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FARMING SYSTEMS THAT OPTIMISE THE CONTROL OF GREYBACK CANEGRUBS BY BIOCANE™

by

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SUMMARY

The carry-over of BioCane from one crop to the next is likely to be affected by soil cultivation during seedbed preparation. *Metarhizium* spores were concentrated in the rows in ratoon crops, either as spores surviving from the original BioCane application or as new spores produced by cadavers that are likely to be aggregated beneath stools. Soil disturbance was analysed using plastic beads as a marker. There was surprisingly little lateral displacement of the beads after cultivation. However, the rows themselves are likely to be displaced when crops are replanted unless minimum tillage planting into the old rows is practiced. In one field site prepared conventionally, spore concentrations were much lower in the new crop than they had been in the old crop.

There was no effect of a trash blanket on grub response to BioCane in bins. Death of grubs following contact with BioCane may be slower at lower temperatures (<24°C), but the apparent difference was small. Spore concentrations in soil were not significantly affected by trash blanketing in most experiments. Overall, we were unable to conclude that trash blanketing will influence the efficacy of BioCane in commercial fields.

There was no deleterious effect of the insecticides suSCon Plus, Confidor CR (controlled release) or Confidor SC (liquid), or of the fertilisers sulphur, gran-am or urea, on the survival of spores on BioCane granules in PVC rings. This is in agreement with field observations, where there have been no complaints from farmers who have applied BioCane close to some of these other products in commercial practice. However, abnormally low concentrations of spores in the rings indicate technical problems with the product that reduce confidence in the results.

The value of combining BioCane with chemical insecticides was investigated. In three trials conducted with suSCon Plus, there was little or no benefit in including BioCane in a combination treatment, with the effect of suSCon being dominant. The combination of BioCane with Confidor as a plant cane treatment gave promising results in the plant crop in one trial. There was no interaction between effects of the two products in the statistical analysis of yields, that is, the effect of each seemed to be additive. Both Confidor and BioCane reduced numbers of live grubs when applied together in pots. This project taken alone has not produced good justification for the use of BioCane combined with chemical insecticides, but other trials are in place to collect additional data.

When grubs are killed by *Metarhizium*, their dead bodies (cadavers) may produce a new crop of spores in the soil. Cadavers are not often recovered from BioCane-treated fields, but this does not mean that treatment has been unsuccessful. We found that cadavers in soil became unrecognisable after 2-3 weeks. A bioassay showed that soil that had contained cadavers remained highly infective to greybacks for at least one year. Annual infection of greybacks and production of cadavers has the potential to top-up spores in soil ('biomagnification'), although probably not enough to prevent a gradual decline in levels.

The distribution of spores produced by cadavers will be highly aggregated in soil, so the role of cadavers in the perpetuation of greyback control is uncertain. A complex and labour-intensive trial to measure the effect of canegrub density on biomagnification and consequent efficacy of *Metarhizium*, which was intended to be a key part of this project, ultimately failed despite two attempts to establish the experimental treatments. It proved

to be impossible to reliably establish the required canegrub densities in the experimental microplots. This trial was abandoned by mutual agreement at the project review in July 2001.

Grub numbers measured in the first and second ratoons of commercial trials established in 1997 did not provide convincing evidence for long-term benefits of BioCane, partly because of the small number of infested trials and also because some did not include untreated strips. There is also the complication that occurs with all greyback trials, that plots protected by pesticides in one year may suffer disproportionate damage in the following year because of the attraction of ovipositing beetles to taller cane. Taken at face value, results suggest reductions in grub numbers of 20-40% in ratoon crops.

A bin experiment to compare different BioCane formulations, while not producing statistically significant differences between treatments, did indicate that a spore suspension sprayed in a band in soil may have comparable efficacy to the standard granular formulation. An experiment with different formulations in tubes also indicated that the more even distribution of spores that results from a liquid formulation should have comparable efficacy to granules. A liquid *Metarhizium* product could be used in novel ways that may allow new grub control strategies.

1.0 BACKGROUND

Greyback canegrub (*Dermolepida albohirtum* (Waterhouse)) is the major insect pest of sugarcane grown from Sarina to the wet tropics of Queensland. In the Burdekin cane-growing district, damage to sugarcane due to greyback canegrub is common on free-draining soils. BioCane™, a rice-based formulation of the entomopathogen *Metarhizium anisopliae* Metschnikoff (Sorokin) manufactured by Bio-Care Technology Pty Ltd, is registered for the control of greyback canegrub. Application of BioCane to plant crops reduces grub numbers by abut 50% by the following March (Samson *et al.* 1999). The resulting cadavers (mummified dead grubs killed by *Metarhizium*) may then produce spores that add to the *Metarhizium* inoculum in the field for subsequent ratoons ('biomagnification'). However, few data are available to estimate the efficacy of BioCane in ratoon cane. In this study, the dynamics of BioCane under current farming practices in the Burdekin were determined.

Farming practices likely to affect the efficacy of BioCane include the level of soil disturbance before planting and in ratoons, trash-retention or trash removal after harvest, and the application of chemical insecticides and fertilisers.

Intensive tillage operations to prepare seedbeds probably disperse and expose *Metarhizium* spores to sunlight, reducing the inoculum available to infect grubs in subsequent generations.

In the wet tropics, fewer grubs are sometimes found under trash-blankets than where trash has been burnt after harvest (Robertson and Walker 1996). Trash blanketing may be associated with higher disease levels in greyback canegrubs in areas such as Tully compared with the Burdekin where no trash is retained and disease levels are low. A difference in disease levels under trash and bare soil may be related to differences in soil temperature, as temperatures under trash blankets are typically cooler than in bare soil.

In the Burdekin, trash cover is rarely retained, weed control in the bare rows is by cultivation and herbicide, and fields are frequently ploughed out and replanted. These factors may militate against long-term BioCane efficacy.

The efficacy of BioCane combined with chemical insecticides such as suSCon Blue or Plus and Confidor is unknown. These chemicals may enhance the efficacy of BioCane, or alternatively, chemical insecticides may reduce any biomagnification of *Metarhizium* by killing infected larvae before sporulation can occur. In addition, chemical insecticides and fertilisers may have a direct effect on viability of *Metarhizium* spores.

Nothing is known of the impact of biomagnification on canegrub control. Canegrub density is likely to be important for biomagnification, as it affects the potential number of cadavers and resulting level of inoculum available to infect future generations. Here we investigate the role of cadavers in the control of canegrubs.

2.0 OBJECTIVES

- Identify farming practices that maximise survival of *Metarhizium* in the Burdekin canegrowing soils.
- Determine how canegrub density influences the control of greyback canegrub by BioCane in the Burdekin.
- Develop and extend guidelines to assist canegrowers to maximise effectiveness of BioCane as an insecticide.
- Integrate these guidelines into the IPM program for canegrubs.

Considerable progress was made with the first of these objectives, with farming practices identified that included cultivation method, compatibility with chemical fertilisers and insecticides, and combinations with chemical insecticides to improve efficacy. However, limited progress was made with the second, due to technical difficulties with the planned experiments. The results with BioCane show that it is most suitable for fields with a low risk of greyback infestation, and it has been proposed for that use at GrubPlan workshops in 2001 and 2002. Work is continuing to better define the risk of greyback infestation (BSS257 - GrubPlan2), and to further develop combinations of BioCane with chemical insecticides and novel use patterns for the product in ratoon crops.

3.0 FARMING PRACTICES AND *METARHIZIUM* SURVIVAL

3.1 Effect of soil disturbance on persistence of *Metarhizium*

3.1.1 Materials and methods

3.1.1.1 Seedbed formation

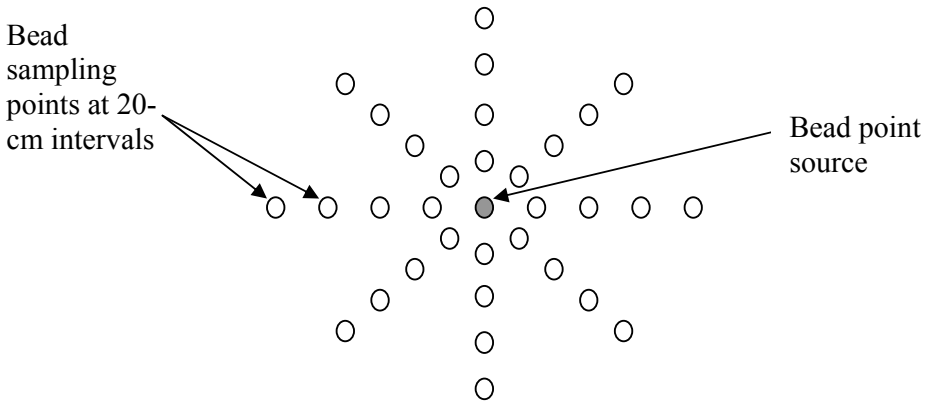

Figure 1. Representation of sampling pattern for plastic beads introduced to soil to study the likely movement of *Metarhizium* in soil during seedbed preparation.

Single columns of plastic beads 40 cm deep were buried in fields to determine the possible dispersal of *Metarhizium* in surface soil with soil preparation for planting (Fig. 1). Beads were buried in two separate columns in a field partly prepared for planting at

the BSES Burdekin. Two columns were buried at Poli's farm, UpRiver Home Hill, in areas to be prepared using either conventional or minimum tillage operations. A column was also added to soil in a minimum tillage seedbed at Mottin's farm, Jarvisfield. After planting beds were formed, the columns of beads were located and 30 mm diameter cores of soil were taken at 20-cm distances to 80 cm in eight different vectors (Fig. 1). The core segments were sieved, and any plastic beads removed and weighed.

The effect of ploughout on carry-over of BioCane from one crop to the next was assessed in a second ratoon field (Fabbro). Soil samples were taken in July 2001 after harvest but before ploughout. Six cores were taken and segmented in 10 cm increments to 40 cm depth. Similar sampling was carried out in 2002 in the new crop when hills were fully formed.

3.1.1.2 Ratoon cultivation

Soil samples for spore counts were taken in five first ratoon fields in 1999. The presence of plastic marker, introduced in the plant crop with BioCane, was used to locate soil samples under the stool. Soil was sampled from the stool, hill shoulder and interrow following harvest of the plant crop and prior to any watering and cultivation. Sites were sampled again after the final cultivation.

3.1.2 Results

3.1.2.1 Seedbed formation

At all sites, most beads were found within 20 cm of the point source, but some were found up to 80 cm away (Fig. 2). The distribution of beads from the minimum tillage and conventional tillage sites at Poli's farm did not differ greatly (Fig. 2). At all sites, some beads were observed on the soil surface up to 2 m away from the point source.

At Fabbro, most spores were found in the top 20 cm of soil in the old crop (Table 1). Levels were much lower in the new crop after replanting, particularly in this surface zone (Table 1).

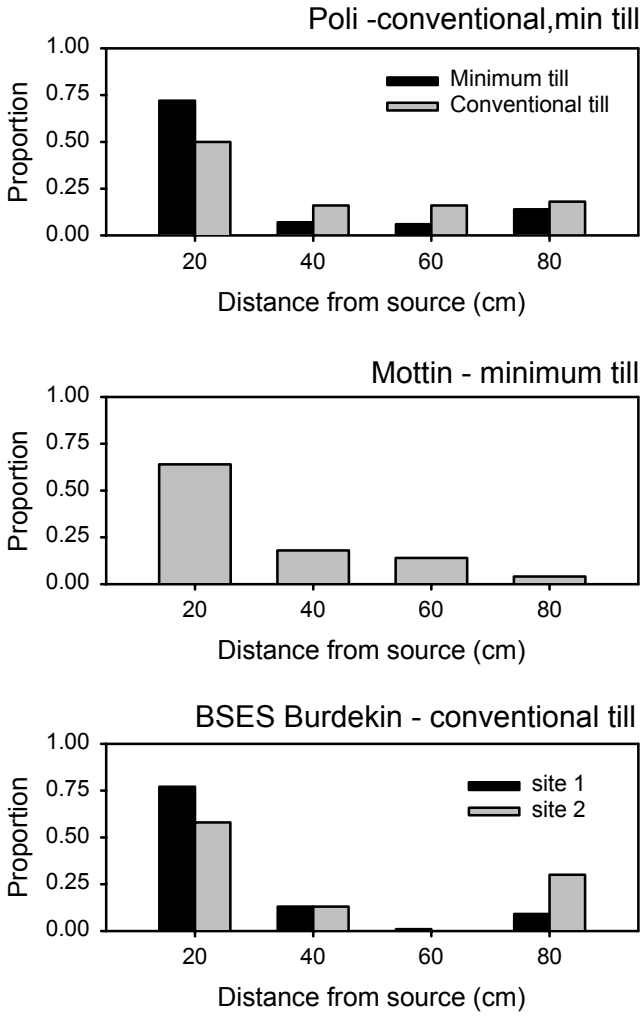

Figure 2. Displacement of plastic beads from a point source under conventional and minimum tillage seedbed preparation. Y-axis is the proportion of mean weight of beads recovered from soil samples for each interval from the source.

TABLE 1
Spore concentrations (x 10^4/g oven dry soil) of FI-1045 in samples taken at different depths from Fabbro before ploughout and after replanting.

Depth	Old crop	New crop
0-10 cm	3.21	0.17
10-20 cm	3.82	0.39
20-30 cm	0.19	0.0
30-40 cm	0.0	1.26

3.1.2.2 Ratoon cultivation

Immediately after harvest, more spores were found in the stool than in the interrow at three of five sites (Table 2). Two sites (Kelly, Schultz) had relatively low counts of spores in the stool. Spore levels after the final working were consistently low for all sites. The cause of the reduction in spore levels after the final working at sites where the initial counts were relatively high is unknown. Plastic marker introduced in the plant crop was found only under the stool and was apparently undisturbed, so it is unlikely that cultivation was the cause of the reduction.

TABLE 2
Spore counts (x 10^3 spores/g wet soil) for samples taken from the stool, hill shoulder and interrow of first ratoons of BioCane-treated crops (the figures for the stool and hill shoulder are means for samples taken at 0-10cm and 10-20cm deep).

Site	Sample location	Before first cultivation	Mid-period	After final cultivation
La Spina	Stool	25	30.5	4
	Shoulder	3	2.5	6.5
	Interrow	0	0	1
Fabbro	Stool	24.5	27	3.5
	Shoulder	1.5		1.65
	Interrow	1	4	1
Schultz	Stool	0.85		3.45
	Shoulder	0.75		0.95
	Interrow	0.4		0.8
Kelly	Stool	8		3.1
	Shoulder	1.15		1.45
	Interrow	37		0
Gabiola	Stool	35.1		
	Shoulder	29		
	Interrow	3		

3.2 Effect of trash-blanketing on efficacy of BioCane

3.2.1 Materials and methods

3.2.1.1 Trash and *Metarhizium* efficacy in microplots

A trial was established at the Burdekin BSES in August 2000. Trash was established on four of eight plots (3 m x 1 row) in a first ratoon crop in July 2000. Plots were enclosed with plastic sheeting. Second instar greybacks were introduced to plots in mid-January 2001, 50 per plot. Plots were sampled for surviving canegrubs 13.5 weeks later in mid-April 2001. The proportions of grubs surviving were compared between treatments by *t*-test following the arc sine (angular) transformation.

3.2.1.2 Trash and *Metarhizium* efficacy in bins

A factorial experiment was established with and without BioCane and with and without trash in large bins (40 cm diameter) containing cane plants, with ten replications. Treatments were applied in November 2001 and first instar greybacks were introduced into the bins on 2 January 2002, 30 per bin. Grub survival and weights were assessed on 9 April 2002. Three rings containing BioCane at a high application rate were buried in two bins with and without trash to assess the effect of trash on spore survival.

3.2.1.3 Temperature and *Metarhizium* efficacy

Trash blankets reduce temperature near the soil surface. Two laboratory experiments were established to determine whether temperature influences the infection rate of second/third instar greyback canegrubs with *Metarhizium*. Inoculation of larvae was standardised by forcing individual larvae to travel down a vertical column of soil within PVC pipe or tube ('Chandler tubes', 150 mm length) with or without BioCane granules. Grubs emerging from the far end of each tube were placed into containers of fresh soil at a constant temperature and fed carrot and grass seedlings, and checked regularly for death or overt infection by *Metarhizium*. In the first experiment in 2001, tubes (25 mm diameter) contained 0.02 g BioCane (usually two or three granules) and grubs were subsequently transferred to four temperatures, 20, 24, 27 and 30° C. In the second experiment in 2002, tubes (30 mm diameter) contained a single granule only; response following passage through tubes containing granules was corrected for untreated response using Abbott's formula.

3.2.1.4 Trash and *Metarhizium* survival in rings

To estimate the effect of trash on *Metarhizium* spore survival, PVC rings containing soil and eight BioCane granules were buried at two sites, Burdekin SES on 27 July 2001 and Kennif on 5 September 2001, with six rings covered with trash and six rings uncovered at each site. Three rings in each treatment were buried 10 cm deep and the other three rings were buried at 20 cm. Rings were retrieved on 16 April 2002 and spore concentrations measured.

3.2.1.5 Trash and spore persistence in field samples

Soil in rows with and without trash was sampled for *Metarhizium* in one first ratoon field, which was sampled again in the second ratoon, and one second ratoon field. Each field had been treated with BioCane at 33 kg/ha in the plant crop, and three replicate strips with and without trash established by raking. Three or five soil samples were taken from each strip by mechanical auger and spore concentrations measured.

3.2.2 Results

3.2.2.1 Trash and *Metarhizium* efficacy in microplots

There was no difference in the proportion of third instars surviving in plots with a trash blanket (mean ± SE = 0.19 ± 0.06) or without (0.13 ± 0.02) (t = 0.95, df = 6, P = 0.38). No cadavers were retrieved from plots.

3.2.2.2 Trash and *Metarhizium* efficacy in bins

Grub survival was very poor even in untreated soil (12%). There were significantly fewer live grubs in pots treated with BioCane (1.8 ± 0.4 SE) than in untreated pots (3.5 ± 0.5 SE) ($P = 0.007$) (Table 3). The response to BioCane was not influenced by the presence or absence of trash ($P_{interaction} = 0.49$), and there was no significant effect of trash on numbers of live grubs ($P = 0.86$). There were significantly more infected grubs where soil was treated with BioCane than in untreated soil (Table 3, $P = 0.038$).

Average weight of grubs was greater in pots covered with trash (4.9 ± 0.1 SE, g) than in bare pots (4.3 ± 0.3 SE, g) ($P = 0.049$) (Table 3). There was no significant effect of BioCane on grub weight ($P = 0.067$), and there was no interaction between effects of BioCane and trash ($P = 0.92$).

Spore concentrations were high in rings buried in the bins, 135 (± 12 SE) x 10^4 without trash and 213 (± 18 SE) x 10^4 with trash; these concentrations were significantly different ($P = 0.022$).

TABLE 3
Numbers of live and infected greyback canegrubs and average weight of live grubs in bins with different combinations of BioCane and trash.

BioCane	Trash	Live grubs (mean ± SE)	Infected grubs (mean ± SE)	Grub weight (mean ± SE)
No	No	3.3 ± 0.7	0.0 ± 0.0	4.1 ± 0.4
	Yes	3.6 ± 0.7	0.0 ± 0.0	4.6 ± 0.2
Yes	No	2.0 ± 0.7	0.3 ± 0.2	4.6 ± 0.2
	Yes	1.5 ± 0.3	0.3 ± 0.2	5.1 ± 0.1

3.2.2.3 Temperature and *Metarhizium* efficacy

In the first experiment, the development of infection with *Metarhizium* was rapid for larvae in all temperature regimes (68-92% infection after 12 days) (Fig. 3). Mortality after 75 days was 94-100% for inoculated larvae. All inoculated larvae that died, died from *Metarhizium* infections. The dose received by larvae was too high for any effect of temperature on infection rate to be apparent. Temperature control failed in the 30° C cabinet and temperatures reached 35° C, resulting in the death of untreated larvae (Fig. 3).

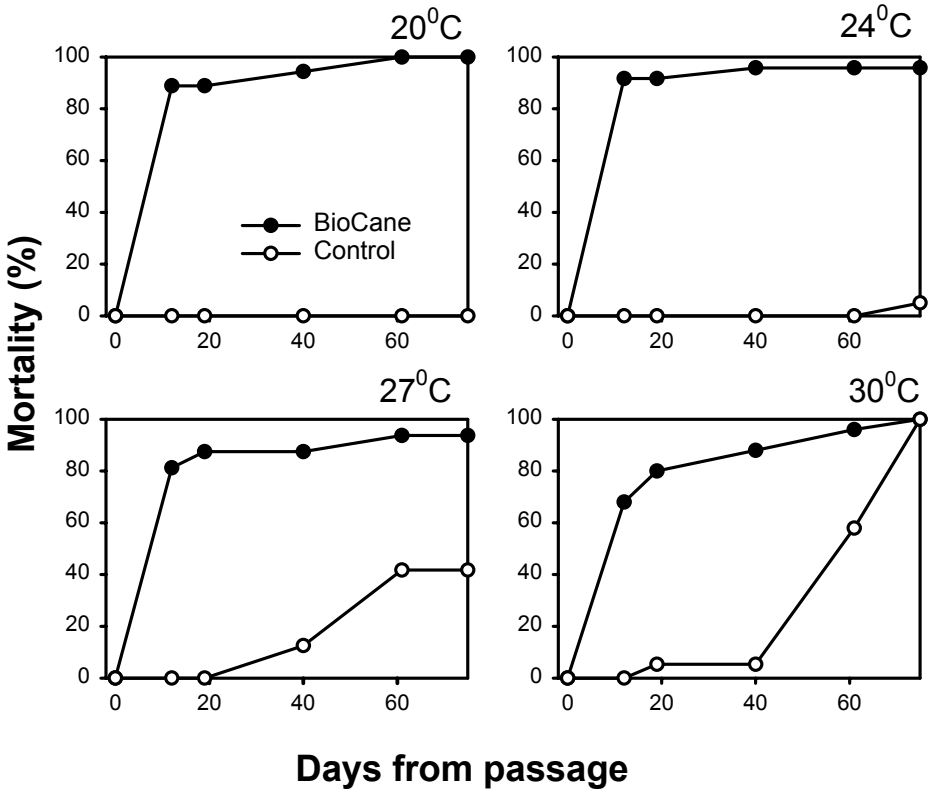

Figure 3. The effect of temperature mortality of young third instar greyback canegrubs after passage through a column of soil with and without 0.02 g of BioCane granules.

In a second bioassay, response seemed slower at 20° and 24°C than at 27°C or 30°C (Fig. 4). The analysis was not continued beyond 53 days as mortality subsequently exceeded 20% in untreated soil at the higher temperatures, reaching 53% and 39% at 27°C and 30°C, respectively, after 73 days, compared with 11% at 24°C. Almost all grubs that died in treated soil showed symptoms of *Metarhizium* infection, with negligible overt infection in untreated soil. Response was slower than in the first bioassay, presumably reflecting the smaller number of BioCane granules within the tubes or the greater diameter of each tube.

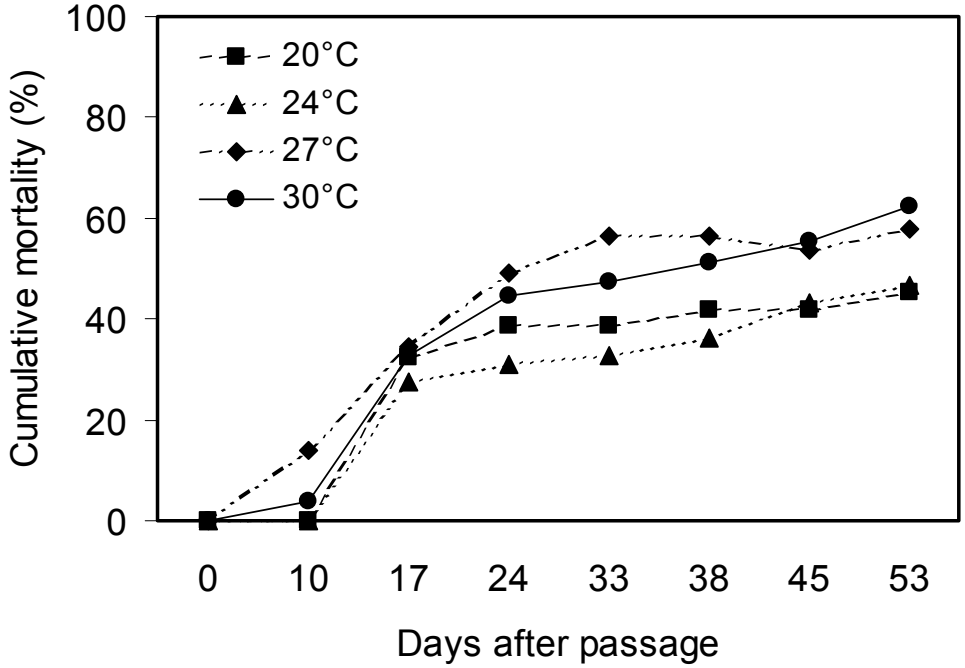

Figure 4. The effect of temperature on mortality (corrected for untreated) of young third instar greyback canegrubs after passage through a column of soil containing one BioCane granule.

3.2.2.4 Trash and *Metarhizium* survival in rings

There was no significant effect of depth (P = 0.77 and 0.50) or trash (P = 0.14 and 0.57) on spore concentrations in rings at Sites 1 and 2, respectively (Table 4), and there was no interaction between effects of depth and trash (P = 0.14 at both). The means across both sites also indicate no effect of trash at 10 cm, where any effect is more likely to be manifested than at 20 cm.

TABLE 4
Spore concentrations (x 10^4/g, mean ± SE) after eight BioCane granules were buried in rings at two depths beneath bare soil or a trash blanket, at two field sites for 7-9 months.

Depth (cm)	Trash	Site 1	Site 2	Mean
10	No	2.5 ± 1.4	4.1 ± 1.3	3.5 ± 0.9
	Yes	2.3 ± 0.5	2.0 ± 0.7	2.2 ± 0.4
20	No	1.3 ± 0.5	2.9 ± 0.5	2.1 ± 0.5
	Yes	6.1 ± 3.2	4.4 ± 1.4	5.3 ± 1.6

Spore concentrations were abnormally low in this experiment, as well as others set up at about the same time (see also Section 3.3.2). If granules have an average weight of 13 mg, and with a spore level in the BioCane product of 2 x 10^9/g, the expected spore concentration with eight fresh granules in 100 g of soil would be about 200 x 10^4/g, and

this would be expected to decline by about half during the time rings were buried in the field. In a similar study of FI-1045 persistence at different initial spore concentrations at Mackay, rings buried with only four BioCane granules contained 25 x 10^4 spores/g after 1 year (Final Report, BSS234).

3.2.2.5 Trash and spore persistence in field samples

In the first ratoon at Fowler's farm, spore counts were higher under trash blankets than under burnt trash, but not significantly so (t = 2.9, df = 2, P = 0.10) (Table 5). Spore counts were low in the second ratoon.

TABLE 5
Spore counts per g wet soil from the BioCane application zone in two blocks (A and B) on Fowler's farm, Upriver Home Hill, in the first ratoon (1R) and second ratoon (2R).

Treatment	Fowler A		Fowler B	
	1R	2R[1]	1R[2]	2R[1]
Trash-blanket	NC	3.9 x 10^3	5.8 x 10^3	4.0 x 10^3
Burnt	NC	Na	3.3 x 10^3	15.7 x 10^3

[1] soil sampled from 0-20 cm.
[2] soil sampled from 10-20 cm

3.3 Effect of fertilisers and insecticides on *Metarhizium*

3.3.1 Materials and methods

The effect of sulphur pastilles, gran-am and urea on BioCane was assessed in PVC rings (50 mm diameter, 30 mm height). Each ring contained eight BioCane granules either in contact with or separated from eight fertiliser granules, and was buried at 200 mm depth. Rings with BioCane alone were set up as Controls. Each treatment was replicated three times at each of two sites, Burdekin SES and Mottin. Rings were buried on 31 July 2001 and 6 August 2001, respectively; they were retrieved on 16 April 2002 and spore concentrations measured. Spore concentrations were transformed as log(x+1) before one-way analysis of variance.

A second experiment was set up using similar methods but with three insecticides, suSCon Plus, controlled-release Confidor and Confidor 200SC liquid. Eight controlled-release granules were placed into each ring, either in contact with or separated from the BioCane, while Confidor liquid was applied at the equivalent of 2.5 L/ha on top of or away from the BioCane. Rings were buried at two sites, Burdekin SES and Mottin, on 10 October 2001, and retrieved on 16 April 2002.

3.3.2 Results

There was a significant effect of fertiliser treatments on spore concentrations in PVC rings at Site 1 (Table 6, P = 0.035). However, interpretation of this apparent effect is difficult as no spores were detected in rings containing BioCane granules only (untreated). Rings containing sulphur granules had higher spore concentrations than other rings. There was

no significant effect of fertiliser treatments on spore concentrations in rings at Site 2 (Table 6, $P = 0.23$), although again rings containing sulphur tended to have higher spore levels. Spore concentrations were abnormally low in all rings, as was observed previously in an experiment established at about the same time to measure the effect of trash (Section 3.2.2.4)

TABLE 6
Spore concentrations (x 10^4/g, mean ± SE) after eight BioCane granules were buried with fertilisers, either in direct contact or separated, in rings at two field sites for 8-9 months.

Treatment	Site 1	Site 2	Mean
Untreated	0.0 ± 0.0 c	1.6 ± 0.8	0.8 ± 0.5
Sulphur, contact	2.1 ± 1.4 ab	3.9 ± 1.9	3.0 ± 1.1
Sulphur, separate	3.1 ± 1.1 a	2.7 ± 0.5	2.9 ± 0.5
Gran-am, contact	0.4 ± 0.3 bc	1.0 ± 0.2	0.7 ± 0.2
Gran-am, separate	0.7 ± 0.3 bc	1.1 ± 0.6	0.9 ± 0.3
Urea, contact	0.1 ± 0.1 c	1.2 ± 0.2	0.7 ± 0.3
Urea, separate	0.7 ± 0.6 bc	1.3 ± 0.3	1.0 ± 0.3

There was a significant effect of insecticide treatments on spore concentrations in PVC rings at the first site (Table 7, $P = 0.0042$). However, the differences between treatments did not follow a logical pattern; spore concentrations with Confidor liquid applied directly to BioCane granules were significantly higher than with BioCane alone, but there was no effect of Confidor CR in direct contact with granules. No insecticide treatments gave a significantly lower spore concentration than untreated BioCane. There was no significant effect of insecticide treatments on spore concentrations at Site 2 (Table 7, $P = 0.38$). As in the fertiliser experiment, spore concentrations were abnormally low.

TABLE 7
Spore concentrations (x 10^4/g, mean ± SE) after eight BioCane granules were buried with insecticides, either in direct contact or separated, in rings at two field sites for 6 months.

Treatment	Site 1	Site 2	Mean
Untreated	2.3 ± 0.5 bc	2.2 ± 0.9	2.3 ± 0.4
suSCon Plus, contact	2.5 ± 0.4 bc	0.6 ± 0.2	1.6 ± 0.5
suSCon Plus, separate	3.7 ± 1.7 ab	0.9 ± 0.5	2.0 ± 0.9
Confidor CR, contact	1.2 ± 0.5 c	4.1 ± 1.8	2.6 ± 1.1
Confidor CR, separate	0.9 ± 0.3 c	0.7 ± 0.3	0.8 ± 0.2
Confidor SC, contact	7.8 ± 1.8 a	2.6 ± 1.8	5.2 ± 1.6
Confidor SC, separate	na	0.8 ± 0.7	na

In summary, there is no evidence that the fertilisers or insecticides tested have any deleterious effect on survival of BioCane, and in fact it appeared that sulphur and Confidor liquid might be beneficial. However, these apparent results should be treated with scepticism; suSCon Plus granules which are almost 50% sulphur did not increase spore concentrations, while the Confidor result applied in only one experiment and only to

the liquid formulation. The extremely low spore concentrations in all rings suggest a problem with the initial BioCane product or with the experimental procedure. These experiments would need repeating to be confident in the results.

4.0 COMBINING INSECTICIDES WITH BIOCANE

4.1 suSCon Plus and BioCane

4.1.1 Materials and methods

4.1.1.1 Lucas field trial

At Lucas' farm, Upriver Home Hill, a plant crop of cultivar Q117 was treated with factorial combinations of rates of suSCon Plus (0, 20, 40 kg/ha) and BioCane (0, 16, 33 kg/ha) on 22 September 1999. Treatments were applied by hand to plots measuring 13.4 m long and five rows wide, and were replicated six times in a fully randomised design. Grub numbers and crop yields were measured annually. Grubs were counted beneath six stools from each plot in the plant crop and four stools in ratoons. Yields were measured at harvest using a weighing machine for two rows from each plot, and levels of ccs were measured in a six-stalk sample from each plot. Grub counts per stool (first ratoon) or per plot (plant crop) were transformed to the power of 0.44 before analysis of variance.

4.1.1.2 Mottin field trial

A replicated small-plot trial was established in Q117 at Mottin's farm on 24 November 2000, with the same treatments and similar methodology as at Lucas. However, the trial design was a randomised complete block, with plots measuring four rows by 10 m replicated five times. Grubs were counted under four stools from each plot, and plot totals were transformed to the power of 0.44 before analysis of variance.

4.1.1.3 Fabbro field trial

A replicated strip trial was established at Fabbro's farm, Airville on 25 September 2000. Treatments were suSCon Plus at 40 kg/ha and suSCon Plus at 20 or 40 kg/ha combined with BioCane at 33 kg/ha, applied by machine. Each treatment was replicated three times in three-row strips, together with a single untreated strip of five rows. Grubs were counted in the plant crop (eight stools, or 16 in the untreated strip) and first ratoon (six stools), with plot totals transformed to the power of 0.44 before analysis of variance, and first ratoon shoots were counted over a 10 m length in each plot after harvest.

4.1.2 Results

4.1.2.1 Lucas field trial

The plant crop at Lucas' farm was lightly infested in March 2000. There was a significant effect of suSCon Plus application on grub numbers ($P < 0.001$), with numbers at both application rates (20 and 40 kg/ha) lower than in untreated Controls (Table 8). No effect

of BioCane was detected (P = 0.48), and there was no interaction between effects of BioCane and suSCon Plus (P = 0.08).

If results were analysed for individual treatments without considering the factorial combinations, there was a highly significant effect of treatment (P < 0.001) but grub numbers at both rates of BioCane alone were not significantly different from the Controls (P = 0.05).

TABLE 8
Grub numbers per stool at Lucas in the plant crop on 14 March 2000 (± SE).

Rate of BioCane (kg/ha)	Rate of suSCon Plus (kg/ha)			
	0	20	40	Mean
0	1.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.6 ± 0.1
16	1.0 ± 0.3	0.3 ± 0.1	0.2 ± 0.1	0.5 ± 0.1
33	0.6 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	0.3 ± 0.1
Mean	1.0 ± 0.1 a	0.3 ± 0.1 b	0.1 ± 0.1 b	0.5 ± 0.1

Mean grub numbers in suSCon Plus treatments followed by the same letter were not significantly different (P = 0.05).

At harvest of the plant crop, the only significant difference between treatments was the effect of suSCon Plus on CCS (P = 0.041); CCS with suSCon applied at 40 kg/ha was lower than with suSCon at 20 kg/ha or untreated (Table 9). All other effects were not significant (P > 0.05).

TABLE 9
Plant crop yields at Lucas on 13 July 2000 (± SE).

Rate of BioCane (kg/ha)	Rate of suSCon Plus (kg/ha)			
	0	20	40	Mean
Cane yield (t/ha)				
0	93.1 ± 7.7	110.2 ± 5.3	99.1 ± 6.2	100.8 ± 3.9
16	102.9 ± 4.7	96.8 ± 8.5	103.3 ± 5.4	101.0 ± 3.6
33	102.2 ± 5.7	102.1 ± 7.0	100.3 ± 5.3	101.5 ± 3.3
Mean	99.4 ± 3.5	103.0 ± 4.1	100.9 ± 3.1	101.1 ± 2.0
CCS				
0	14.7 ± 0.4	15.0 ± 0.5	14.0 ± 0.4	14.6 ± 0.3
16	14.1 ± 0.3	14.5 ± 0.2	13.9 ± 0.3	14.2 ± 0.1
33	14.9 ± 0.2	14.5 ± 0.1	14.2 ± 0.2	14.5 ± 0.1
Mean	14.6 ± 0.2 a	14.7 ± 0.2 a	14.0 ± 0.2 b	14.4 ± 0.1
Sugar yield (t/ha)				
0	13.5 ± 0.8	16.5 ± 1.0	13.9 ± 0.8	14.6 ± 0.6
16	14.5 ± 0.6	14.1 ± 1.2	14.3 ± 0.5	14.3 ± 0.5
33	15.3 ± 0.9	14.5 ± 1.1	14.2 ± 0.7	14.7 ± 0.5
Mean	14.4 ± 0.5	15.1 ± 0.7	14.1 ± 0.4	14.5 ± 0.3

Mean CCS levels in suSCon Plus treatments followed by the same letter were not significantly different (P = 0.05).

In the first ratoon, there was no interaction between effects of suSCon Plus and BioCane on grub numbers ($P = 0.17$) but effects of both products were significant ($P < 0.001$ and $P = 0.013$, respectively). There were fewer grubs in suSCon-treated plots than in untreated plots, with both application rates being similarly effective (Table 10). Apparent effects of BioCane were confusing, with the higher rate appearing to have more grubs than the lower (Table 10).

TABLE 10
Grub numbers per stool at Lucas in the first ratoon crop on 13 March 2001 (± SE).

Rate of BioCane (kg/ha)	Rate of suSCon Plus (kg/ha)			
	0	20	40	Mean
0	1.5 ± 0.2	0.8 ± 0.2	0.4 ± 0.1	0.9 ± 0.1 ab
16	1.3 ± 0.2	0.4 ± 0.1	0.3 ± 0.1	0.7 ± 0.1 b
33	1.7 ± 0.3	0.8 ± 0.2	1.1 ± 0.2	1.2 ± 0.1 a
Mean	1.5 ± 0.2 a	0.7 ± 0.1 b	0.6 ± 0.1 b	1.1 ± 0.1

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

There was a significant effect of suSCon Plus application on first ratoon yields in July 2001 ($P = 0.032$), with yields at both application rates (20 and 40 kg/ha) higher than in untreated Controls (Table 11). No effect of BioCane was detected ($P = 0.92$), and there was no interaction between effects of BioCane and suSCon Plus ($P = 0.71$).

TABLE 11
First ratoon yields of Q117 at Lucas on 18 July 2001 (t/ha ± SE).

Rate of BioCane (kg/ha)	Rate of suSCon Plus (kg/ha)			
	0	20	40	Mean
0	69.8 ± 7.4	94.5 ± 7.9	89.3 ± 4.6	84.5 ± 4.5
16	79.9 ± 7.2	83.4 ± 10.5	90.8 ± 6.8	84.7 ± 4.6
33	77.9 ± 7.1	92.0 ± 6.6	90.5 ± 6.8	86.8 ± 4.0
Mean	75.9 ± 4.1 b	90.0 ± 4.8 a	90.2 ± 3.3 a	85.4 ± 2.5

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

Grub numbers were low in the second ratoon on 4 March 2002, with only four found under 24 stools in untreated plots and very few under 8-16 stools from each treated plot, so sampling was not completed.

4.1.2.2 Mottin field trial

The trial at Mottin's farm was lightly infested with less than one grub per stool in the plant crop in March 2001. There was a significant effect of suSCon Plus application on grub numbers ($P < 0.001$), with numbers lower at 20 kg/ha and lower again at 40 kg/ha compared with untreated Controls (Table 12). No effect of BioCane was detected ($P = 0.20$), and there was no interaction between effects of BioCane and suSCon Plus ($P = 0.15$).

TABLE 12
Grub numbers per stool at Mottin in the plant crop on 20 March 2001 (± SE).

Rate of BioCane (kg/ha)	Rate of suSCon Plus (kg/ha)			
	0	20	40	Mean
0	0.7 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.3 ± 0.1
16	0.4 ± 0.2	0.2 ± 0.1	0.0 ± 0.0	0.2 ± 0.1
33	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0
Mean	0.4 ± 0.1 a	0.1 ± 0.1 b	0.0 ± 0.0 c	0.2 ± 0.0

Mean grub numbers in suSCon Plus treatments followed by the same letter were not significantly different ($P = 0.05$).

Analysis of the plant crop data in this trial without considering the factorial combinations showed a highly significant effect of treatment on grub numbers ($P < 0.001$). All treatments including both half and full rates of BioCane had fewer live grubs than untreated plots (Fig. 5).

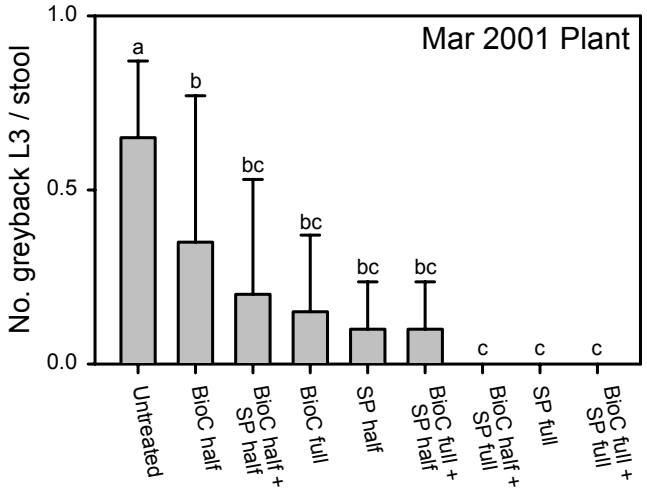

Figure 5. Effect of BioCane and suSCon Plus on numbers of greyback canegrub in the plant crop at Mottin (mean ± SD). Bars with the same letter were not significantly different ($P = 0.05$).

At the plant crop harvest in July 2001, there were no significant effects on cane or sugar yield of suSCon Plus ($P = 0.16$ and 0.36) or BioCane ($P = 0.98$ and 1.00), nor was there any interaction between effects of the two products ($P = 0.38$ and 0.43) (Table 13). There was a significant reduction in CCS where suSCon Plus was applied ($P = 0.011$) but no effect of BioCane ($P = 0.50$) and no interaction ($P = 0.10$).

In the first ratoon in March 2002, there were no significant effects on grub numbers of suSCon Plus ($P = 0.28$) or BioCane ($P = 0.78$), nor was there any interaction between effects of the two products ($P = 0.83$) in a factorial analysis (Table 14). There was also no significant effect of treatment in a non-factorial analysis of variance ($P = 0.79$).

TABLE 13
Plant crop yields of Q117 at Mottin (± SE).

Rate of BioCane (kg/ha)	Rate of suSCon Plus (kg/ha)			
	0	**20**	**40**	**Mean**
Cane yield (t/ha)				
0	113.1 ± 8.5	133.6 ± 3.6	124.4 ± 6.8	123.7 ± 4.2
16	118.9 ± 8.3	131.3 ± 7.4	121.2 ± 4.7	123.8 ± 4.0
33	122.0 ± 2.9	120.3 ± 7.2	131.5 ± 6.5	124.6 ± 3.4
Mean	118.0 ± 3.9	128.4 ± 3.7	125.7 ± 3.5	124.0 ± 2.2
CCS				
0	17.2 ± 0.1	16.4 ± 0.2	16.4 ± 0.2	16.7 ± 0.1
16	16.8 ± 0.2	16.9 ± 0.3	16.1 ± 0.3	16.6 ± 0.2
33	16.6 ± 0.2	16.5 ± 0.2	16.4 ± 0.2	16.5 ± 0.1
Mean	16.9 ± 0.1 a	16.6 ± 0.1 ab	16.3 ± 0.1 b	16.6 ± 0.1
Sugar yield (t/ha)				
0	19.5 ± 1.5	21.8 ± 0.4	20.4 ± 1.1	20.6 ± 0.7
16	20.0 ± 1.5	22.1 ± 1.1	19.5 ± 0.9	20.5 ± 0.7
33	20.2 ± 0.5	19.9 ± 1.4	21.6 ± 1.3	20.5 ± 0.6
Mean	19.9 ± 0.7	21.3 ± 0.6	20.5 ± 0.6	20.6 ± 0.4

Mean CCS levels in suSCon Plus treatments followed by the same letter were not significantly different ($P = 0.05$).

TABLE 14
Numbers of live grubs per stool at Mottin in the first ratoon on 12 March 2002 (± SE).

Rate of BioCane (kg/ha)	Rate of suSCon Plus (kg/ha)			
	0	**20**	**40**	**Mean**
0	0.4 ± 0.2	0.6 ± 0.4	0.2 ± 0.1	0.4 ± 0.2
16	0.3 ± 0.1	0.5 ± 0.2	0.1 ± 0.1	0.3 ± 0.1
33	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Mean	0.3 ± 0.1	0.4 ± 0.2	0.2 ± 0.0	0.3 ± 0.1

4.1.2.3 Fabbro field trial

In the plant crop at Fabbro's farm, there was no significant effect of treatment on grub numbers on 21 March 2001 ($P = 0.08$), although there was a strong indication that treatment was beneficial (note the untreated strip was unreplicated) (Table 15). After harvest, there were significantly more shoots in all treatments compared with the untreated strip in the first ratoon on 11 October 2001 (Table 15, $P = 0.047$). There was no significant effect of treatment on grub numbers in the first ratoon on 18 March 2002 ($P = 0.41$) (Table 15).

TABLE 15
Effect of BioCane and suSCon Plus on numbers of grubs in a strip trial at Fabbro (± SE).

Treatment	Grubs/stool P	Shoots/m 1R	Grubs/stool 1R
BioCane 33kg/ha + SP 40kg/ha	0.5 ± 0.2	31.2 ± 0.9 a	3.3 ± 0.8
BioCane 33kg/ha + SP 20kg/ha	0.8 ± 0.3	35.6 ± 2.3 a	3.8 ± 0.3
suSCon Plus 40kg/ha	0.5 ± 0.2	32.5 ± 2.3 a	4.7 ± 0.1
Untreated	3.1	20.5 b	3.8

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

4.2 Confidor and BioCane

4.2.1 Materials and methods

4.2.1.1 Field trials

Two field trials were established with factorial combinations of BioCane (0, 33, 66 kg/ha) and Confidor 200SC (0, 1, 2 L/ha). Trials were laid out as randomised complete block designs, with five replicates of plots measuring four rows x 15 m (Sgarbossa, Q117) or four rows by 20 m (McDonald, Q189[A]). BioCane was applied in a band during fill-in of the planting furrows. At Sgarbossa, Confidor was applied over the top of the BioCane, also in a band, before covering both with soil (12 November 2001). At McDonald, BioCane was applied in September 2001 and Confidor was then applied on 20 November 2001 using an interrow implement. Grubs were counted in March 2002 under four stools from each plot, and the individual counts were transformed to the power of 0.44 before analysis of variance.

4.2.1.2 Pot trial

An experiment was set up in 300 mm diameter pots containing small sugarcane plants, with factorial combinations of BioCane (0, 33, 66 kg/ha) and Confidor 200SC (0, 0.5, 1, 2 L/ha) applied on 16 November 2001, with 12 replications. Twenty first-instar greybacks were introduced into each pot on 19-20 December 2001. The number of surviving grubs and above-ground fresh weight of cane were recorded on 19-20 March 2002.

4.2.2 Results

4.2.2.1 Field trials

At Sgarbossa, numbers of grubs were very low when checked on 1 March 2002, with no grubs found under 16 stools from the untreated plots and only two grubs under 68 treated stools. Sampling was not completed.

Numbers of grubs at McDonald were low in the plant crop (Table 16). There were no significant effects of Confidor ($P = 0.47$) or BioCane ($P = 0.14$), nor was there any interaction between effects of the two products ($P = 0.96$).

TABLE 16
Numbers of live grubs per stool at McDonald in the plant crop on 8 March 2002 (± SE).

Rate of BioCane (kg/ha)	Rate of Confidor (L/ha)			
	0	1	2	Mean
0	0.7 ± 0.2	0.8 ± 0.3	0.7 ± 0.3	0.7 ± 0.2
33	0.6 ± 0.3	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.1
66	0.4 ± 0.2	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.1
Mean	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.1

Despite the low grub numbers at McDonald, treatment did affect plant crop yields (Table 17). Cane yield was increased by both Confidor (P = 0.030) and BioCane (P = 0.010), with no interaction (P = 0.054). There was no difference in response between the two application rates of either product (Table 17). For sugar yield, Confidor and BioCane again acted independently ($P_{interaction}$ = 0.23), and each significantly increased yield (P = 0.017 and 0.006, respectively) (Table 17). Effects on CCS were more complex, as there was a significant interaction between effects of the two products (P = 0.011). It appears from Table 17 that each product increased CCS only when the alternative product was not applied.

TABLE 17
Plant crop yields at McDonald on 7 July 2002 (± SE).

Rate of BioCane (kg/ha)	Rate of Confidor (L/ha)			
	0	1	2	Mean
Cane yield (t/ha)				
0	145.4 ± 8.0	148.9 ± 8.1	160.3 ± 2.3	151.5 ± 4.0 b
33	159.5 ± 1.2	159.3 ± 6.9	169.2 ± 7.5	162.7 ± 3.4 a
66	152.6 ± 4.2	183.9 ± 5.5	165.7 ± 5.4	167.4 ± 4.4 a
Mean	152.5 ± 3.2 b	164.0 ± 5.4 a	165.1 ± 3.1 a	160.5 ± 2.4
CCS				
0	14.5 ± 0.3	14.9 ± 0.1	15.6 ± 0.2	15.0 ± 0.2
33	14.7 ± 0.2	15.3 ± 0.3	15.0 ± 0.1	15.0 ± 0.1
66	15.4 ± 0.1	14.9 ± 0.3	15.6 ± 0.3	15.3 ± 0.2
Mean	14.8 ± 0.2	15.0 ± 0.2	15.4 ± 0.1	15.1 ± 0.1
Sugar yield (t/ha)				
0	21.1 ± 1.4	22.1 ± 1.6	25.0 ± 0.5	22.8 ± 0.8 b
33	23.5 ± 0.5	23.6 ± 0.7	25.4 ± 1.2	24.2 ± 0.5 ab
66	23.5 ± 1.1	27.4 ± 1.0	25.9 ± 1.0	25.9 ± 0.7 a
Mean	22.6 ± 0.7 b	24.6 ± 0.9 ab	25.4 ± 0.5 a	24.3 ± 0.4

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

There was no relationship between grub numbers and cane yield at McDonald (P = 0.18 by linear regression), and it has already been noted that treatment effects on grub numbers were not significant. Crop yields appear to have been a better indicator of the effect of treatment than grub counts.

4.2.2.2 Pot trial

Grub survival was extremely poor in the pot trial, with an average of only 0.8 grubs/pot retrieved from untreated soil (ie 4% survival). The reason is unknown, although the very hot conditions over summer may have been responsible. Despite this, there were significant differences between treatments for grub numbers (Table 18). There was a significant interaction between effects of Confidor and BioCane when results were analysed as both the raw grub counts ($P_{interaction}$ = 0.017) and after transformation as log(x+1) ($P_{interaction}$ = 0.006). The likely cause is the complete mortality at the highest rate of Confidor, such that no additional response could be achieved by addition of BioCane. Unfortunately, grub numbers are too low to critically interpret the interaction. Ignoring the interaction, numbers of live grubs were significantly affected by both Confidor (P = 0.013) and BioCane ($P < 0.001$) (Table 18). Only one *Metarhizium*-infected grub cadaver was found over all pots. There were no significant treatment effects on cane weights (Table 18, $P > 0.05$).

TABLE 18
Numbers of live greyback canegrubs and above-ground fresh weight of cane (mean ± SE) in pots treated with different combinations of BioCane and Confidor.

BioCane (kg/ha)	Confidor (L/ha)				
	0	**0.5**	**1**	**2**	**Mean**
Live grubs					
0	0.8 ± 0.3	1.3 ± 0.3	1.3 ± 0.4	0.2 ± 0.2	0.9 ± 0.2 a
33	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0 b
66	0.2 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.1 ± 0.0 b
Mean	0.4 ± 0.1 a	0.5 ± 0.1 a	0.5 ± 0.2 a	0.1 ± 0.1 b	0.3 ± 0.1
log(grubs+1)					
0	0.195	0.330	0.285	0.040	0.212
33	0.050	0.025	0.025	0.000	0.025
66	0.050	0.000	0.050	0.000	0.025
Mean	0.098	0.119	0.120	0.013	0.088
Cane wt (kg)					
0	2.1 ± 0.2	2.6 ± 0.5	2.9 ± 0.5	2.9 ± 0.3	2.6 ± 0.2
33	2.6 ± 0.1	2.6 ± 0.2	2.9 ± 0.4	2.4 ± 0.1	2.6 ± 0.1
66	2.5 ± 0.1	2.7 ± 0.2	3.0 ± 0.2	3.1 ± 0.2	2.8 ± 0.1
Mean	2.4 ± 0.1	2.6 ± 0.2	2.9 ± 0.2	2.8 ± 0.1	2.7 ± 0.1

For live grubs, Confidor or BioCane means in either columns or rows followed by the same letter were not significantly different (P = 0.05).

5.0 CANEGRUB DENSITY AND CONTROL BY BIOCANE

5.1 Canegrub density trial

5.1.1 Materials and methods

A trial was designed in microplots to measure the biomagnification of *Metarhizium* as affected by annual canegrub density within the plots. Factorial combinations of three densities, zero, moderate and high, were to be established in the first and second years. The infectivity of inoculum in each microplot following the different histories of grub infestation was to be tested annually by a bioassay *in situ*, that is a moderate density of grubs would be added to the microplots and the numbers of surviving grubs counted subsequently (Table 19).

In the first attempt at this trial, ten rows of cultivar Q165 were planted at the Burdekin BSES in April 1998. Seven rows of the block were treated with BioCane at 33 kg/ha in mid-August 1998. Sheet metal enclosures (400 mm wide, 300 mm deep) were placed around individual stools in December. A mixture of field-collected and lab-reared larvae was introduced to enclosures in mid-February 1999. Enclosures were sampled 8 weeks after larvae were introduced. Few canegrubs were found in or immediately below enclosures (12% of introduced canegrubs), and there was no effect of BioCane on canegrub density. Spore counts from soil taken around marker beads in the BioCane-treated rows were extremely low ($9 \times 10^2 - 2 \times 10^3$ spores/g soil). BioCane applied to the trial site came from one of the batches ultimately rejected for the 1998 semi-commercial trials due to inconsistent spore viability. Consequently, this trial was abandoned. The plant crop was harvested and ploughed out in June 1999 and the trial re-established.

TABLE 19
Experimental plan for history of canegrub infestation x BioCane efficacy trial. 'Bioassay' indicates that treatments would be destructively sampled to estimate grub survival; otherwise pots and grubs were not disturbed.

Treatment		Planned date of introduction and number of introduced larvae/enclosure			Planned sample date
		Plant (Feb 00)	1R (Feb 01)	2R (Feb 02)	
BioCane	1	25 (bioassay)			P(Apr 00)
	2	0	25 (bioassay)		1R (Apr 01)
	3	25	25 (bioassay)		1R (Apr 01)
	4	100	25 (bioassay)		1R (Apr 01)
	5	0	0	25 (bioassay)	2R (Apr 02)
	6	0	25	25 (bioassay)	2R (Apr 02)
	7	0	100	25 (bioassay)	2R (Apr 02)
	8	25	0	25 (bioassay)	2R (Apr 02)
	9	25	25	25 (bioassay)	2R (Apr 02)
	10	25	100	25 (bioassay)	2R (Apr 02)
	11	100	0	25 (bioassay)	2R (Apr 02)
	12	100	25	25 (bioassay)	2R (Apr 02)
	13	100	100	25 (bioassay)	2R (Apr 02)
Untreated	14	25 (bioassay)	25 (bioassay)	25 (bioassay)	All crops (Apr)

The new trial was planted in July 1999, and BioCane was applied to seven rows in November. In December, trenches 600 mm deep in the interrow and across the row were dug with the aid of ditchwitches and a Bobcat. Plastic sheeting was installed in the trenches to provide enclosures 3 m long x 1 row wide x 0.6 m deep for the introduction of canegrubs. Plots were hand-infested with laboratory-reared eggs and first instars in December 1999 and January 2000. High-density plots received 120 eggs and first instars; low-density plots received 35 eggs and first instars. The plant cane plots were sampled in late March 2000. Very few larvae were retrieved and no reliable estimate of mortality due to BioCane in the plant crop was made. Therefore, third instars collected from the field were introduced to plots as replacements in late March. High-density plots received 24 larvae per plot and low density plots received 8 larvae per plot. Plots were not sampled for surviving larvae. However, emergence holes made by greyback beetles were counted in December 2000.

Field-collected second and early third instar greyback larvae were introduced to first ratoon plots in the field trial in early February 2001. Plots were sampled for surviving canegrubs 9 weeks later in early April.

A greenhouse trial was run concurrently with the plant crop to estimate survival of larvae introduced as first or third instars in 0.2 m^2 concrete cubicles with and without BioCane. First-instar larvae were introduced at 25 per cubicle to six cubicles in January 2000. Third instars were introduced at 12 per cubicle to four cubicles in March 2000. Half of the cubicles had been treated with BioCane at approximately 33kg/ha in late December. Cubicles were checked for larvae in April 2000.

5.1.2 Results

Low numbers of beetle emergence holes were found in the young first ratoon crop with maximum survival of larvae to beetles being 25%. Most holes were found in plots that had the highest numbers of larvae introduced (Table 20).

TABLE 20
Greyback beetle emergence holes in plots in canegrub density trial, December 2000.

Grubs introduced to plant plots (Mar 00)	Mean ± SD holes (Dec 00)	Range
0	0.2 ± 0.4	0-1
8	0.3 ± 0.5	0-1
24	1.4 ± 1.9	0-6

There was no difference in the number of surviving larvae per treatment in the first ratoon crop in March ($F_{3,16} = 0.66$, $P = 0.58$) following different infestation levels in the plant crop (Table 21). The trial area was naturally infested with about 1 grub per stool.

TABLE 21
Infestation history, number of cadavers infected with *Metarhizium*, and numbers of recovered larvae in a first ratoon crop treated with BioCane

Treatment (see Table 19)	Grubs introduced to plant plots (Mar 00)	Grubs introduced to 1R plots (Feb 01)	Grubs retrieved in 1R (Apr 01) Mean ± SD	Cadavers in 1R (Apr 01) Mean ± SD
BioCane 2	0	40	6.2 ± 5.3	1.6 ± 0.6
BioCane 3	8	40	5.8 ± 2.4	1.0 ± 0.7
BioCane 4	24	40	3.8 ± 2.6	1.8 ± 0.5
Untreated	0	40	6.2 ± 3.0	0

In a greenhouse trial, all larvae found in cubicles in April 2000 were third instars. More third instars were retrieved from untreated cubicles than from BioCane-treated cubicles (Table 22), but the difference was not statistically significant (L1: χ^2=2.45, P = 0.12; L3: χ^2=1.5, P = 0.22). Survival of larvae introduced as first instars was less than survival of larvae introduced as third instars (χ^2=75.3, P < 0.001, Table 22). The poor survival of first instars confirms experience in the field plots.

TABLE 22
Survival of greyback larvae introduced at different ages to soil with and without BioCane in a greenhouse

Stage at introduction	Sample day after introduction	Percent survival	
		Untreated	BioCane
L1	87	11	4
L3	32	75	58

A decision was made at the project review in July 2001 not to persevere with this experiment, due to ongoing technical difficulties.

5.2 Role of sporulated cadavers in control of canegrubs

5.2.1 Materials and methods

5.2.1.1 Persistence of cadavers in soil

Cadavers of greyback canegrubs with *Metarhizium* are potentially a source of infection for greyback canegrubs of future generations. As BioCane kills about 50% of greyback canegrubs by March, cadavers should occur as frequently as grubs, but they are not often seen. We measured the period during which cadavers remain intact and recognisable, for cadavers in laboratory containers (550 mL at 27°C) and for fresh cadavers buried in a sugarcane field in April-May. These data may be useful in understanding the role of cadavers in future infections.

5.2.1.2 Persistence of spores on cadavers

This experiment aimed to determine whether the persistence of spores on cadavers was similar to that of spores on BioCane granules. The trial site was a canefield at Mackay. The trial design was a single treatment, ie a fresh greyback cadaver infected with FI-1045, with five replicates of five times of retrieval, 0, 1, 3, 6 and 12 months. Each PVC ring was a 51 mm internal diameter cylinder consisting of two 25 mm halves. The two halves were packed with native soil (total 100 g), a cavity was moulded in the centre, a cadaver was placed in the cavity, and the halves were taped together and then buried on 8 June 2001.

5.2.1.3 Distribution of cadavers in soil

A first ratoon block on Sgarbossa's farm, Upriver Home Hill was sampled in July 2000. The plant crop had been treated with BioCane in 1998, and was not damaged by canegrubs; canegrub numbers were <0.5 per stool. However, the first ratoon block was damaged and heavily infested with canegrubs. Trenches (n = 22) approximately 80 cm wide were excavated in the first ratoon block and the number of greyback canegrubs and cadavers of canegrubs infected with *Metarhizium* counted to 70 cm depth in 10 cm increments.

5.2.1.4 Bioassay of cadavers

A bioassay was established with Chandler tubes to determine mortality of larvae passing through soil with a field-aged cadaver or with a single BioCane granule. Cadavers were from a bioassay of BioCane in 2000. Cadavers and BioCane granules were introduced to soil in rings in November 2000 and retrieved in February 2001 and January 2002. Larvae were introduced to tubes (n = 25 per treatment) and those that passed through were kept in an incubator at 27°C and fed carrot sections. Survival was checked at weekly intervals for 6 weeks.

5.2.1.5 Effect of cadavers on canegrub mortality in bins

The effect of *Metarhizium*-infected cadavers on mortality of greyback canegrub was assessed using garbage bins (75 L) buried in soil. Bins were placed in a line of six in each of five trenches. Each bin had drainage holes in the base covered by netting to prevent escape of larvae and was half-filled with coarse river sand to promote drainage. The remainder of the bin was filled with a loamy-sand and planted with billets of Q117. Factorial combinations of BioCane (0 and 33 kg/ha) and cadaver density (0, 1 and 4/bin) were randomly assigned to bins in each row. Cadavers were from a bioassay of BioCane and had been stored in the laboratory for 8 months. BioCane and cadavers were placed on the soil surface approximately 10 cm above planted billets and then covered with 10-15 cm of soil. Larvae were introduced to bins in February at 16 per bin. After 9 weeks, bins were removed from trenches and the sand and soil checked for *Metarhizium*-infected cadavers and surviving larvae.

5.2.2 Results

5.2.2.1 Persistence of cadavers in soil

Most cadavers had disintegrated and were no longer recognisable after 2 weeks at 27°C or in soil in the field (Fig. 6). All cadavers had disintegrated after 6 weeks.

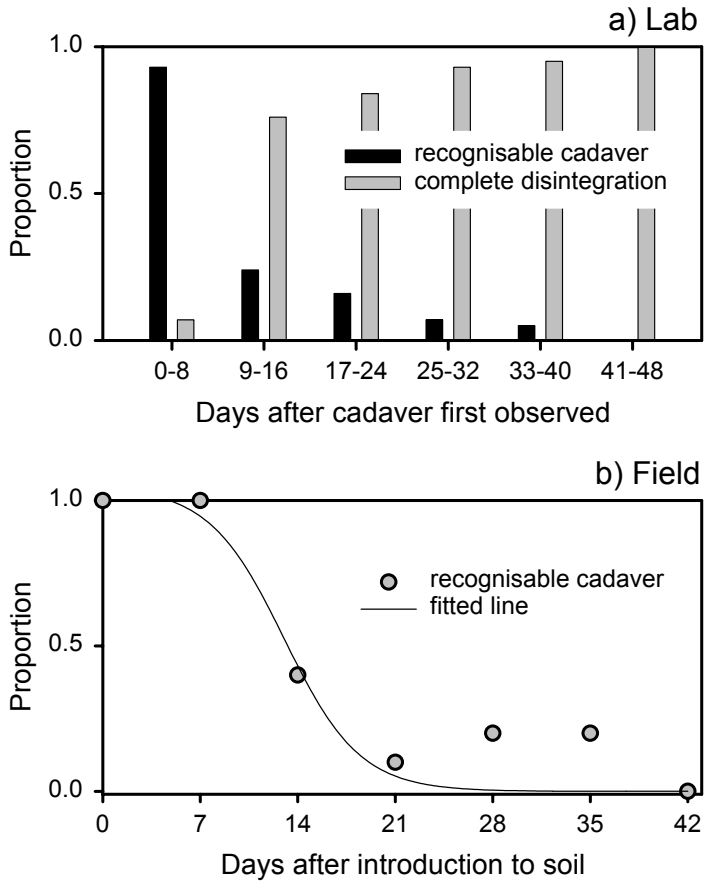

Figure 6. Persistence of *Metarhizium*-infected cadavers in a recognisable state in a) soil in containers and b) in soil in the field.

5.2.2.2 Persistence of spores on cadavers

The rate of decline of spore levels around cadavers was relatively fast (Fig. 7), compared with similar measurements on BioCane granules (Final Report, BSS246). The annual survival rate was only 14%, compared with about 50% in experiments with BioCane. It is possible that decomposition of the cadaver affects persistence, but we would need to carry out a longer-term experiment with a simultaneous comparison of cadavers and BioCane granules to confirm this.

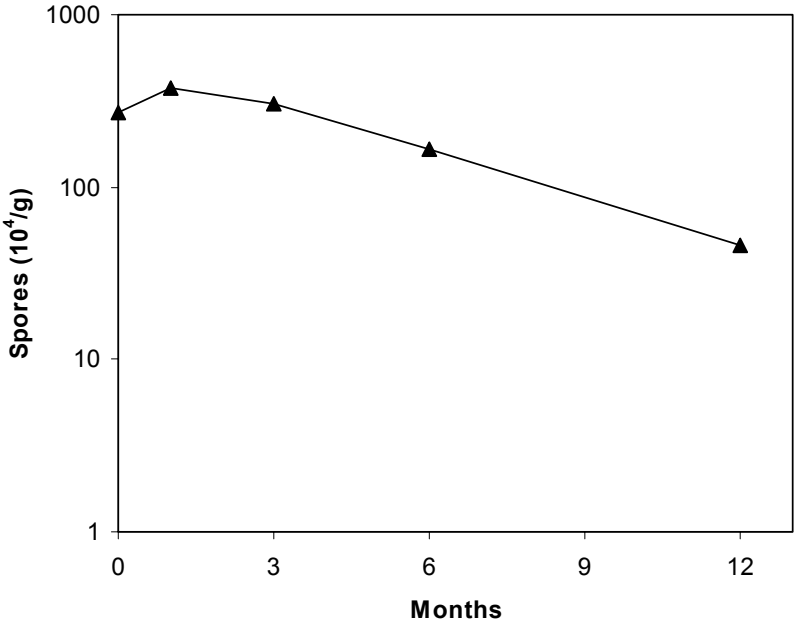

Figure 7. Spore concentrations in PVC rings containing greyback canegrub cadavers infected with FI-1045.

5.2.2.3 Distribution of cadavers in soil

In July 2000, cadavers were found from <10 cm deep to as deep as 60 cm (Fig. 8). Many cadavers had not sporulated, but did so later in the laboratory. Peak frequency of spores, cadavers and third instars coincided at 30-40 cm.

In other occasional observations, cadavers of first and second instars greybacks with *Metarhizium* were found <10 cm deep in cane in January. In February and March, cadavers of third instars with *Metarhizium* were found within the sampling zone (0-30 cm).

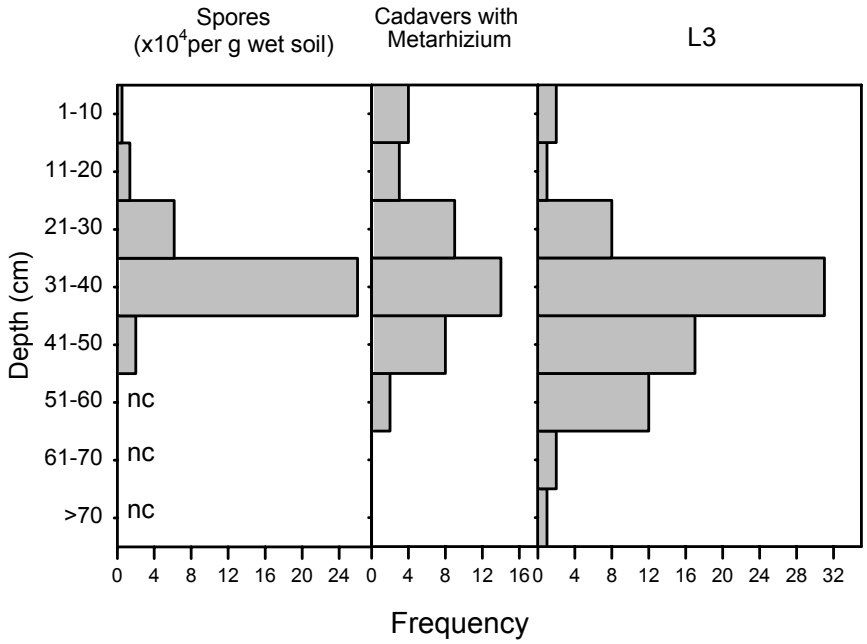

Figure 8. Distribution of *Metarhizium* spores, cadavers with *Metarhizium* and late third instar larvae of greyback canegrub in the soil profile in July 2000.

5.2.2.4 Bioassay of cadavers

When cadavers and BioCane granules in tubes were retrieved from fields after 3 months, cadavers were highly infective to grubs, whereas few grubs responded in tubes containing BioCane (Fig. 9).

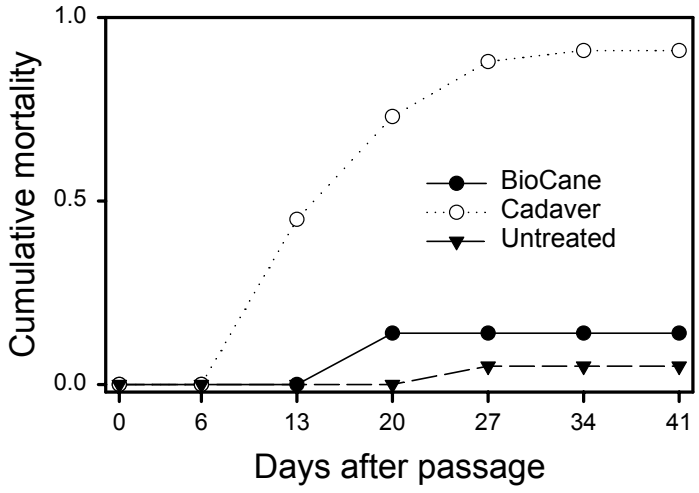

Figure 9. Mortality of greyback third instars after passage through Chandler tubes with either a field-aged cadaver, a BioCane granule or soil only.

Soil containing a field-aged cadaver was still infective to grubs in February 2002 (Fig. 10). Soil containing a single field-aged BioCane granule was not infective. All dead grubs in both treatments showed symptoms of *Metarhizium* infection, while mortality in untreated soil appeared due to other causes.

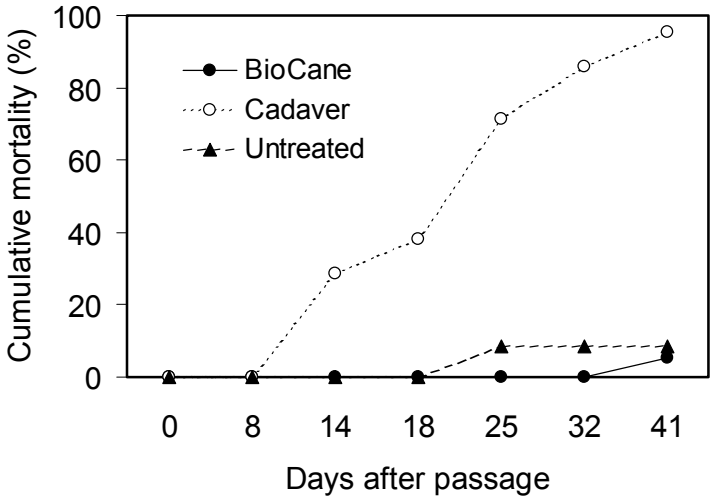

Figure 10. Mortality of greyback third instars after passage through Chandler tubes with either a cadaver or a BioCane granule, both field-aged for 15 months, or soil only.

5.2.2.5 Effect of cadavers on canegrub mortality in bins

TABLE 23
Number of surviving greyback larvae and total *Metarhizium*-infected cadavers 9 weeks after larvae were introduced to bins with combinations of BioCane and *Metarhizium*-infected cadavers.

Treatment	Live grubs ± SE	Total new cadavers in bins	Additional cadavers after 18 days in lab.
BioCane	6.2 ± 0.9	10	17
1 cadaver	10.2 ± 0.8	0	3
BioCane + 1 cadaver	5.8 ± 1.2	10	14
4 cadavers	9.0 ± 0.8	0	0
BioCane + 4 cadavers	4.2 ± 1.2	11	7
Untreated	8.6 ± 1.1	2	5

Bins with BioCane had fewer living grubs than those without ($F_{1,20}$ = 26.4, P < 0.001) (Table 23). There was no significant effect of cadavers on numbers of surviving grubs ($F_{2,20}$ = 1.2, P = 0.33), and no interaction ($F_{2,20}$ = 1.0, P = 0.40) Most new cadavers were recovered from treatments with BioCane (Table 23). Additional larvae retrieved from bins then died of *Metarhizium* in the laboratory, including some larvae from control bins (Table 23). The original cadavers seem to have had no effect on canegrub mortality, but they had been stored for a long period since death of the host.

6.0 COMMERCIAL SCALE TRIALS OF BIOCANE

6.1 Materials and methods

Data for infestation of ratoon crops treated with *Metarhizium* in the plant crop were collected as part of this project and BSS134. First ratoon blocks (n = 25) treated with BioCane during 1997 were sampled for greyback canegrubs in 1999. Six trials were sufficiently infested to justify complete sampling. Two of the six trials had been infested in the plant crop.

Second ratoon blocks (n = 18) treated in 1997 with BioCane were sampled in March 2000. Only four had reasonable grub numbers (≥1/stool for one or more treatments).

6.2 Results

In the first ratoon, average grub numbers in six fields that had been treated with BioCane at 5 g/m were 40% lower than in untreated fields (Table 24). However, two of the farms had no untreated cane. If only the farms with untreated strips were considered, then the apparent reduction in grub numbers was 24%.

TABLE 24
Mean grub numbers/stool for first ratoon crops treated with BioCane or suSCon Blue in 1997.

Farm	Untreated	BioCane 5 g/m	BioCane 10 g/m	suSCon Blue
Cjetanovic		0.9	0.8	1.0
Fabbro		0.4		1.2
Fowler	1.9	0.5	1.7	
McCubbin	0.9	1.7	0.5	
Menso	2.2	2.0	0.2	
Pilchowski	2.8	1.7	1.1	
Average	2.0	1.2	0.9	1.1

TABLE 25
Mean grub numbers/stool for second ratoon crops treated with BioCane or suSCon Blue in 1997.

Farm	Untreated	BioCane 5 g/m	BioCane 10 g/m	suSCon Blue
Felesina	2	0.9	0.6	
Lyons		1.53	1.3	1.4
Pilchowski	1.7	0.6	0.4	1.2
Swindley	1.2	1.06	1.5	1.9
Average	1.63	1.0225	0.95	1.5

In the second ratoon, the apparent reduction in grub numbers was 37% as an overall average, or 48% considering only the three farms with untreated strips (Table 25).

7.0 BIOCANE FORMULATIONS

7.1 Tube experiment

7.1.1 Materials and methods

This experiment aimed to determine the number of *Metarhizium* spores required to cause a satisfactory infection of greyback canegrubs when all spores were present at a single point in soil, with a view to designing an effective granule containing fewer spores than current rice-based granules. Response to the point source would be compared with the effect of spores mixed evenly through soil.

The isolate used was FI-1045, tested against greyback canegrub. Grubs were forced to contact the point source of spores by allowing them to pass through an open-ended vertical tube with the point at its centre. Different numbers of spores, in either water or a powder carrier or on rice, were placed at a single point within each tube, or the same number of spores in water was mixed throughout the soil. Grubs that emerged from the tube were then confined in clean medium.

The trial design was:

- aqueous dilution point source (0.05 mL) of 2 x 10^7, 2 x 10^6, 2 x 10^5, 2 x 10^4 spores;
- solid (peat) dilution point source (10 mg) of 2 x 10^7, 2 x 10^6, 2 x 10^5, 2 x 10^4 spores;
- aqueous dilution mix (2 mL) of 2 x 10^7, 2 x 10^6, 2 x 10^5, 2 x 10^4 spores (/110 g soil);
- single rice-based granule (ca. 14 mg, or about 5 x 10^7 spores if 3.5 x 10^9/g);
- untreated control;

= 14 treatments x 20 reps
= 280 tubes.

Each bioassay container consisted of a PVC tube, 150 mm long x 29 mm id, containing soil and placed vertically into a 150 mL cup containing peat. Each tube was split in half and the halves joined by plastic pipe, to facilitate dosing at the centre.

A grub was released in the top of each tube and the tube capped. The finished tube was stood in a cup of peat on top of a slice of carrot. Tubes were removed after grubs had emerged into the cup beneath. Grubs were then observed weekly at 28°C.

7.1.2 Results

Some grubs had not emerged from the bottom of the tubes within 1 week. The tubes were then dismantled and remaining grubs transferred to peat, but many of the unemerged grubs subsequently died with no evidence of *Metarhizium* infection. Unemerged grubs were therefore omitted from analyses.

Response of grubs to treatments was slow, even at the highest doses (Fig. 11). There was little evidence that the artificial point sources were particularly effective compared with a spore mixture through soil or single BioCane granules. For some reason, only a small proportion of dead grubs exhibited overt symptoms of *Metarhizium* infection in this experiment (Fig. 11).

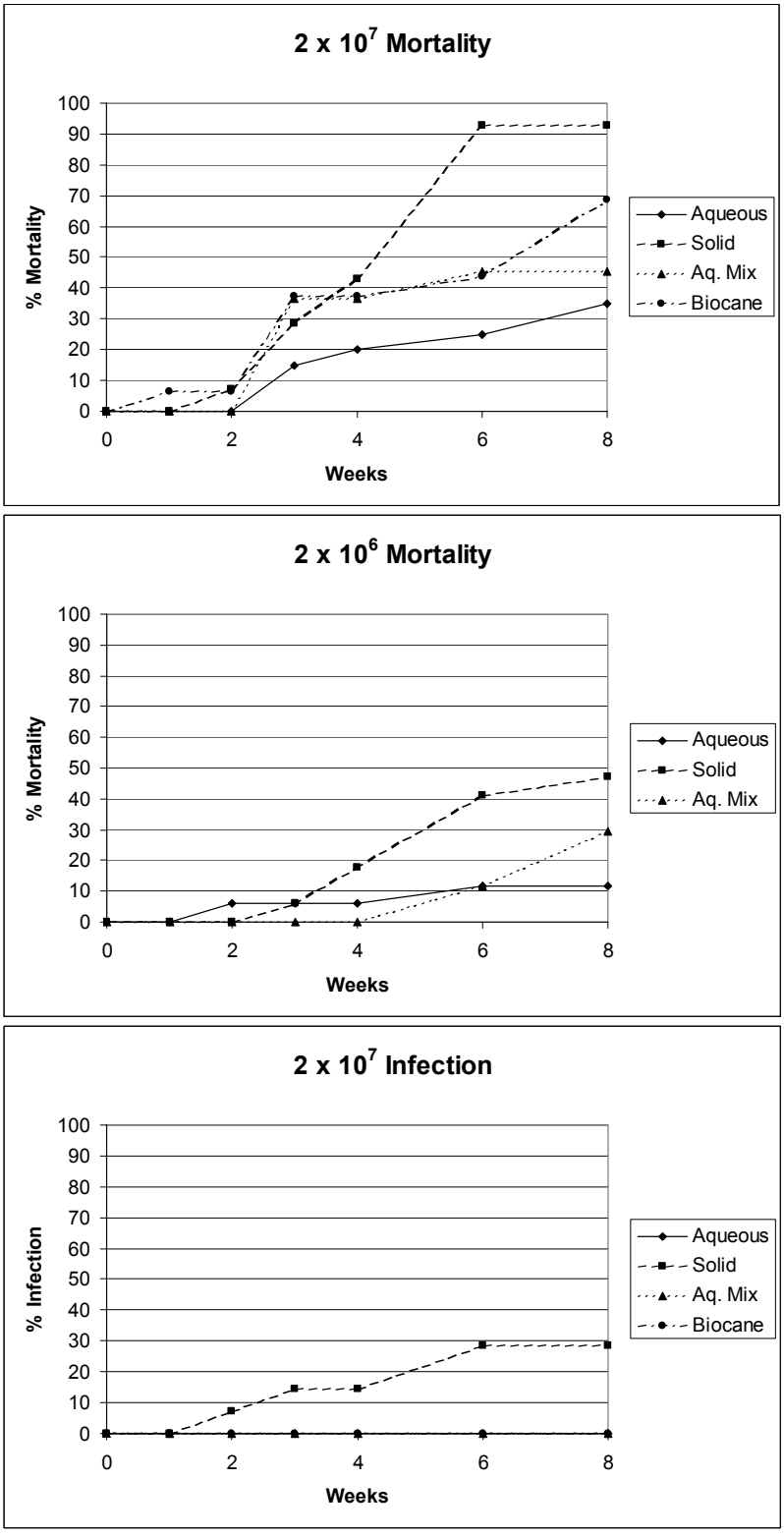

Figure 11. Response of greyback canegrubs after exposure to point sources or mixtures of FI-1045 in soil in hollow tubes; highest doses only. Control response was zero.

7.2 Bin experiment

7.2.1 Materials and methods

An experiment was established in large bins to investigate different formulations of BioCane, with the aim of optimising efficacy for a given number of spores. Granular formulations were standard BioCane granules, or granules with either a high spore load (5x standard, obtained by grouping five standard granules together in soil) or a light spore load (1/5x standard, obtained by rubbing spores from standard granules), and these were used with different frequencies of granules per metre (Table 26). In addition, a suspension of spores in water sprayed onto soil was applied at two rates. Treatments were applied on 14 November 2001 and replicated five times; 30 first instar greybacks were added to each bin on 13 December 2001 and were sampled and cane tops weighed on 20-21 March 2002.

TABLE 26
Treatment combinations in bins; standard (std) treatment corresponds with BioCane at 5 g/m.

Trt #	Granules/m	Spores/granule	Spores/m
1	1/5 x std	5 x std	std
2		std	1/5 x std
3	std	std	std
4		1/5 x std	1/5 x std
5	5 x std	1/5 x std	std
6	spray	na	std
7			1/5 x std
8	Untreated		

7.2.2 Results

TABLE 27
Numbers of live and infected greyback canegrubs and above-ground fresh weight of cane in bins treated with different BioCane formulations and application rates (std = standard). Treatments are ranked in order of decreasing numbers of live grubs.

Spores per m	Granules per m	Spores per granule	Live grubs (mean ± se)	Infected grubs (mean ± se)	Cane (kg) (mean ± se)
1/5 x std	std	1/5 x std	6.0 ± 2.0	0.0 ± 0.0	5.9 ± 1.0
Nil			5.4 ± 1.4	0.0 ± 0.0	4.4 ± 0.7
1/5 x std	1/5 x std	std	4.2 ± 1.5	0.0 ± 0.0	3.9 ± 0.2
std	5 x std	1/5 x std	3.6 ± 1.1	0.2 ± 0.2	6.0 ± 0.7
1/5 x std	spray		3.6 ± 1.1	2.0 ± 1.3	4.8 ± 2.1
std	1/5 x std	5 x std	2.6 ± 0.9	1.2 ± 1.0	5.9 ± 0.8
std	spray		1.8 ± 0.7	0.4 ± 0.2	7.1 ± 1.2
std	std	std	1.4 ± 0.7	0.0 ± 0.0	6.4 ± 0.9

Unfortunately, grub survival was poor even in untreated pots (27%), which would have reduced the precision of this experiment. The number of live grubs did not differ significantly between treatments (P = 0.092). The treatments are ranked in Table 27. Taking the results at face value and ignoring the statistics, the standard BioCane application – standard granules at the standard application rate – was one of the most effective treatments. Changing the formulation to either make the frequency of granules higher or to make each granule 'larger', while maintaining the same number of spores/m, did not improve efficacy. A spore suspension sprayed on to soil seemed quite effective, but only when using the standard rate of spores per m of row. The number of infected grubs and the weight of cane did not differ significantly between treatments (P = 0.18 and 0.37, respectively).

8.0 DISCUSSION

8.1 Effect of farming practices on BioCane

Metarhizium spores were concentrated in the rows in ratoon crops (3.1.2.2), either as surviving spores from the original BioCane application or as new spores produced by cadavers that are likely to be aggregated beneath stools. The displacement of plastic beads suggests that minimal lateral movement of spores may result from seedbed preparation. However, the rows themselves are likely to be displaced when crops are replanted unless minimum tillage planting into the old rows is practiced. In one field site prepared conventionally, spore concentrations were much lower in the new crop than they had been in the old crop (3.1.2.1).

There was no effect of a trash blanket on grub response to BioCane in microplots or in bins. There is some concern with the microplot experiment (3.2.2.1), as no cadavers were recovered and there were no Control plots without BioCane to ensure that the product was viable. However, the bin experiment (3.2.2.2) did show an effect of BioCane that was not affected by the presence or absence of trash. Death of grubs following contact with BioCane may be slower at lower temperatures (<24°C), but the apparent difference was small (3.2.2.3). Spore concentrations in soil were not significantly affected by trash blanketing in most experiments (3.2.2). Overall, we were unable to conclude that trash blanketing will influence the efficacy of BioCane in commercial fields.

There was no deleterious effect of the insecticides suSCon Plus, Confidor CR (controlled release) or Confidor SC (liquid), or of the fertilisers sulphur, gran-am or urea, on the survival of spores on BioCane granules in PVC rings (3.3.2). This is in agreement with field observations, where there have been no complaints from farmers who have applied BioCane close to some of these other products in commercial practice. However, abnormally low concentrations of spores in the rings indicate technical problems with the product that reduce confidence in the results.

8.2 Combinations with insecticides

In the three trials conducted with suSCon Plus, there was little or no benefit from including BioCane in a combination treatment. BioCane was ineffective at Lucas (4.1.2.1) in both the plant and first ratoon crops, for unknown reasons; there may have

been problems with the product or with its application. At Mottin (4.1.2.2), BioCane at the commercial rate of 33 kg/ha reduced grub numbers in the plant crop with no suSCon applied, but its effect in combination was masked by the much stronger effect of suSCon. Grub numbers were low in the first ratoon and neither product had a detectable effect. At Fabbro (4.1.2.3), the addition of BioCane to the full rate of suSCon Plus did not improve grub control in the plant crop or the first ratoon. A half rate of suSCon Plus together with BioCane was equally effective as the full rate alone, but the efficacy of the half rate without BioCane was not tested.

The combination of BioCane with Confidor as a plant cane treatment gave promising results in the plant crop in one trial at McDonald (4.2.2.1). There was no interaction between effects of the two products in the statistical analysis of yields, that is, the effect of each seemed to be additive. Both Confidor and BioCane reduced numbers of live grubs when applied together in pots (4.2.2.2).

In summary, this project taken alone has not produced good justification for the use of BioCane combined with chemical insecticides. However, other trials are in place to collect additional data. Available evidence suggests only additive effects, although synergy that might lead to an interaction in commercial use has been demonstrated experimentally for *Metarhizium* and Confidor against some other insects.

8.3 Role of biomagnification

A complex and labour-intensive trial to measure the effect of canegrub density on biomagnification and consequent efficacy of *Metarhizium*, which was intended to be a key part of this project (see Project Proposal), ultimately failed despite two attempts to establish the experimental treatments (5.1.2). It proved to be impossible to reliably establish the required canegrub densities in the experimental microplots. We suspected the first attempt had failed due to escape of grubs from the microplots, so more elaborate enclosures were used in the second. However, grub establishment was again poor, and high mortality of first instars seemed to have been the cause. This trial was abandoned by mutual agreement at the project review in July 2001.

Cadavers are not often recovered from BioCane-treated fields because they are no longer recognisable after 2-3 weeks (5.2.2.1). It is not certain whether spores around cadavers are as persistent as those around BioCane granules (5.2.2.2). However, a bioassay showed that soil that had contained cadavers remained highly infective to greybacks for at least one year (5.2.2.4). Work within Project BSS246 showed that each cadaver from a third instar greyback grub produced about 3.9×10^9 spores ($n = 10$). The commercial application rate of BioCane per metre of cane row, 5 g, is 2×10^{10} spores or the equivalent of five cadavers. Thus there is the potential for cadavers to add a significant amount of inoculum to canefields, eg 50% infection of a grub population of 10 per metre (five per stool) should produce the same number of spores as an application of BioCane. This infestation density would cause unacceptable cane damage, but annual infection at lower densities has the potential to top-up spores in soil, although probably not enough to prevent a gradual decline in levels.

An unknown factor is whether the highly aggregated distribution of spores around cadavers is likely to result in significant infection of new hosts. Placement of cadavers in

bins resulted in negligible infection of grubs (5.2.2.5), but cadavers were old and the viability of spores is unknown. In one field in July, most cadavers were found 30-40 cm deep, reflecting the distribution of healthy grubs (late third instar) at that time of year (5.2.2.3). Spores produced at that depth are unlikely to contact young grubs of the next generation. However, cadavers produced during summer-autumn may be much shallower in the soil.

8.4 Long-term results of BioCane in commercial scale trials

Grub numbers measured in the first and second ratoons of commercial trials established in 1997 did not provide convincing evidence for long-term benefits of BioCane, partly because of the small number of infested trials and also because some did not include untreated strips. There is also the complication that occurs with all greyback trials, that plots protected by pesticides in one year may suffer disproportionate damage in the following year because of the attraction of ovipositing beetles to taller cane. Taken at face value, results suggest reductions in grub numbers of 20-40% in ratoon crops (6.2).

8.5 BioCane formulations

A bin experiment to compare different BioCane formulations and consequent changes in spore distribution in soil, while not producing statistically significant differences between treatments, did indicate that a spore suspension sprayed in a band in soil may have comparable efficacy to the standard granular formulation (7.2.2). An experiment with different formulations in tubes also indicated that the more even distribution of spores that results from a liquid formulation should have comparable efficacy to granules (7.1.2). Studies within BSS134 showed that long-term spore survival was as good for a spore suspension as for BioCane granules. A liquid *Metarhizium* product could be used in novel ways that may allow new grub control strategies, eg treatment with vertical bands in ratoon crops.

9.0 RECOMMENDATIONS FOR FURTHER R, D & E

Further R&D with BioCane should focus on developing new uses for the product in its current form or in new formulations. BioCane has advantages that would allow it to be part of novel control techniques:

- the spores are long-lived in soil, with an annual survival rate of almost 50%;
- the spores are self replicating if sufficient grubs are present;
- the spores can be formulated in various ways, as their survival rate is not dependent on formulation;
- the action of *Metarhizium* against certain pests can be synergised by chemicals, particularly imidacloprid (Confidor).

There is potential for BioCane to be used in new ways to improve grub methods in ratoon crops. There are two approaches:

1. Applying BioCane in combination with complementary chemical pesticides to plant crops, with the chemical intended to improve control in the plant crop and with BioCane intended to improve control in subsequent ratoons, in comparison with each product separately.

2. Developing a method for applying liquid formulations of BioCane or of BioCane/chemical combinations to ratoon crops.

Further extension of current BioCane practice should include its promotion in districts outside the Burdekin, as its use is currently restricted almost entirely to that region, and continuing risk assessment within the GrubPlan program to allow BioCane to be targeted at low-risk fields.

10.0 REFERENCES

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