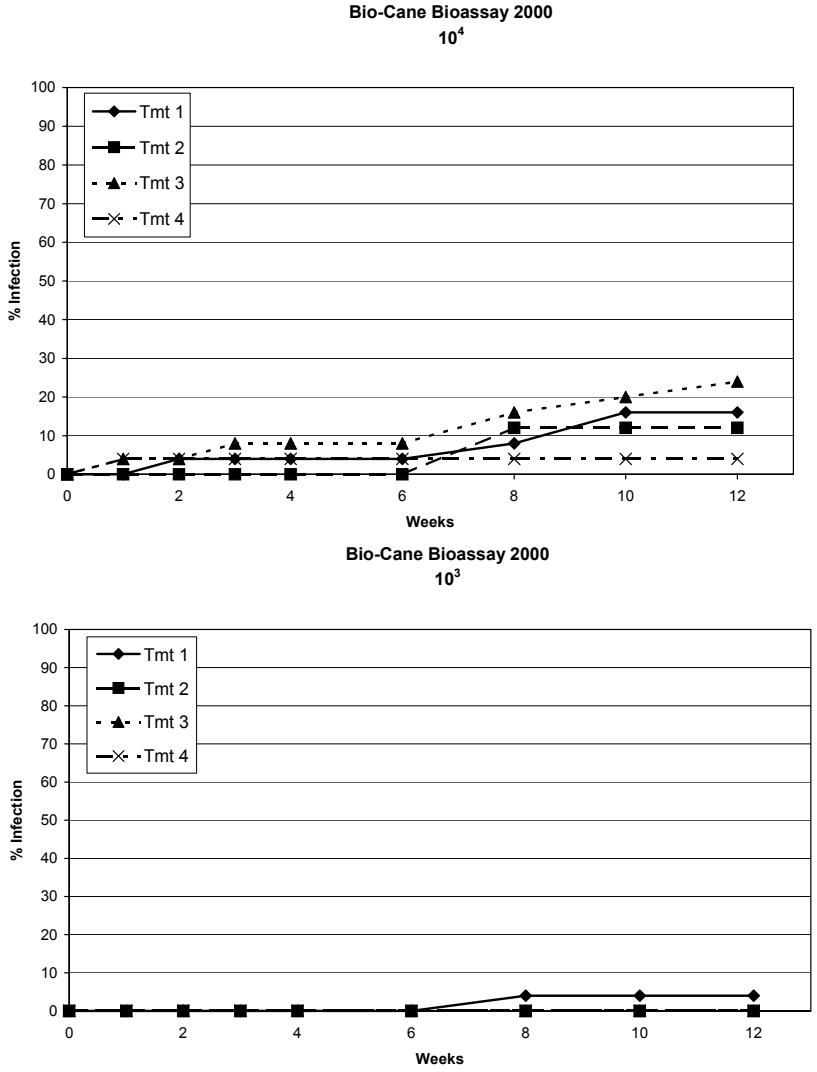


Figure 6. Response of third instar greyback canegrubs to spores from four *Metarhizium* cultures of FI-1045, at concentrations in peat from 10^7 to 10^3/g, in 2000

15.2 2001

Maintenance bioassays on BioCane were carried out as in 2000. FI-1045 cultures with different histories were used. In addition, southern one-year canegrub was included, both as a test subject and as a host for passage of FI-1045 before bioassay.

Treatments were as follows:

1. CSIRO FI-1045 grub: CSIRO -70°C FI-1045 spores on CSIRO SDAYE slope, then to BCT (Bio-Care Technology), then to larvae, then spores directly off larvae to bioassay.

2. CSIRO FI-1045 media: CSIRO -70°C FI-1045 spores on CSIRO SDAYE slope, then to BCT, then to larvae, then to BCT antibiotic media, then spores off media direct to bioassay.

3. CSIRO FI-1045 rice: As in treatment 1 above but after collection of spores from larvae, then grow spores on rice, then some rice culture to bioassay.

4. BC623PC: FI-1045 ampoule (1997) cultured on BCT media in 2000, then to larvae, then to antibiotic media, then to freeze dried ampoules, then in 2001 ampoule cultured to BCT media, then to larvae, then to antibiotic media, then to BCT media, then to rice production, then some rice culture to bioassay (NOTE: this culture was passaged through greyback twice).

5. BC623GC: FI-1045 ampoule (1997) cultured on BCT media in 1999, then to rice production, then seven-month-old culture to larvae in March 2000, then to BCT antibiotic media, then to freeze dried ampoules, then to BCT media in 2001, then to larvae, then to BCT antibiotic media, then to rice production, then some rice culture to bioassay (NOTE: this culture was passaged through greyback twice).

6. BioCane old: Some 2000 produced BioCane (FI-1045 ex 1997 ampoule stock but passaged through greyback, then to freeze dried ampoules (=BC623PC) before use in 2000 production) batch M192 (made 28.9.00) direct to bioassay (=older spores off rice).

7. CSIRO FI-1045 SOY: CSIRO -70°C spores on slope to BCT, then to southern one-year larvae, then to BCT antibiotic media, then spores off media direct to bioassay.

8. CSIRO FI-1045 media (2. above): Bioassay using southern one-year canegrub

.

9. CSIRO FI-1045 SOY (7. above): Bioassay using southern one-year canegrub.

10. BSES Burdekin grubs: Spores directly off cadavers from Burdekin to bioassay.

Each culture was tested at two or three spore concentrations, 10^5, 10^6 and, sometimes, 10^7/g peat, with 25 grubs at each concentration, plus 50 grubs as untreated controls.

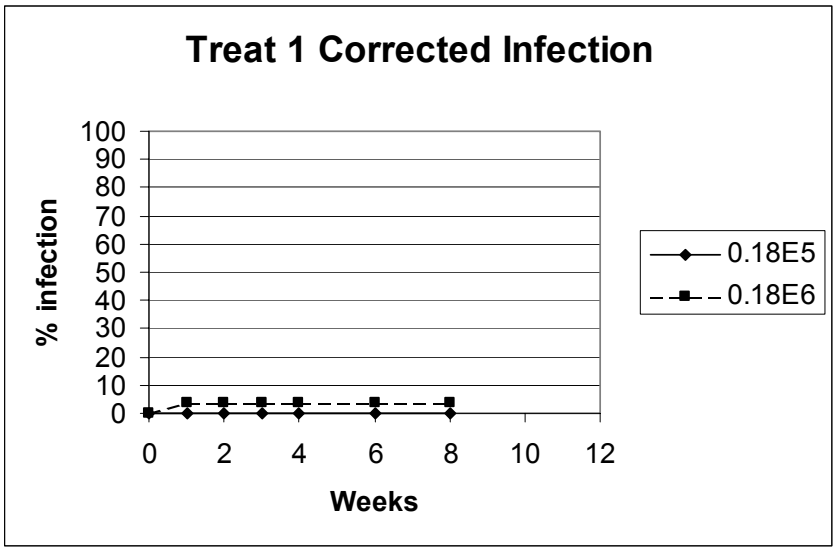
Table 63
Spore concentrations and viability of suspensions prepared for treating peat in BioCane bioassays in 2001

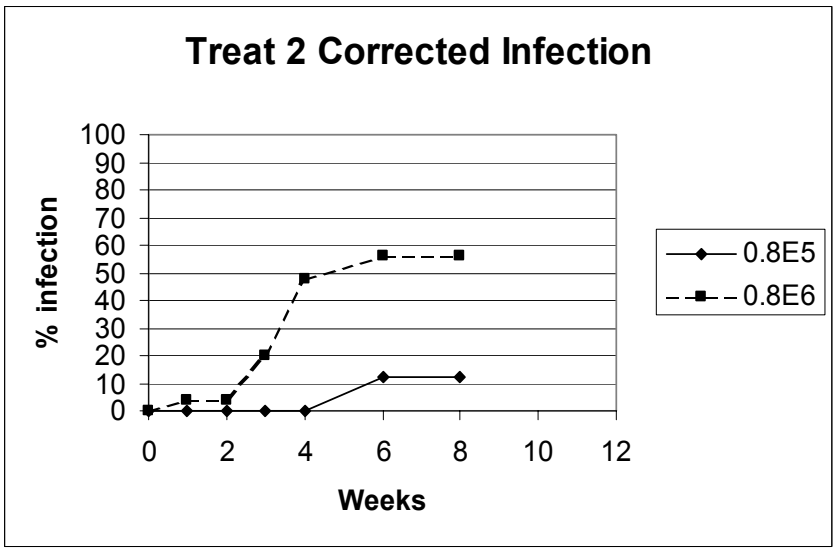
Treatment	Germination %	Total spore count/mL	Expected spore count/mL
1	28	0.63×10^6	10^6
2	100	0.76×10^6	10^6
3	96	0.42×10^7	10^7
4	99	0.61×10^7	10^7
5	100	0.65×10^7	10^7
6	93	1.6×10^7	10^7
7	100	1.1×10^6	10^6
8 (=2)	100	0.98×10^6	10^6
9 (=7)	100	1.1×10^6	10^6
10	98	2.2×10^7	10^7

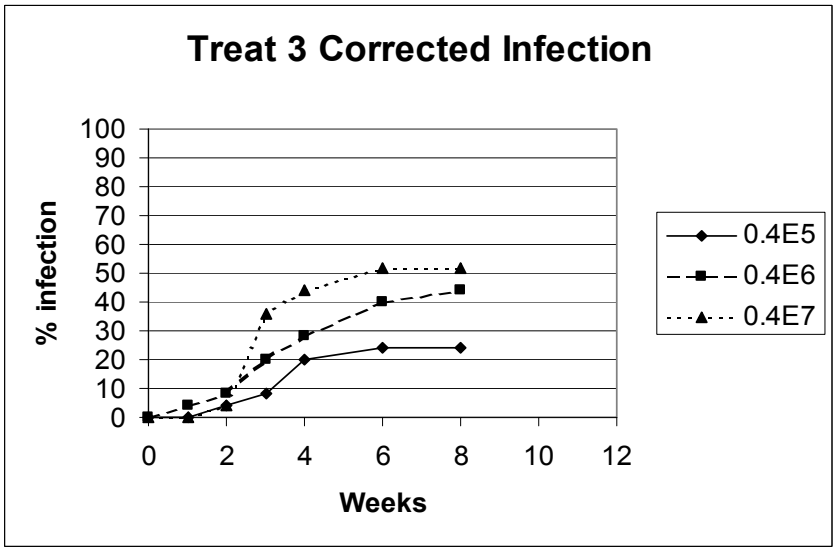
Suspensions for dosing peat were produced by weighing out quantities of granules or spores, based on spore counts and viability for these materials. Samples of suspensions of all treatments except #1, spores from a grub at Bio-Care Technology, averaged >90% viability (Table 63). Treatment 1 averaged only 28%. Total spore counts were often very different from expected counts (Table 63), suggesting some sampling error with the counts done on materials before suspensions were prepared.

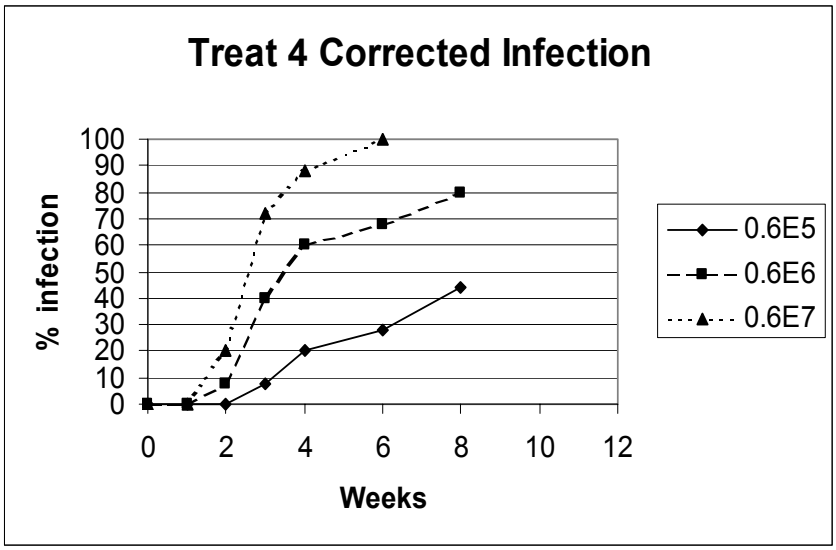
Numbers of spores produced from cadavers were estimated as 1.14×10^9 for the two cadavers from Bio-Care Technology and 3.92×10^9 for the ten cadavers from BSES Burdekin. The latter were fresh whereas the former had been stored since 2000.

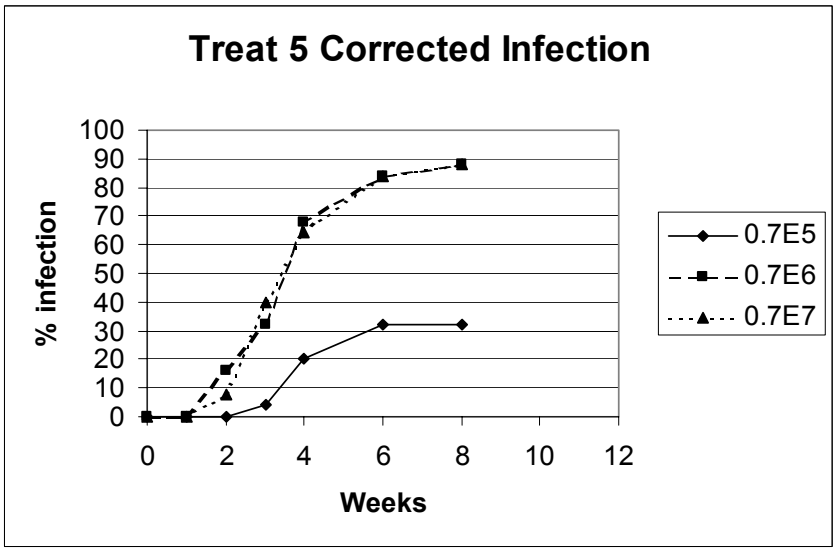
Southern one-year canegrubs responded more strongly to FI-1045 than did greyback canegrub (Treatments 8 and 9, Figure 8, compared with 2 and 7, Figure 7, respectively). The culture in Treatment 4 was used for BioCane production in 2001.

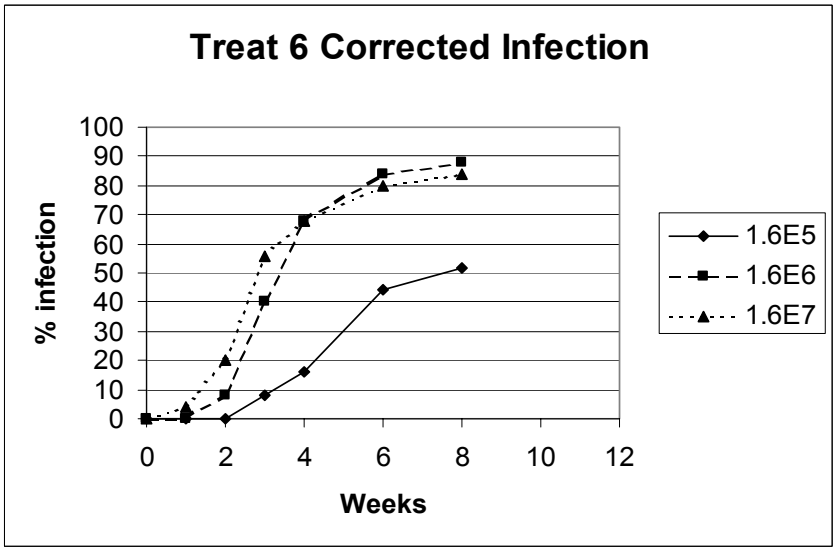

Treat 1 Corrected Infection
% infection
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
Weeks
0.18E5
0.18E6


Treat 2 Corrected Infection
% infection
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
Weeks
0.8E5
0.8E6


Treat 3 Corrected Infection
% infection
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
Weeks
0.4E5
0.4E6
0.4E7


Treat 4 Corrected Infection
% infection
Weeks
0.6E5
0.6E6
0.6E7


Treat 5 Corrected Infection
% infection
Weeks
0.7E5
0.7E6
0.7E7


Treat 6 Corrected Infection
% infection
Weeks
1.6E5
1.6E6
1.6E7

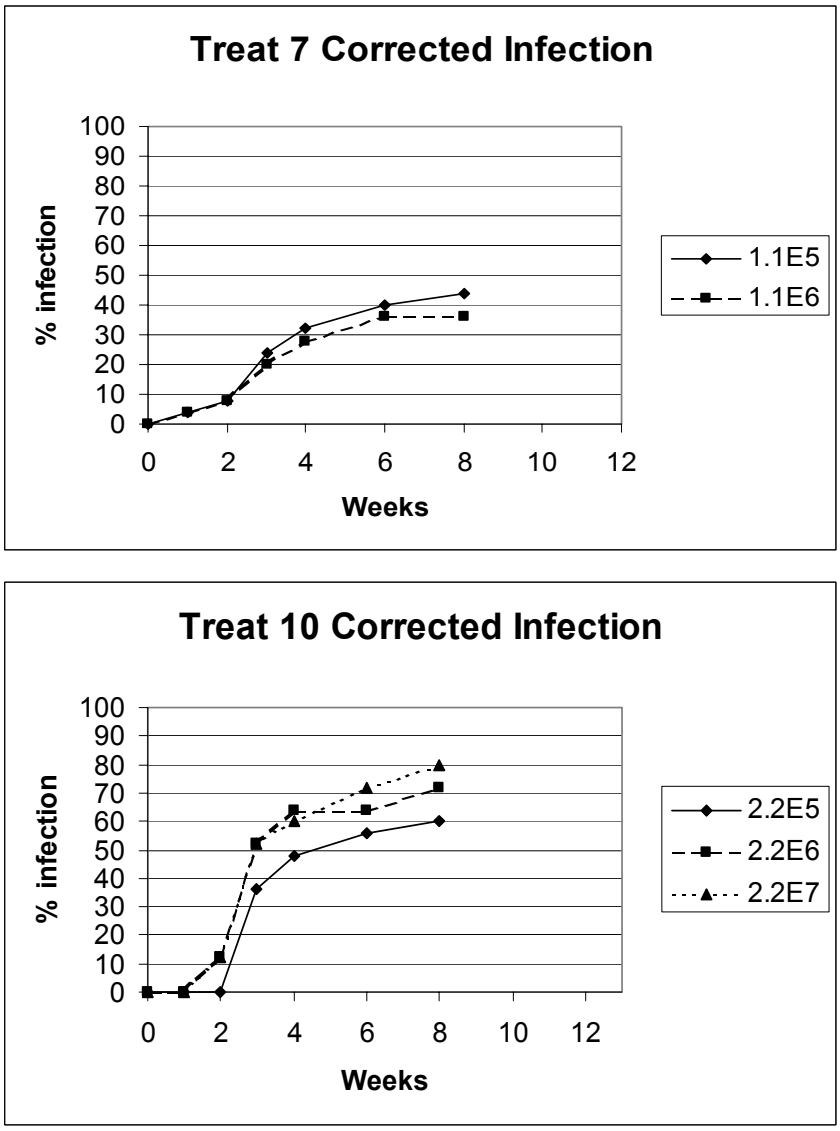


Figure 7. Response of third instar greyback canegrubs to spores from different FI-1045 cultures in 2001

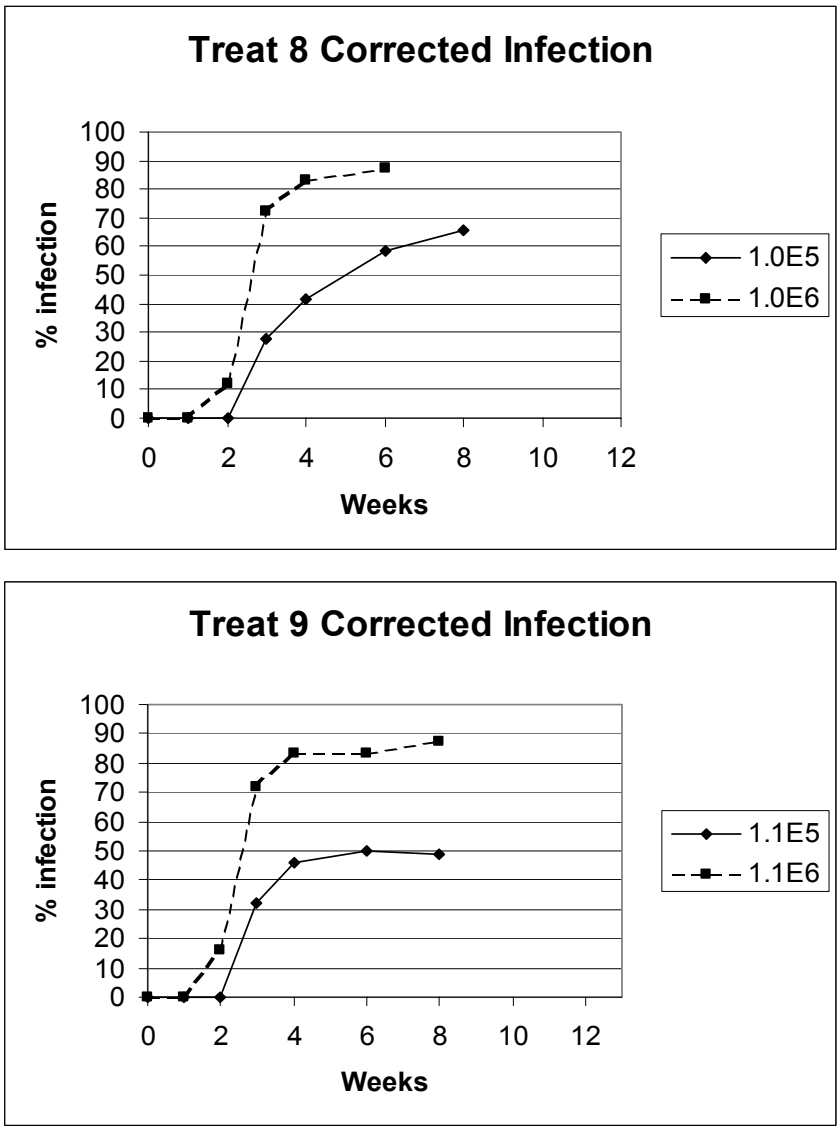
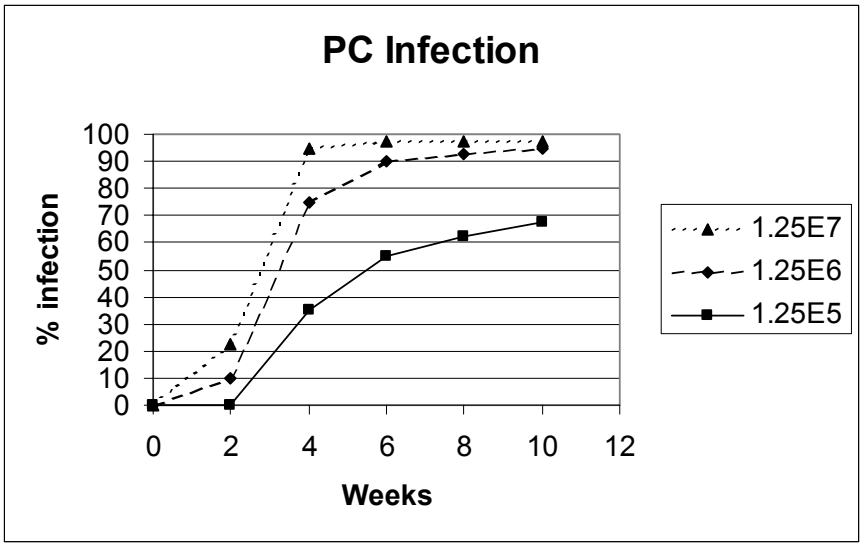


Figure 8. Response of southern one-year canegrubs to spores from different FI-1045 cultures in 2001

15.3 2002

Only two cultures were bioassayed in 2002, with three spore concentrations in peat and 40 greyback grubs per concentration. Results as overt infection are given in Figure 9. The corresponding response in untreated controls was zero on all occasions. Both cultures had good virulence but the GC culture was chosen for 2002 BioCane production due to better sporulation on rice medium.



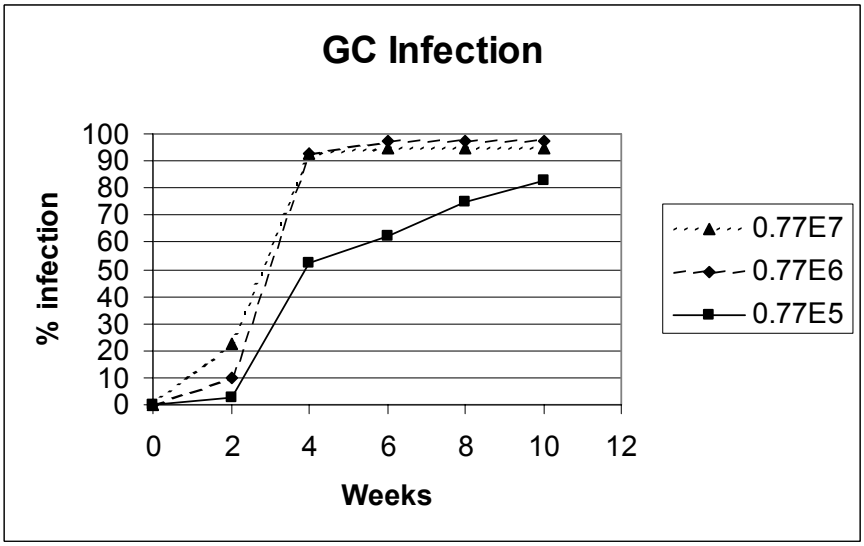


Figure 9. Response of greyback canegrubs to two cultures of FI-1045 (BC623PC and BC623 GC) in 2002 bioassays

16.0 REFERENCES

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Pung, S.H., Rath, A.C., Wright, P.J. and Headlam, N. (1993) Effect of pesticides on germination, growth and infectivity of the entomogenous fungus *Metarhizium anisopliae*. *Pest Control and Sustainable Agriculture* (ed Corey, S.A., Dall, D.J. and Milne, W.M.), 264-267. CSIRO, Melbourne.

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APPENDIX 1

SAMPLING DATES

1997 *Metarhizium* canegrub trials (southern) – sampling.

Trial	Farm	Type	Applied	3 week	6 month	1 year	2 year	3 year	Grubs
ES97-6	McCarthy	Cons.-P	16/9/1997	6/10/1997	16/3/1998	19/1/1999	8/2/2000	4/1/2001	11/2/1999 25/2/2000 14/3/2001*
ES97-7	Petersen	Cons.-P	17/9/1997	6/10/1997	17/3/1998	22/2/1999	9/2/2000	3/1/2001	5/3/1999 9/2/2000 6/4/2001
ES97-10	DePaoli	Cons.-P	13/10/1997 19/12/1997	3/11/1997	13/4/1998 19/6/1998	18/1/1999	8/2/2000	4/1/2001	18/2/1999 24/2/2000 6/3/2001*

* full sample for canegrubs, otherwise untreated plots only.

1998 *Metarhizium* canegrub trials (southern) – sampling.

Trial	Farm	Type	Applied	3 week	6 month	1 year	2 year	Grubs
ES98-9	Laufer	Negatoria-P	13/10/98	12/11/98	20/4/99	abandon	-	13/12/99
ES98-10	Bunn	Childers-P	16/10/98	12/11/98	30/4/99	15/11/99	3/1/2001	2/12/99*
ES98-11	Campbell	Negatoria-P	6/11/98	8/12/98	22/4/99	7/2/00	2/1/2001	30/11/99 14/12/00 28/11/01
ES98-15	Laufer	Negatoria-R	4/12/98	11/1/99	22/4/99	abandon	-	13/12/99
ES98-16	Hetherington	Negatoria-P	11/12/98	11/1/99	27/4/99	abandon	-	8/12/99*

* full sample for canegrubs, otherwise untreated plots only.

1999 *Metarhizium* canegrub trials (southern) – sampling.

Trial	Farm	Type	Applied	3 week	6 month	12 month	2 year	Grubs
ES99-4	Sellers	Consanguineus-P	11/08/1999	8/09/1999	8/02/2000	4/01/2001	7/02/2002	6/04/2001 7/3/2002
ES99-6	Steemson	Picticollis-P	18/08/1999	6/09/1999	7/02/2000	5/01/2001	16/10/2001	27/2*,12/4/2001* 18/04/2002
ES99-12	Hetherington	Negatoria-P	26/10/1999	15/11/1999	10/05/2000	2/01/2001	6/02/2002	12/12/2000 11/12/2001
ES99-18	Chapman	Negatoria-P	24/11/1999	9/02/2000	10/05/2000	4/01/2001	5/02/2002	14/12/2000 28/11/2001

* full sample for canegrubs, otherwise untreated plots only.

2000 *Metarhizium* canegrub trials (southern) – sampling.

Trial	Farm	Type	Applied	3 week	6 month	12 month	2 year	Grubs
ES00-3	Rasmussen	Consanguineus-P	9/03/2000	10/05/2000	3/10/2000	29/03/2001	7/02/2002	19/03/2002
ES00-4	Harney	Negatoria-P	31/03/2000	10/05/2000	3/10/2000	28/03/2001	5/02/2002	11/12/2001
ES00-7	Peterson	Negatoria-P	18/08/2000	3/10/2000	28/03/2001	5/02/2002**		11/12/2001
ES00-8	McCarthy	Consanguineus-P	24/08/2000	4/10/2000	na	16/10/2001		2/04/2002*
ES00-9	Frederickson	Negatoria-P	25/08/2000	4/10/2000	29/03/2001	6/02/2002**		12/12/2001
ES00-11	Cook	Rugulosus-P	15/09/2000	5/10/2000	27/03/2001			17/05/2002

* full sample for canegrubs, otherwise untreated plots only.

** 18 month.

2001 *Metarhizium* canegrub trials (southern) – sampling.

Trial	Farm	Type	Applied	3 week	6 month	Grubs
ES01-4	Mizzi	Consanguineus-P	22/08/2001	17/10/2001	-	2/04/2002
ES01-5	Taylor	Consanguineus-P	31/08/2001	17/10/2001	-	1/03/2002*
ES01-6	Maeyke	Consanguineus-P	7/09/2001	17/10/2001	-	18/03/2002
ES01-7	Ricciardi	Consanguineus-P	11/09/2001	17/10/2001	-	25/03/2002*

* full sample for canegrubs, otherwise untreated plots only.

1998 *Metarhizium* canegrub trials (Central) – sampling.

Trial	Farm	Type	Applied	3 week	6 month	12 month	2 year	3 year	Grubs
EC98-2	Schembri	Frenchi-P	3/08/1998	25/08/1998	23/03/1999	8/10/1999	20/09/2000	9/01/2002	14/12/1999 21/12/2000 18/12/2001
EC98-3	Pace	Frenchi-P	11/08/1998	7/09/1998	18/03/1999	14/09/1999	na	na	14/12/1999
EC98-4	Scibberas	Frenchi-P	6/11/1998	23/11/1998	31/05/1999	5/11/1999	7/11/2000	17/12/2001	15/12/1999 19/12/2000* 21/01/2002
EC98-5	Azzopardi	Frenchi-1R	16/10/1998	30/10/1998	22/04/1999	5/11/1999	7/11/2000	na	13/12/1999 4/12/2000
EC98-9	Kane	Frenchi-P	18/11/1998	9/12/1998	31/05/1999	15/11/1999	19/10/2000	15/01/2002	30/11/1999 11/12/2000 18/12/2001

* full sample for canegrubs, otherwise untreated plots only.

1999 *Metarhizium* canegrub trials (Central) – sampling.

Trial	Farm	Type	Applied	3 week	6 month	12 month	2 year	Grubs
EC99-1	Woodman	Frenchi-P	12/08/1999	31/08/1999	13/03/2000	9/01/2001	16/11/2001	21/12/2000 23/01/2002
EC99-2	Woodman	Frenchi-1R	2/09/1999	23/09/1999	15/03/2000	9/01/2001	16/11/2001	13/12/1999 21/12/2000 23/01/2002
EC99-3	Lamb	Frenchi-1R	16/09/1999	14/10/1999	too wet	19/10/2000	24/01/2002	30/11/1999 18/12/2001
EC99-4	Sciberras	Frenchi-1R	29/10/1999	19/11/1999	16/05/2000	20/10/2000	17/12/2001	27/1/2000* 19/12/2000* 21/1/2002
EC99-5	Zarb	Frenchi-P	2/11/1999	30/11/1999	15/05/2000	28/11/2000	10/01/2002	11/12/2000 18/12/2001
EC99-6	Simpson	Frenchi-P	18/11/1999	13/12/1999	19/05/2000	8/12/2000	23/01/2002	12/12/2000* 26/2/2001* 28/01/2002
EC99-7	Finn	Frenchi-P	10/12/1999	21/12/1999	6/06/2000	28/11/2000	24/01/2002	11/12/2000 18/12/2001

* full sample for canegrubs, otherwise untreated plots only.

2000 *Metarhizium* canegrub trials (Central) – sampling.

Trial	Farm	Type	Applied	3 week	6 month	12 month	Grubs
EC00-1	Currie	Frenchi-3R	25/10/2000	28/11/2000	22/05/2001	14/01/2002	14/01/2002
EC00-3	Muscat	Frenchi-1R	1/11/2000	29/11/2000	17/05/2001	15/01/2002	18/12/2001
EC00-4	Galea	Frenchi-P	4/12/2000	20/12/2000	22/05/2001	12/12/2001	21/01/2002
EC00-6	Zarb	Frenchi-P	16/11/2000	29/11/2000	17/05/2001	10/01/2002	18/12/2001

* full sample for canegrubs, otherwise untreated plots only.

2001 *Metarhizium* canegrub trials (Central) – sampling.

Trial	Farm	Type	Applied	3 week	6 month
EC01-11	Galea	Frenchi-1R	11/10/2001	15/11/2001	12/04/2002

* full sample for canegrubs, otherwise untreated plots only.